

Effect of soil disturbance and biocides on nematode communities and extracellular enzyme activity in soybean cyst nematode suppressive soil

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Summary – Soybean cyst nematode (SCN), *Heterodera glycines*, remains a major yield limiting pathogen of soybean. Natural suppression of SCN exists and becomes increasingly attractive; however, ecological mechanisms leading to the suppressive state are rarely studied. A glasshouse experiment was performed to determine the effects of soil disturbance and biocides on nematode community and extracellular enzyme activities in the SCN-suppressive soil collected in 2007 and 2008. Soil disturbance was simulated by passing soil through a sieve (aperture 5 mm) and compared with no-disturbance (non-sieve) treatment. Composition of microbial communities was manipulated by applying captan (fungicide), streptomycin (bactericide), captan plus streptomycin, or no biocide. SCN egg population density, proportion of second-stage juveniles (J2) parasitised by fungi, nematode communities in the soil, and plant weight in each pot were determined 70 days after planting soybean. In addition, the activities of six selected hydrolytic and oxidative extracellular enzymes representing cellulose, chitinase, serine protease, collagenase and peroxidase were measured. Soil disturbance resulted in an increase in SCN egg population density and reduction in the proportion of J2 parasitised by fungi. Biocide treatments increased SCN egg population density and the proportion of J2 parasitised by fungi at the end of experiment. Values of nematode community diversity index decreased and dominance and maturity indices increased in the disturbed soil compared with the no-disturbance treatment. Biocide treatments reduced maturity index values exclusively. With soil disturbance, the activity of extracellular enzyme L-proline aminopeptidase activity declined to less than half of that under no-disturbance in 2007. This experiment showed that both bacteria and fungi were potentially involved in the soil suppressiveness to SCN: soil disturbance and biocide application may reduce natural soil suppressiveness that was potentially associated with soil nematode community diversity and microbial enzyme activities.

Keywords – biocontrol, extracellular enzyme assay, *Heterodera glycines*, nematode diversity, soil suppression.

Soybean cyst nematode (SCN), *Heterodera glycines*, is a major yield limiting factor in soybean production (Chen *et al.*, 2001). Estimated losses attributed to this pathogen exceed US\$ 1 billion annually in the USA (Wrather & Koenning, 2006). Numerous aspects of the biology of the nematode enable this organism to be a successful and highly virulent pathogen, including the variation in virulence phenotypes, a narrow host range, a synchronisation of host and parasite life cycles and the evolution of strategies for survival (Niblack & Riggs, 2004). Nematicides, resistant cultivars and non-host crop rotations are the current major management tactics for SCN. Although nematicides have been used successfully to control the nematodes on soybean, economic and

environmental issues have increasingly limited their use (Johnson & Feldmesser, 1987; Rich *et al.*, 2004). Some SCN-resistant cultivars may not be durable or may not be of a sufficiently high level to avoid crop yield loss. Thus, the impetus to develop commercial biological products to control SCN may be increasing today.

Modern agriculture, emphasising intensive and high input production practices, may at times be in conflict with environmental considerations and sustainability. Recently, high input production practices have been decreasing, and a survey conducted in 2004 revealed that 41% of all cropland in the USA is under conservative tillage (Towery, 2004). A number of studies have reported on the effects of tillage on SCN in the USA. In the south-

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ern USA, no-tillage reduced the SCN population density compared with conventional or minimum tillage (Hersman & Bachi, 1995; Koenig *et al.*, 1995). Research in the north-central USA demonstrated inconsistent effects of tillage on SCN in the region. Workneh *et al.* (1999) reported that of all fields infested with SCN, the population densities were smaller in no-tillage fields than in fields that had received some type of tillage. Increased SCN reproduction was reported in no-tillage soils as compared with conventional tillage in Illinois (Noel & Wax, 2003). However, no effect of tillage on SCN egg population density was observed in Minnesota (Chen *et al.*, 2001; Chen, 2007a). There is a need to determine whether or not conservation tillage (no-till, ridge till and mulch till) improves soil health and soil environmental quality compared with conventional tillage. The long-term direct and indirect impact of conservative tillage on soil microfauna and microflora is not fully understood (Duncan & Noling, 1998).

Natural suppression of SCN has been documented worldwide (Hartwig, 1981; Carris *et al.*, 1989; Liu & Wu, 1993; Kim & Riggs, 1994; Chen *et al.*, 1996; Sun & Liu, 2000; Chen, 2004, 2007b). Soil suppression can be general suppression or general suppression plus specific suppression (Weller *et al.*, 2002; Westphal, 2005). General suppression may develop over a long period of time with some cultural practices. Many groups of organisms may be involved in regulation of nematode populations through predation, parasitism and competition for food in general suppression. In contrast to general suppression, specific suppression is caused by one or few major antagonistic organisms. Fungi and bacteria have been the most widely tested microorganisms for biological control of plant-parasitic nematodes (Kerry, 2000). A glasshouse study demonstrated that both *Hirsutella rhossiliensis* and *H. minnesotensis*, two nematode-endoparasitic fungi, had potential as biological control agents of SCN (Chen & Liu, 2005). *Pasteuria* species, a group of obligatory parasitic, endospore- and mycelium-forming bacteria, have been observed in the SCN-suppressive soils in Japan (Sayre *et al.*, 1991) and Illinois in the USA (Atibalentja *et al.*, 1998). In spite of the knowledge about some microorganisms associated with suppression of nematode populations in natural fields (Chen & Dickson, 2004), the ecological interactions of soil nematode communities and how the mechanisms leading to the suppressive state operate are rarely studied.

Extracellular enzymes are excreted and employed by soil microbes to access their food by decomposing complex organic compounds. Certain hydrolytic enzymes

might be used by nematophagous bacteria and fungi to digest and penetrate the nematode cuticle and eggshell at an early stage of infection (Meyer & Wergin, 1998). Histochemical studies support the hypothesis that hydrolytic enzymes are involved in the penetration of nematode cuticles by fungi (Lopez-Llorca *et al.*, 2002). Serine protease, chitinase and collagenase are reported to play a part in the infection of plant-parasitic nematodes (Huang *et al.*, 2004). Serine proteases are a family of enzymes that utilise a uniquely activated serine residue in the substrate-binding site to hydrolyse peptide bonds catalytically (Siezen & Jam, 1997). Serine proteases and collagenases have been detected and partly identified from different nematode-trapping fungi (Tosi *et al.*, 2001; Wang *et al.*, 2006). Chitin is a key component of the middle layer of nematode eggshells (Bird & Self, 1995). As shown in scanning electron micrographs, papain and chitinase have destructive effects on the cuticle of plant-parasitic nematodes (Miller & Sands, 1977). Collagen is the main constitutive component of the nematode cuticle. Collagenases are enzymes that can catalyse the hydrolysis of collagen and gelatin rather than other proteinaceous substrates (MacLennan *et al.*, 1953). Measures of cellulose and peroxidase activity can reflect rates of production, decomposition and nutrient turnover, which are included as a general indicator of microbial activity (Sinsabaugh *et al.*, 2002).

A previous glasshouse study (Chen, 2007b) demonstrated that the soil from a field with a long-term monoculture of soybean and no-till practice was suppressive to SCN. Both formalin and autoclave treatments removed suppressive factors and increased the nematode egg population density as compared with non-treated soil, indicating that biological factors were involved in the nematode suppression. *Hirsutella rhossiliensis* was observed in the soils and parasitised a large percentage of SCN second-stage juveniles (J2). The parasitism of J2 by the fungus may be a factor for the suppression of the nematode population but other biological factors cannot be ruled out. The objective of this study was to determine further the factors involved in the SCN suppression in this soil. Specifically, we tested the hypothesis that soil disturbance may affect the nematode suppression, and that fungicide and bactericide treatments may differentiate the level of suppression; we also investigated the potential association of extracellular enzyme activities and nematode community indices with soil suppressiveness to SCN.

1 **Materials and methods**

3 **SITE SELECTION AND SOIL SAMPLING**

5 An SCN-suppressive field was identified at Southern
6 Research and Outreach Center Research Farm, Waseca,
7 MN, USA (Chen, 2007b). This field has been planted
8 with soybean continuously for 36 years with no-till
9 management during the past 10 years. Soil cores were
10 taken in early July from the field by driving 15 cm
11 long segments of 10 cm diam. plastic (PVC) pipe into
12 soil to a depth of 15 cm. The top 2 cm layer of loose
13 surface dry soil was removed in each pipe to have space
14 for watering during the glasshouse experiment. The soil
15 samples were processed and the experiment was set up
16 within the following few days. Each of the soil cores
17 was an experimental unit, and eight soil cores from
18 the same area were used as the experimental units in
19 a block (replicate) in the glasshouse experiment. Initial
20 SCN egg population densities in individual soil cores
21 were not determined to avoid any soil disturbance in
22 the 'undisturbance' treatment. However, the SCN field
23 population densities were monitored every year and the
24 nematode egg population densities in autumn generally
25 were below 10 000 (10-year average 6000) eggs 100 cm⁻³
26 soil (Chen, 2007b).

28 **EXPERIMENTAL DESIGN**

30 Treatments were combined factorially and experimen-
31 tal units were arranged as a randomised complete block
32 design. One factor had two levels of soil disturbance (no,
33 yes) to represent the practice of no-till and conventional
34 tillage, respectively. The second factor had four levels
35 of biocide treatments (none, the antibiotic streptomycin
36 alone, the fungicide captan alone, and streptomycin plus
37 captan) to manipulate the composition of microbial com-
38 munities (streptomycin for suppressing bacteria and cap-
39 tan for suppressing fungi, respectively). Each treatment
40 combination was replicated four times. To simulate dis-
41 turbance, soils was removed and passed through a sieve
42 (aperture 0.5 cm), and then repacked into the pipe (Young,
43 1987). Six SCN-susceptible soybean (cv. Pioneer 92B02)
44 seeds were sown in each pot. Immediately after planting,
45 the streptomycin/captan treatments were imposed. Cap-
46 tan at 200 ppm and/or streptomycin solution at 150 ppm
47 or water control were added to soil at 200 ml (1 soil)⁻¹
48 (about field capacity) once per week. After 1 week the
49 plants were thinned to provide three plants per pot. The
50 pots were arranged randomly on a bench by block, and

51 maintained in the glasshouse at 25-30°C. Water was sup-
52 plied to about the field capacity every day. No fertiliser
53 was used. The complete experiment was conducted in
54 2007 and 2008 with the same procedures except the appli-
55 cation of 'streptomycin plus captan' (streptomycin + cap-
56 tan) treatment. In 2007, the captan and streptomycin in the
57 streptomycin + captan treatment were mixed together at
58 the beginning of the experiment and stored in the refriger-
59 ator before being applied weekly. However, we observed
60 that captan and streptomycin alone were more effective
61 in lowering the soil suppressiveness than the two agents
62 mixed together. It is possible that the two agents reacted
63 chemically when applied together or during the storage of
64 the mixture. For this reason, we applied streptomycin 3
65 days after application of captan every week in 2008.

67 **PLANT WEIGHT**

68 After 70 days the experiment was terminated. The plant
69 shoots were cut near the soil surface and total shoot dry
70 weight of the plants in each pot was measured.

73 **SCN EGG DENSITY AND FUNGAL PARASITISM OF J2**

74 The SCN egg density was determined from a subsample
75 of 100 cm³ of soil. Cysts (females) were extracted from
76 the soil by hand decanting and centrifugation in 63%
77 (wt/vol) sucrose solution for 5 min at 1500 g. Eggs
78 were released from the cysts by breaking the cysts with
79 a mechanical device (Faghihi & Ferris, 2000), and then
80 collected in a 50 ml tube. They were stored at 4°C before
81 being counted within 2 weeks. J2 were extracted from a
82 subsample of 100 cm³ of soil with centrifugation-flotation
83 in sucrose solution (Jenkins, 1964). The percentage of J2
84 parasitised by fungi was determined from 100 randomly
85 selected J2 (Liu & Chen, 2000). Those J2 with an attached
86 fungal spore or colonised with fungal mycelium are
87 considered as being parasitised by fungi.

89 **EXTRACTION AND IDENTIFICATION OF NEMATODES 90 FOR COMMUNITY ANALYSIS**

91 Nematodes were extracted from 250 g soil using a mod-
92 ified Cobb's decanting and sieving method 70 days after
93 planting (Whitehead & Hemming, 1965). Soil was satu-
94 rated with water, gently stirred for 30 s, and then decanted
95 with duplicate passes over a series of sieves in the order of
96 600, 250, 150, 75 and 44 μm. Nematodes and soil debris
97 caught on the sieves were backwashed into a metal basin
98 and poured onto a cotton filter suspended above a collect-
99 ing tray. After 48 h, the cotton filter was removed and the
100

water beneath the filter was collected, settled and adjusted into 100 ml in bottles. All nematodes were counted in 10 ml taken from a well-mixed 100 ml solution and assumed to be 10% of the initial sample. Nematodes from the original sample were allowed to settle and concentrated into a 15 ml conical tube, and then *ca* 10 ml boiling 8% formalin was added to the concentrated nematode sample to kill and preserve the nematodes. Depending on the concentration of nematodes and debris in a sample, about five microslides were prepared by placing small aliquots of concentrated nematodes onto slides prepared with a paraffin wax ring that secured a cover slip when melted gently and cooled. For each soil sample, generally 150 nematodes were identified to family (2007) or genus (2008) according to Bongers (1987, 1999), Hunt (1993), Nickle (1991), Goodey (1963), Maggenti (1983, 1991) and Maggenti *et al.* (1987). Voucher specimens of observed genera were created by slow evaporation of formalin-preserved nematodes in dilute glycerin solution and mounted in anhydrous glycerin. Formula for calculating the values of indices, Shannon's diversity, Simpson's dominance, richness and maturity index are summarised in Table 1.

EXTRACELLULAR ENZYME ASSAYS

Approximately 1 g of soil subsamples were taken from each nematode sample and transferred into a 100 ml screw-cap Nalgene bottle. These samples were immedi-

ately frozen and stored at -70°C until processed to determine microbial activity at the time of sampling. For processing, 100 ml of 50 mM sodium acetate buffer at pH 6.0 was added and blended with a polytron (Kinematica, Littau-Lucern, Switzerland) at high speed for 1 min. The soil slurry was immediately added to a wide-mouthed glass bowl; 200 μl subsamples were taken from the blended slurry for enzyme analysis while the magnetic bar was stirring. Activities of four hydrolytic and two oxidative enzymes (Table 2) were quantified using the modified protocol of Saiya-Cork *et al.* (2002) with the following modifications in methodology. A 50 mM citrate buffer solution was made by mixing sodium citrate with deionised water and stored at 4°C between analyses. Buffer pH was adjusted to 6.0 to match the mean soil pH of the study site. The activities of four hydrolytic enzymes were quantified fluorometrically using a methylumbelliferone (MUB)-linked substrate. Each MUB-linked substrate was a 200 μM solution in DI water and stored in bottles at 4°C . The reference standard MUB was a 10 μM solution and treated the same as MUB-linked substrates. Multiwell-plates were designed to hold a series of three samples with controls including plate (250 μl buffer), standard (200 μl sample suspension and 50 μl standard), substrate (200 μl sample suspension and 50 μl substrate), and sample (200 μl sample suspension and 50 μl standard). The microplates were covered and incubated in the dark at

Table 1. Indices used in nematode community analysis.

Name	Equation	Reference
Shannon's diversity	$H' = -\sum(p_i \ln p_i)$	Shannon & Weaver (1949)
Simpson's dominance	$D = \sum p_i^2$	Simpson (1949)
Richness	$R = S$	Hill (1973)
Maturity index	$\sum \text{MI} = \sum(v_i \times f_i)/n$	Yeates (1994)

p_i is the relative abundance of taxon i ; S is the total number of taxon present in 200 g fresh soil; $v_i = c-p$ value assigned to family, $f_i =$ frequency of taxon i in sample; $n =$ total number of individuals in a sample.

Table 2. Extracellular enzymes assayed in soil samples.

Enzyme class	Enzyme subclass	Enzyme	Major substrate
Hydrolytic	Glycosidases	β -1,4-glucosidase	Cellulose
		β -1,4-N-acetylglucosaminidase	Chitin
	Aminopeptidases	L-proline aminopeptidase	Collagen
		L-serine aminopeptidase	Protein
Oxidative	Phenyl oxygenases	Phenol oxidase	Lignin
		Peroxidase	Lignin

20°C overnight. To stop the reaction, a 10 μ l aliquot of 1.0 M NaOH solution was added to increase pH beyond 10, maximising MUB fluorescence. Fluorescence was quantified using a microplate fluorometer (FLx800, Bio-Tek Instruments, Winooski, VT, USA) with 360 nm excitation and 460 nm emission filters. Two of the six enzymes assayed were oxidative enzymes (peroxidase and phenol oxidase) and, thus, quantified spectrophotometrically in clear polystyrene 96-well, 300 μ l microplates. The substrate consisted of 10 mM l-3,4-dihydroxyphenylalanine (L-DOPA) and 0.3% hydrogen peroxide. After 1.5 h of incubation at 20°C, absorbance was read on a microplate spectrophotometer (Bio-Tek) with a 460 nm filter. Enzyme activities were expressed in units of $\text{nmol h}^{-1} \text{g}^{-1}$.

STATISTICAL ANALYSIS

A full model two-way analysis of variance (ANOVA) was performed using the MIXED procedure in SAS software version 9 (Statistical Analysis System Institute, 1990). The model consisted of dependent variables including SCN egg population density, proportion of SCN J2 parasitised by fungi in nematode community, extracellular enzyme activity, nematode community diversity, richness, dominance and maturity indices. Independent variables were soil disturbance and biocide treatments. Multiple comparisons between means were performed using Fisher's protected Least Significant Difference (LSD) Test at $P < 0.05$. To meet assumptions of normality and equality of variances, SCN egg population density was transformed as \log_{10} and the proportion of SCN J2 parasitised by fungi was transformed to degree-arc-sin ($x^{0.5}$) prior to analysis. Canonical correspondence analysis (CCA) was employed to identify multidimensional patterns among abundance of nematodes by family or genus and contrasting levels of disturbance and biocides. CCA was performed using Canoco Version 4.5 software (ter Braak & Šmilauer, 2002).

Results

PLANT WEIGHTS

There was no significant difference in plant shoot weight among the four biocide treatments and between the two levels of soil disturbance treatments.

SCN EGGS ABUNDANCE

With the treatment of soil disturbance, SCN egg population density increased 300% in 2007 (Fig. 1A) but not in 2008 (Fig. 1C). SCN egg population density was greater with streptomycin alone, captan alone or streptomycin + captan applied than with no biocide in 2008 (Fig. 1D). The highest egg population density was observed in the treatment of streptomycin + captan. By contrast, only captan alone increased SCN egg numbers in 2007 (Fig. 1B). There was no significant interactive effect of soil disturbance and biocide application on the SCN egg population density.

FUNGAL PARASITISM OF J2

At the end of the experiment, the proportion of SCN J2 parasitised by fungi was low. Soil disturbance lowered percentage of SCN J2 parasitised by fungi in 2007 (Fig. 2A) but not in 2008 (Fig. 2C). All biocide treatments increased the proportion of J2 parasitised by fungi in 2008 (Fig. 2D) but not in 2007 (Fig. 2B). There was no significant interactive effect of soil disturbance and biocide application on fungal parasitism of J2.

NEMATODE COMMUNITY COMPOSITION

Within nematode communities, the most abundant genera were *Helicotylenchus*, *Cephalenchus*, *Aphelenchus*, *Heterodera* (the SCN J2), *Eucephalobus* and *Chronogaster* for all soil samples in both years. Soil disturbance reduced nematode community diversity values in 2007 ($P = 0.0016$) and 2008 ($P = 0.0470$), and increased the values of dominance ($P = 0.0029$) and maturity ($P = 0.032$) indices in nematode communities only in 2007 (Table 3). Index values of dominance and maturity indices were similar between physically disturbed and undisturbed soil in 2008 (Table 3). Greater values of maturity index values were observed in soils treated with biocides compared to soils without biocides in both 2007 and 2008, with the exception of streptomycin in 2008 (Table 3). Index values of richness were similar for soil disturbance and biocide treatments in both years (Table 3).

Generally, canonical correspondence analysis results showed that soil physical disturbance explained more variance than biocides, captan and streptomycin, in both 2007 and 2008 (Figs 3, 4). The occurrence of SCN J2 (*Heterodera*) explained greater variance associated with no-till practices than biocide application in 2007, whereas the opposite was true in 2008 (Figs 3, 4). Common fungivorous genera, *i.e.*, *Aphelenchus* and *Aphelenchoides*,

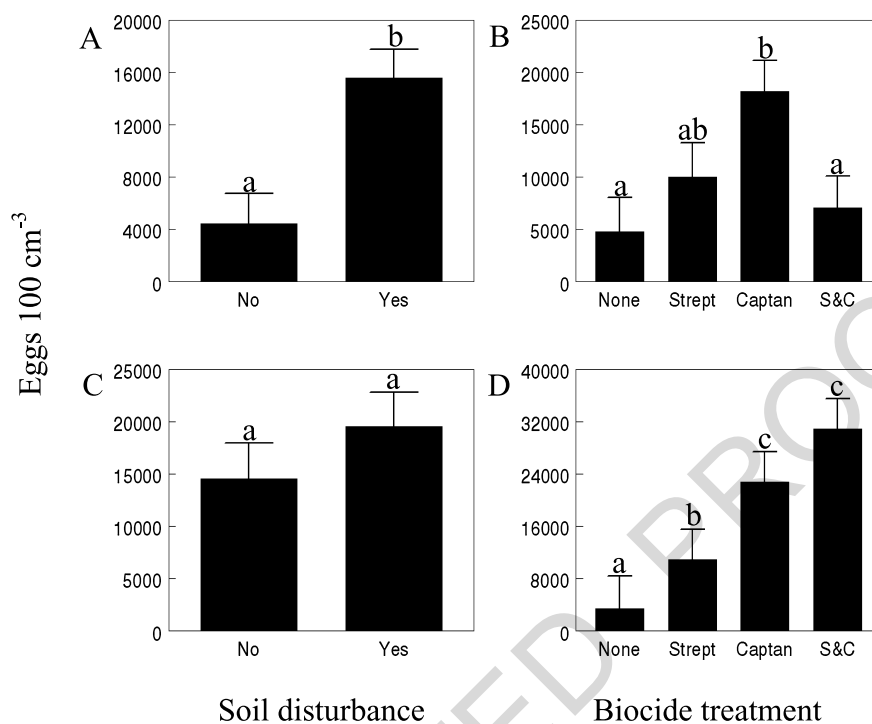


Fig. 1. Effects of soil disturbance and biocide treatments on SCN egg population density in 2007 (A, B) and 2008 (C, D). Means with different letters indicate significant differences were observed ($P < 0.05$).

were more abundant in undisturbed than disturbed soils in both 2007 and 2008 (Figs 3, 4). Consistent positive associations were observed between the abundance of SCN J2 (*Heterodera*) and application of biocides in both 2007 and 2008 (Figs 3, 4). Captan affected the nematode community composition more in 2008 than in 2007 (Figs 3, 4).

EXTRACELLULAR ENZYME ACTIVITY

There were no significant differences in the activities of extracellular enzymes among soil disturbance and biocides treatments, except L-proline aminopeptidase in 2007 (Table 4). Specifically, soil disturbance treatment reduced the activity of L-proline aminopeptidase more than 50% in 2007 (Table 4). In addition, there was a trend for biocide treatments to reduce the activities of L-proline aminopeptidase in 2007 in descending order of a mixture of streptomycin and captan, captan alone, and streptomycin alone, although the differences were not statistically significant (Table 4). Unlike 2007, the activities of L-proline aminopeptidase were similar between soil disturbance treatments in 2008 (Table 4).

Discussion

Most of the studies of naturally occurring nematode-suppressive soils have focused on the identification of the functional biological entities that contribute to observed disease suppression (Kerry *et al.*, 1982; Kim & Riggs, 1994; Chen *et al.*, 1996; Atibalentja *et al.*, 1998). In this study, we attempted to gain an understanding of the ecological factors enabling these organisms to persist, compete and function in soil community with suppression of nematodes.

Our experiment demonstrated that the SCN egg population densities increased and the proportion of SCN J2 parasitised by fungi decreased when the soil was disturbed, which indicated that suppressiveness to SCN could be reduced through intensive soil disturbance. The result corresponds to those of Freckman and Ettema (1993) and Wasilewska (1998), who reported that the proportion of plant-parasitic and bacterivorous nematodes increase, both the proportion of fungivorous and predaceous nematodes decrease, and genus richness decreases after perturbation. By contrast, a recent field study suggested that tillage had little or no effect on percentage of SCN J2 parasitised by *Hirsutella* spp. (Chen & Liu, 2007).

Soil disturbance effect on nematode communities in suppressive soil

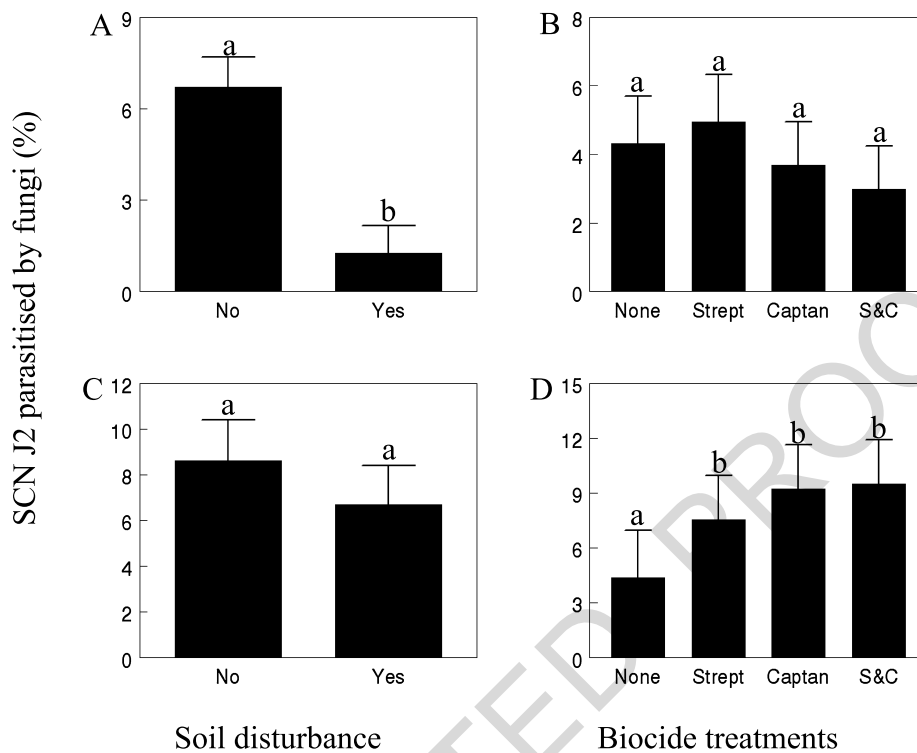


Fig. 2. Effects of disturbance and biocide treatments on the proportion of SCN second-stage juveniles parasitised by fungi in 2007 (A, B) and 2008 (C, D). Means with different letters indicate significant differences were observed ($P < 0.05$).

Table 3. Effects of disturbance and biocide treatments on nematode community indices.

Year	Treatment	Treatment level	Community index			
			Diversity	Dominance	Richness	$\sum MI$
2007	Disturbance	No	1.23 a	0.43 a	9.02 a	2.67 a
		Yes	0.84 b	0.62 b	7.69 a	2.83 b
	Biocide	None	1.27 a	0.41 a	8.38 a	2.56 a
		Streptomycin	0.99 a	0.55 a	7.79 a	2.81 b
		Captan	0.93 a	0.57 a	8.50 a	2.82 b
	Streptomycin + captan	0.96 a	0.56 a	8.75 a	2.82 b	
2008	Disturbance	No	1.31 a	0.40 a	10.56 a	2.60 a
		Yes	1.09 b	0.50 a	10.06 a	2.68 a
	Biocide	None	1.11 a	0.48 a	9.50 a	2.63 a
		Streptomycin	1.38 a	0.36 a	11.38 a	2.55 a
		Captan	1.03 a	0.54 a	9.88 a	2.74 b
	Streptomycin + captan	1.27 a	0.43 a	10.50 a	2.63 b	

Means with different letter indicate significantly different means within an effect ($P < 0.05$).

The increased SCN egg population under biocides treatments in both years supported the presence of antagonistic agents that attack either egg, juvenile or adult nematode stage in the non-treated suppressive soils (Chen,

2007b). Within the biocide treatments, the application of captan seemed to be more effective than streptomycin for reducing suppressiveness, thus increasing the SCN egg population density, suggesting that fungal antagonists

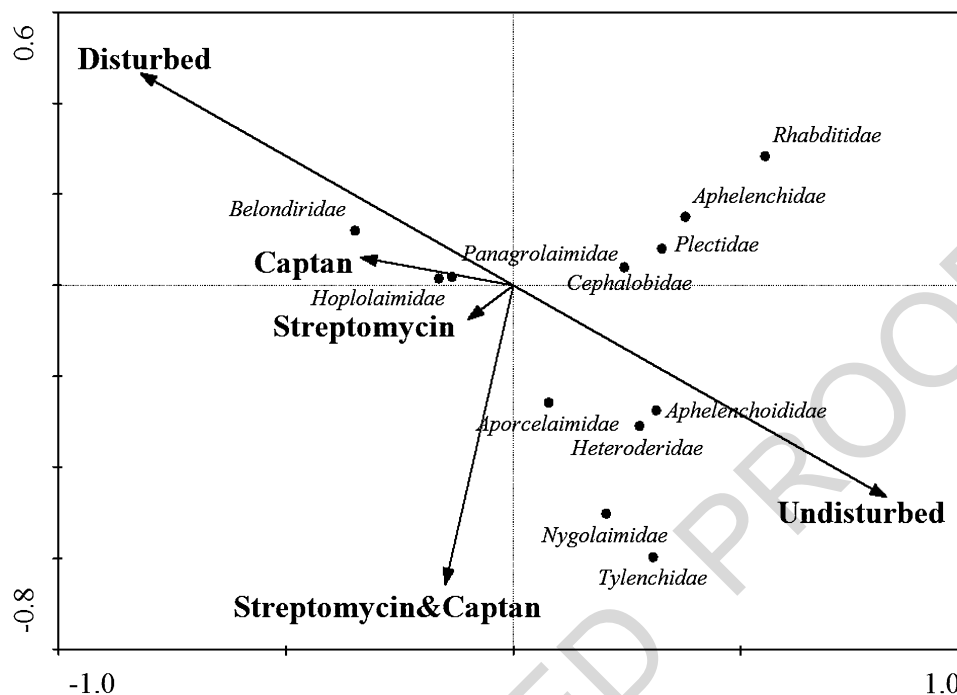


Fig. 3. Nematode community composition from SCN glasshouse experiment 2007. Canonical correspondence bi-plot of CCA1 (x-axis, effect of disturbance) and CCA2 (y-axis, effect of biocides) are shown. Environmental vectors represent treatment combination of tillage and biocides treatments. Points represent relative abundance of nematode genera. Eigenvalues (λ) are 0.091 ($P = 0.0180$), 0.027, 0.008 and 0.002 for first (horizontal), second (vertical), third and fourth axes, respectively.

may play a major role in the SCN suppression. The result is similar to a glasshouse experiment in Arkansas, which suggested that the sterile fungus ARF18 was responsible for the nematode suppression (Kim & Riggs, 1991). In the soil used in this study, the fungus *H. rhossiliensis* was frequently encountered on SCN J2. Although the fungi parasitising the J2 in this study were not isolated and identified, based on previous studies *H. rhossiliensis* was probably the major fungal species (Chen, 1997, 2007b). This fungus may play partial role in suppression of SCN population density. However, we found a significant increase in the percentage of parasitised SCN J2 at the end of experiment treated by biocides in 2008, which could be a result of the higher SCN populations (Chen & Liu, 2005) because the fungal parasitism of nematodes is density-dependent (Jaffee, 1992). Presumably, different mechanisms or antagonistic agents were involved in the suppressive soil to attack SCN eggs and J2, respectively. Furthermore, non-target effects of streptomycin and captan on soil microbial organisms remain a point of discussion, which may have affected the interpretation of our data. In a Georgia no-till agroecosystem, applications of streptomycin to soil reduced bacterial abundance; cap-

tan was effective in reducing fungal activity and total fungal biomass without directly affecting any non-target group (Ingham *et al.*, 1991). By contrast, a previous study showed that streptomycin decreased the numbers of bacteria that grew on tryptone agar but also reduced fungal hyphae with respect to controls (Ingham & Crossley, 1984).

This is the first attempt to incorporate the nematode community analysis into investigation of soil suppressiveness to SCN. Nematode community indices had been developed and applied to investigate the soil biological conditions and ecological processes in various ecosystems (Bongers, 1990; Bongers & Ferris, 1999; Neher, 2001). Our glasshouse experiment supports the concept that intensive tillage reduces soil suppressiveness, which may be associated with reduction of the nematode community diversity compared to non-till soil (Kiewnick *et al.*, 1997). Although Freckman and Ettema (1993) hypothesised that soil nematode communities in never cultivated or no-till agricultural systems were successional more mature than in frequently cultivated agricultural soils, we found an increase in maturity index value with soil disturbance in 2007 but no effect in 2008. We also ob-

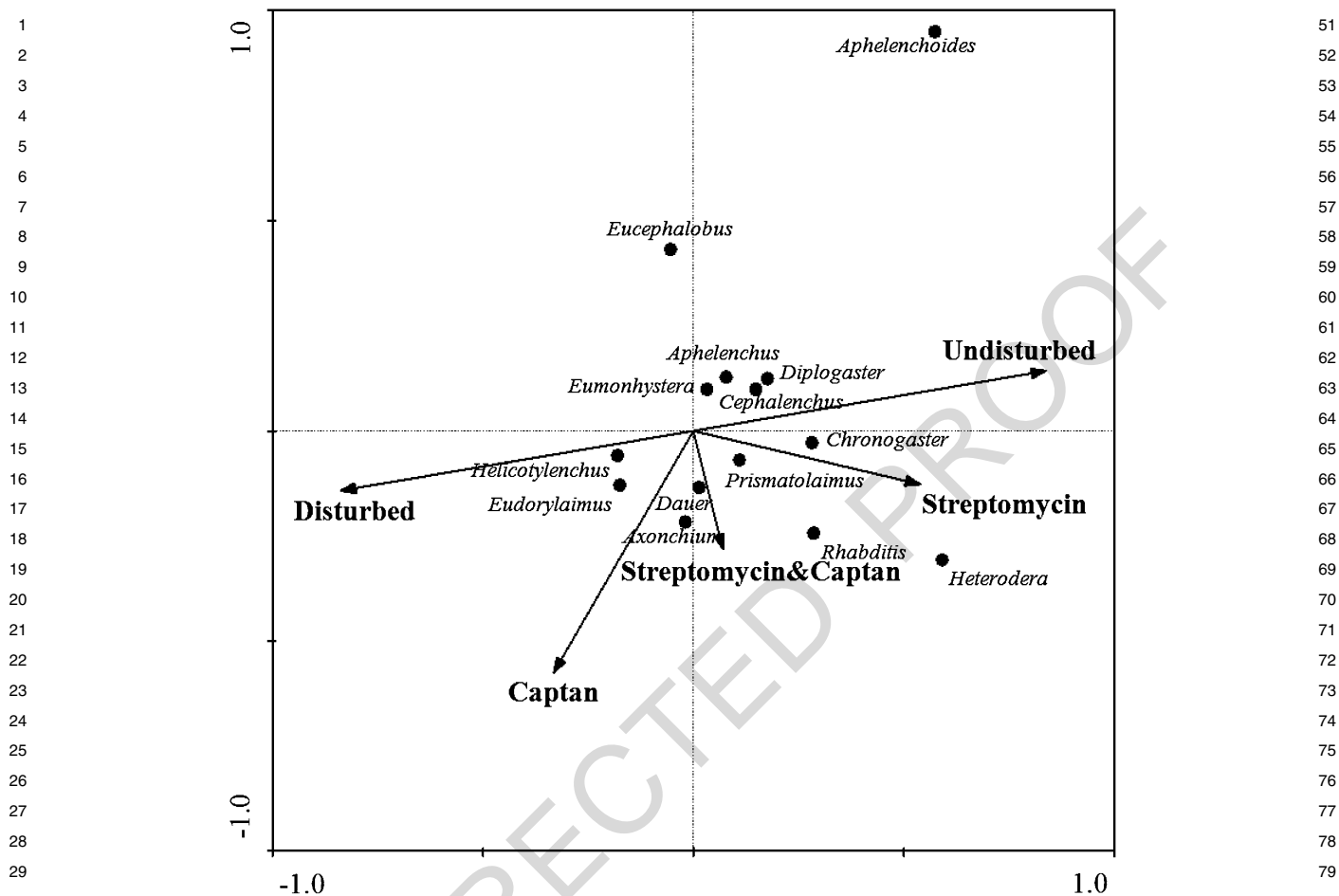


Fig. 4. Nematode community composition from SCN glasshouse experiment 2008. Canonical correspondence bi-plot of CCA1 (x-axis, effect of disturbance) and CCA2 (y-axis, effect of biocides) are shown. Environmental vectors represent treatment combination of tillage and biocides treatments. Points represent relative abundance of nematode genera. Eigenvalues (λ) are 0.047 ($P = 0.0320$), 0.021, 0.012 and 0.005 for first (horizontal), second (vertical), third and fourth axes, respectively.

served that the intensive soil disturbance reduced fungal-feeding nematodes including *Aphelenchus* and *Aphelenchoides*, which were abundant in the soils without disturbance. Therefore, it is possible that the loss of *Aphelenchus* and *Aphelenchoides* with a comparatively small $c-p$ value ($c-p = 2$) when soil was disturbed or treated with biocides at least partially explain the greater values of maturity index observed. These results concur with those of Porazinska and Coleman (1995), who measured similar MI values for cucumber plantings grown under synthetic fertiliser and organic poultry manure regimes. However, Yeates *et al.* (1997) reported greater combined maturity index ($\sum MI$) values for organically managed systems than for conventionally managed systems in silt

and loam soils but not sandy soils. Because our experiments were conducted in a glasshouse, the soil samples with no-disturbance treatment actually had been disturbed slightly when they were removed from no-till suppressive fields to the glasshouse. A further study conducted in the original SCN-suppressive fields will confirm whether or not soils with nematode-suppressiveness are more ecologically mature than when disturbed by intensive tillage. Most of our findings in CCA bi-plot were compatible with previous studies: *Aphelenchus* and *Aphelenchoides* (fungivorous nematodes) increased most rapidly in the no-till field plots amended with materials with relatively high ratios of carbon to nitrogen (Ferris & Matute, 2003); *Aphelenchoides* was one of the genera most sensitive to the

Table 4. Effects of disturbance and biocide treatments on extracellular enzyme activity.

Year	Treatment	Treatment level	Extracellular enzyme activity (nmol h ⁻¹ g ⁻¹)					
			β -glu	NAG	Pro	Ser	PO	Pero
2007	Disturbance	No	56.26 a	12.20 a	34.62 a	51.50 a	N/A	N/A
		Yes	42.19 a	12.13 a	15.49 b	44.68 a	N/A	N/A
	Biocide	None	38.20 a	11.65 a	32.32 a	46.78 a	N/A	N/A
		Streptomycin	56.62 a	13.58 a	25.25 a	46.25 a	N/A	N/A
		Captan	55.32 a	12.00 a	23.02 a	46.40 a	N/A	N/A
		Streptomycin + captan	46.75 a	10.18 a	19.62 a	52.92 a	N/A	N/A
2008	Disturbance	No	30.52 a	11.43 a	1.61 a	2.46 a	7.09 a	10.70 a
		Yes	34.00 a	12.32 a	1.65 a	2.54 a	6.44 a	12.43 a
	Biocide	None	32.65 a	11.01 a	1.65 a	2.13 a	8.70 a	11.38 a
		Streptomycin	31.57 a	11.48 a	1.88 a	2.59 a	6.62 a	12.18 a
		Captan	30.75 a	14.18 a	1.56 a	2.70 a	6.14 a	11.24 a
		Streptomycin + captan	34.07 a	10.82 a	1.43 a	2.59 a	5.60 a	11.47 a

β -glu, β -1,4-glucosaminidase; NAG, β -1,4-N-acetylglucosaminidase; Pro, L-proline aminopeptidase; Ser, L-serine aminopeptidase; PO, phenol oxidase; Pero, peroxidase. Means with different letters indicate significant differences ($P < 0.05$). N/A indicates the activities of PO and Pero were not measured.

direct effects of tillage (Fiscus & Neher, 2002); *Eudorylaimus* and *Enmonhystera* were sensitive to indirect effects of both tillage and chemical treatments (Fiscus & Neher, 2002).

This glasshouse experiment also represents a first attempt to detect the extracellular enzyme activity as a mechanism to explain nematode suppressiveness. Although the effects of soil disturbance and biocide on extracellular enzyme activities were inconsistent, we obtained useful information from the general trends. For example, the suppression of L-proline aminopeptidase activity by the soil disturbance treatment and/or biocides in 2007 indicates that biological suppression of SCN is possibly related to collagenase. Available nitrate and ammonium were quantified at mid-season in the field's soil. Relatively abundant nitrogen (43.7 ± 9.47 mg nitrate and 7.3 ± 0.34 mg ammonium (kg soil)⁻¹) at least partially explains why both phenol oxidase and peroxidase were less active in the field sites than we anticipated. Probably, chitinase is another important extracellular enzyme contributing to the soil suppressiveness. However, we did not find any significant differences in the activity of chitinase between soil disturbance treatments and/or biocides applications. Neither soil disturbance nor biocide treatment affected NAG activity, a major chitinase, so we conclude that chitinase is not a major antagonist mechanism used by soil microbes in the soil used in this glasshouse study. However, it is possible that the enzyme activities in the glasshouse pots

may differ from that under field conditions. Nonetheless, extracellular enzyme analysis provides a means of assessing potential changes in activities of enzymes relative to treatments.

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