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Steinernematidae) Natural Populations and Its Dispersal in the Field 【Research report】

蟲生線蟲Steinernema carpocapsae (線蟲綱：Steinernematidae) 在不同作物系統中之自然棲群及在田間之移動【研究報告】

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Abstract

The effect of cropping practices on population levels of the entomopathogenic nematode, Steinernema carpocapsae, in 3 field crops was examined in these studies. Populations of S. carpocapsae in fallow and bare plots were very low in February and March 1988, due to snow which fell on Feburary 9, 1988. Higher populations of S. carpocapsae were found in no-tillage corn plots than in conventional-tillage plots from October 1988 to August 1989. Populations of S. carpocapsae in no-tillage and conventional tillage corn, sorghum, and soybean fields decreased over time from October 1988 to April 1989. Five months after application, no infective juveniles were recovered from most of the plots, except for those with conventional-tillage corn treatments. The mumber of S. carpocapsae in soil covered with corn and sorghum debris was significantly lower after 30 d at 25℃. In the horizontal movement study, S. carpocapsae was capable of moving 3.5 cm/day in bare soil plots and 7.5 cm in rye mulch-covered plots, indicating that the mulch permits enhanced movement of S. carpocapsae in agriculture systems.

摘要

本試驗在三種糧食作物上研究作物耕作系統對蟲生線蟲Steinernema carpocapsae 自然棲群之影響。在美國喬治亞州休及裸露的試驗小區因1988年2月的下雪造成所粹取出的線蟲數極低。自1988年10月至1989年8月驗期間發現在不耕犛玉米試區的蟲生線蟲數目比傳統耕犛之試區多。蟲生線蟲的棲群在不耕犛或傳統耕犛的玉米、高粱、及大豆試區在1988年10月至1989年4月期間，皆隨試驗時間之進行而下降。在施用後五個月，除了傳統耕犛玉米區外，大部份的試區都無法粹取出線蟲。施用蟲生線蟲於土表後再以玉米及高梁殘株覆蓋，移往25℃的溫箱下。30天後Steinernema carpocapsae 線蟲數顯著下降。在田間水平移動試驗中，不覆蓋黑麥草的情形下S. carpocapsae可移動3.5公分/ 天，覆蓋牧草下線蟲可移動7.5公分 / 天。由此結果指出牧草覆蓋可增進 S. carpocapsae 可移動3.5公分/天，覆蓋牧草下線蟲可移動7.5公分/天。由此結果指出牧草覆蓋可增進S. carpocapsae 的移動。

Key words: Steinermema carpocapsae, no-tillage, conventional-tillage, corn, soybean, sorghum.
關鍵詞: Steinernema carpocapsae ,不耕犛，傳統耕犛，玉米，高梁，大豆

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Survey of the Entomopathogenic Nematode, *Steinernema carpocapsae*: (Rhabditida: Steinernematidae) Natural Populations and Its Dispersal in the Field

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ABSTRACT

The effect of cropping practices on population levels of the entomopathogenic nematode, *Steinernema carpocapsae*, in 3 field crops was examined in these studies. Populations of *S. carpocapsae* in fallow and bare plots were very low in February and March 1988, due to snow which fell on Feburary 9, 1988. Higher populations of *S. carpocapsae* were found in no-tillage corn plots than in conventional-tillage plots from October 1988 to August 1989. Populations of *S. carpocapsae* in no-tillage and conventional tillage corn, sorghum, and soybean fields decreased over time from October 1988 to April 1989. Five months after application, no infective juveniles were recovered from most of the plots, except for those with conventional-tillage corn treatments. The number of *S. carpocapsae* in soil covered with corn and sorghum debris was significantly lower after 30 d at 25℃. In the horizontal movement study, *S. carpocapsae* was capable of moving 3.5 cm / day in bare soil plots and 7.5 cm in rye mulch-covered plots, indicating that the mulch permits enhanced movement of *S. carpocapsae* in agriculture systems.

Key words: *Steinernema carpocapsae*, no-tillage, conventional-tillage, corn, soybean, sorghum.

Introduction

The entomopathogenic nematode, *Steinernema carpocapsae* (Rhabditida: Steinernematidae), has a broad host spectrum, can be mass produced in vitro, posseses the ability to seek out its host, and kill its host rapidly, is environmentally safe, and has been exempted from registration by the U.S. Environmental Protection Agency (Klein, 1990; Kaya and Gaugler, 1993). This combination of attributes has generated an intense interest in the development of this nematode for use against soil insect pests (Klein, 1990).

The soil environment offers an excellent site for insect-nematode interactions; more than 90% of insect pests spend their life cycle in the soil, and soil is the natural reservoir of Steinernematid nematodes (Gaugler, 1988; Georgis and Poinar, 1989). However, field trials with *S. carpocapsae* to control crop pests have had

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erratic results, from no infection to 99% control (Begley, 1990; Klein, 1990). This erratic control is probably due to a poor understanding of the soil biology of this nematode (Poinar, 1979). Information on ecological niches, habitat preference, overwintering biology, nematode-host interaction, nematode-plant interaction, and interactions with other soil micro-organisms is meager. Therefore, details of the environmental biology of *S. carpocapsae* need to be characterized to improve its efficacy in the field.

Greater knowledge is needed on the biology of the free-living (infective juveniles) stage of *S. carpocapsae* in order to utilize them effectively to control insect pests in agricultural environments. The purpose of these studies was to evaluate selected agricultural practices for developing cropping environments that conserve *S. carpocapsae* populations. The population density of *S. carpocapsae* was monitored under different agricultural practices and combinations, e.g., no-tillage, plow tillage, conventional tillage, and the effect of crop debris on the survival and pathogenicity of *S. carpocapsae* were also conducted in the laboratory and in the field.

Materials and Methods

(A) Survey of natural populations under different cropping systems

Populations of infective juveniles of *S. carpocapsae* were monitored during the 1987-1989 season in microplots established in selected cropping systems in Georgia, USA. Sorghum, corn, soybean, and grapes (*Vitis labrusca*) were used as test crops. Cropping practices included no tillage, conventional tillage, burning before planting, and use of pesticides. In the vineyard plot, 4 soil samples were taken from chlorpyrifos (Lorsban[R], 1.0 kgAI / ha) microplots and non-treated plots.

To study changes in the population of *S. carpocapsae* at the seedling stage and after harvest, samples were taken from 3 crops (corn, sorghum, and soybeans) in both conventional and no-tillage areas. The experiment was set up as a randomized complete block split-split plot design. Tillage treatments were split plots and crop plantings were split-split plots. Tillage plots covered 0.025 ha and individual crop plantings were 0.008 ha; each treatment was replicated 4 times.

Twenty soil cores (10 cm deep) were taken each month at random in each plot with a soil auger. Soil from each treatment was bulked (or mixed) and 100 g sample was extracted with a Baermann funnel to determine the number of *S. carpocapsae*. The extracted nematodes were used in bioassays with *Galleria* larvae. Nematodes that emerged from the cadavers were identified to ensure them to be the causal agent of the death of *Galleria* larvae.

Air temperature and relative humidity were recorded at the Plant Science Farm weather station of the University of Georgia, Athens. Soil moisture was determined by gravimetric methods.

(B) Study of overwintering biology

Overwintering biology of nematodes was studied from December 1987 to June 1988 in a continuous no-tillage corn field (0.5 ha), and a fallow field (previously planted with soybeans) (0.25 ha). In a 2nd experiment, a plowed field and no-tillage field planted with plots of soybean, corn, and sorghum were studied from September 1988 to May 1989. The field design was a split-plot in a randomized complete block with tillage as split plots and crops as split-split plots. Coarse sandy loam was placed in 0.912-l cups (diam. 11.5 cm, height 14 cm) with drain holes. Each cup was first soaked in tap water to raise the moisture content to the saturation point. Approximately 10,000 infective juveniles of *S. carpocapsae* were added 1 cm beneath the soil surface of the cup. Each treatment was replicated 4 times, and each replicate consisted of 10 cups. The cups were set 90 cm a part within a row. The distance between replicates was 2 crop rows (120 cm).

At monthly intervals, 5 cups per treatment were brought to the laboratory and nematodes were extracted by the Baermann funnel technique. The nematodes were counted and used in the *Galleria* infectivity bioassay described above. The nematodes were examined under the microscope for parasitization and any anatomical or other physical changes that may have occurred during the winter.

A seasonality test of *S. carpocapsae* was conducted in a corn field planted in no-tillage and conventional-tillage systems at the Plant Science Farm from October 1988 to August 1989. Tillage blocks were 0.025 ha and each was replicated 4 times. Coarse sandy loam was placed in 0.285-1 cups. Cup preparation and placement in the field, sample collection, nematode extraction, and bioassay were the same as in the overwintering experiment described above.

(C) Impact of crop debris on *S. carpocapsae* survival

This experiment evaluated the effect of corn and sorghum debris on survival and development of *S. carpocapsae*. Crop debris was collected from a no-tillage field located at the UGA Plant Science Farm on May 16, 1988. Debris had accumulated on the soil surface from the previous year. One kilogram coarse sandy loam was placed into a 0.912-1 cup (diam. 11.5 cm; depth 14 cm) and 100g of water was added to attain 10% soil moisture-(W / W). Approximately 10,000 infective juveniles of *S. carpocapsae* were added 1 cm beneath the soil surface. Five grams of crop debris was placed on the soil surface. Cups were placed in an incubator (25℃). At 30, 60, and 90 d after nematode application, the cups were removed and the soil was extracted by Baermann funnel, and nematodes were counted. Nematode infectivity in Galleria larvae was also evaluated.

Another test was conducted using a 0.285-1 cup, adding 3 gm of debris on the surface and covering each cup with aluminum foil to retard moisture loss. The soil was extracted with a Baermann funnel at 7, 14, 21, 28, 30, 60, and 90 d after applications and nematode numbers were recorded.

(D) Dispersal of *S. carpocapsae* in the field

A field experiment was conducted from May 7to June 4, 1987 to study horizontal movement using 5x5 m^2 plots. The field was plowed earlier and surface soil was covered with a polyethelene sheet and sterilized with 500 gm methylbromide. Seven days after fumigation, the field was irrigated with 3 cm of water. Following irrigation, a nematode suspension containing about 10^6 infective juveniles / 100 ml was added to the center of 4 plots. Two plots were not inoculated and served as controls.

Soil samples were collected at a depth of 15 cm 1 wk after nematodes were added. Sampling continued at weekly intervals for four weeks. Four samples were taken at 25-cm intervals at 0°, 90°, 180°, and 270° positions from the center of the plots to a distance of 125 cm. Soil samples collected at the same distance from the center were mixed in a plastic bag and returned to the laboratory. Nematodes were extracted using a Baermann funnnel, counted, and tested to determine infectivity on *Galleria* larvae. Larval cadavers were dissected to verify the presence of nematodes. Analysis of variance and Duncan's multiple range test were performed on log-transformed data.

The effect of mulch (rye) on the dispersal of *S. carpocapsae* was conducted in the field from October 23 to December 4, 1989. Eight 60 cm x 60 cm microplots were established for no-mulch and mulch-covered plots. Each treatment was replicated 4 times. Ten centimeters of mulch was put on the surface of microplots. Soil samples were taken 15-45 cm (at 0°, 90°, 180°, and 270° positions) from the center of the plots.

Approximately 10,000 infective juveniles in a 1-ml formalin solution (0.1%) were added to the center of each plot. Four soil samples were collected 4, 8, 12, and 16 d after nematode inoculation in a 1st test, and at 7, 14, and 21 d after nematode inoculation in a 2nd test. Soil samples were mixed together and nematode extracted using Baermann funnel. Soil temperature was determined using a soil thermometer, and soil moisture was determined by gravimetric methods.

Analysis of variance, t-test, and Duncan's multiple range test were used to analyze data from each test (SAS Institute, 1985).

Results and Discussion

(A) Survey of natural populations under different cropping systems

The number of nematodes recovered from control grape plots (no chlorpyrifos) was significantly higher than numbers recovered from chlorpyrifos-treated plots (Table 1). This experiment indicates that chlorpyrifos is detrimental to *S. carpocapsae*. Marban-Mendoz and Viglierchio (1980) found that carbofuran could reduce the motility and dispersal of plant-

Table 1. Mean number of *S. carpocapsae* collected in a grape vineyard and its pathogenicity to *Galleria* larvae[a]

Treatment	Mean no. nematodes / rep.[b]	Mortality (%)	Moisture (%)
Control	10 a[c]	3 a	9.55
Chlorpyrofos	3 b	0 a	10.39

[a] Samples were collected on Oct. 29, 1987.

[b] Each treatment has 4 replications.

[c] Means within a column with the same letter are not significantly different (P<0.5) with t-test (SAS Institute, 1985).

Table 2. Mean number of *S. carpocapsae* extracted from soil collected in plots planted with 3 crops in no-tillage and conventional-tillage systems[a]

Crop	Mean no. of nematodes		Mortality(%)	
	S	H	S	H
		No-tillage		
Corn	19aA	5aB	18aA	3aB[b]
Soybean	14aA	3aB	17aA	0aB
Sorghum	15aA	8aB	15aA	5aB
		Conventional-tillage		
Corn	19aA	16aA	10aA	10aA
Soybean	18aA	0bB	10aA	0aB
Sorghum	13aA	10aA	8aA	7aA

[a] S=soil sampled in the later crop seedling stage on Aug. 26; and H = soil sampled at harvest period on Nov. 2, 1987. Each treatment has 4 replicates.

[b] Means with the same letter in a column are not significantly different (P<0.05) in Duncan's multiple range test. Means with the same capital letter in a row are not significantly different (P<0.05) in t-test(SAS Institute 1985).

parasitic nematodes. The bioassay resulted in zero mortality of *Galleria* due to the low numbers of nematodes in the chlorpyrifos plots.

In the field crop experiment, the number of infective juveniles recovered during the seedling stage was higher than that recovered after harvest from all 3 crops (corn, sorghum, and soybean) planted in no-tillage and soybean in conventional tillage systems (Table 2). Generally, in the crop seedling stage, there were no significant differences in the number of nematodes in the 3 crops regardless of tillage. The number of nematodes in conventional-tillage soybeans was significantly lower than in no-tillage at harvest. The mortality of *Galleria* larvae was higher in crops in the seedling stage as compared with that at harvest in the no-tillage plots.

(B) Study of overwintering biology

Numbers of *S. carpocapsae* in fallow plots were very low in February and March and increased in April and May, 1988 (Fig. 1). This was probably caused by detrimental effects of UV or the snow which occurred on February 9, 1988. In the no-tillage corn plots, numbers of *S. carpocapsae* increased during January to March and declined from April.

The number of *S. carpocapsae* in the fields decreased with time (Fig. 2). No infective juveniles of *S. carpocapsae* were found in microplots after February 1989 in no-tillage sorghum, no-tillage soybean, and conventional-tillage sorghum. No juveniles of *S. carpocapsae* were found in conventional-tillage soybean after December 1988 (Fig. 2).

Numbers of *S. carpocapsae* decreased with time in both tillage systems (Fig. 3). There were more *S. carpocapsae* in no-tillage than in conventional-tillage plots, and this may be due to higher moisture and reduced UV light in no-tillage as compared with conventional tillage. Gaugler and Bouch (1978) reported the deleterious effect of UV light on the survival of *S. carpocapsae*.

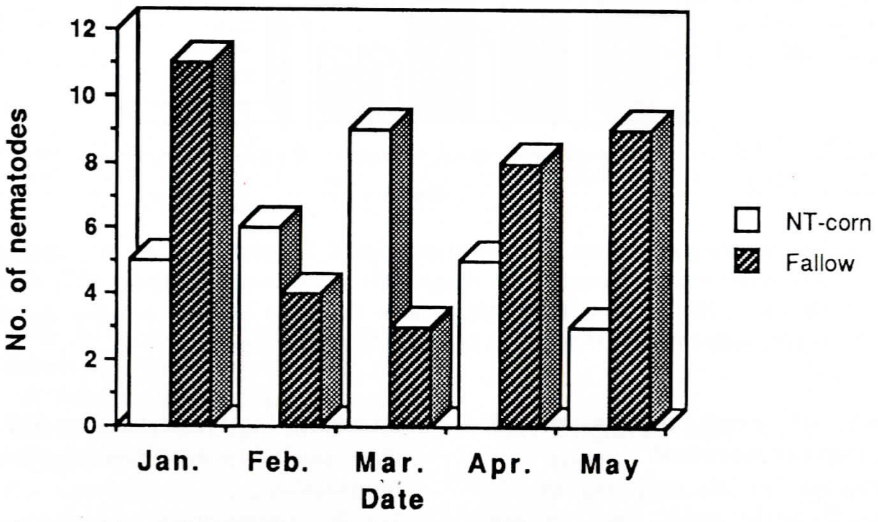

Fig. 1. Overwintering population of infective juveniles of *S. carpocapsae* in a fallow and a no-tillage corn field during January to May 1988. Means with the same letter in the figure are not significantly different ($P<0.05$) in Duncan's Multiple Range Test.

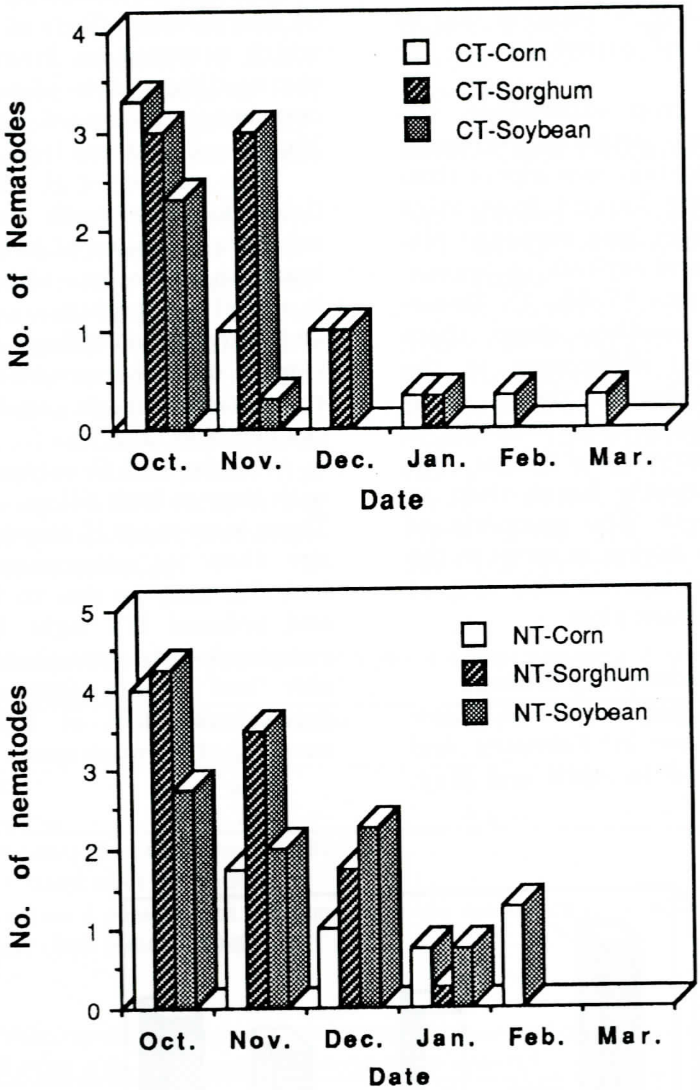

Fig. 2. Overwintering population of infective juveniles of *S. carpocapsae* in corn, sorghum, and soybean plots planted in no- tillage (NT) and conventional-tillage (CT) during October 1988 to April 1989. Means with the same letter in a figure are not significantly different (P<0.05) in Duncan's Multiple Range Test.

(C) Impact of crop debris on *S. carpocapsae* survival

The number of *S. carpocapsae* decreased significantly with time in soil covered with corn and sorghum debris (Figs. 4 and 5). No juveniles were recovered after 3 m, except in the treatment with sorghum debris. The loss of moisture (soil moisture = 0.7%) in the cups probably resulted in elimination of nematodes.

S. carpocapsae numbers decreased significantly 30 d after application in cups with soil and in soil covered with corn debris (Table 3). However, there were significantly more nematodes as-

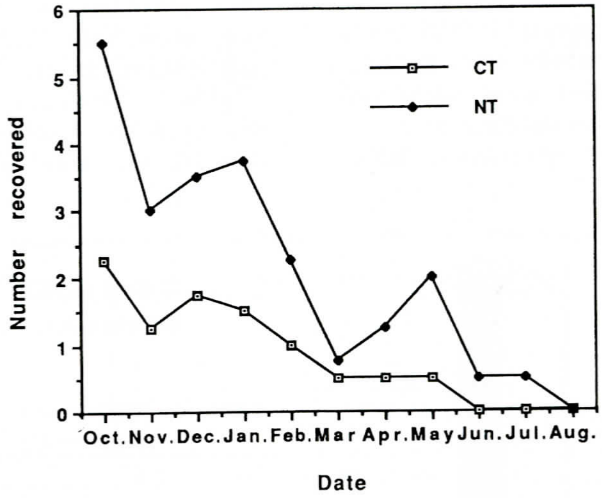

Fig. 3. Seasonal incidence of infective juveniles of *S. carpocapsae* in no-tillage (NT) and conventional-tillage (CT) corn plots during October 1988 to August 1989.

Table 3. Influence of corn debris on the survival of *S. carpocapsae* (S.c.) infective juveniles in sandy loam soil

Day	Mean no. nematodes			Mortality(%)		
	Debris	Soil+S.c.	Soil+debris +S.c.	Debris	Soil+S.c.	Soil+debris +S.c.
7	0	630a	511a	0	100a	100a[a]
14	0	598a	320b	0	100a	100a
21	0	512a	183c	0	100a	100a
28	0	503a	110c	0	100a	100a
30	0	524a	107c	0	100a	95a
60	0	1b	0d	0	0b	0b
90	0	0b	0d	0	0b	0b

[a] Means with the same letter in a column are not significantly different ($P<0.05$) in Duncan's multiple range test (SAS Institute, 1985).

sociated with bare soil treatments than were in debris-covered cups during the first 30 d. A fungus, *Aspergillus sp.*, was found in debris-covered cups 10 d after nematode application and it may have inhibited the survival of *S. carpocapsae*.

In cups covered with aluminum foil, the number of *S. carpocapsae* was greater than in those covered with debris, due to better retention of moisture levels conduce to nematode survival (Figs. 4 and 5).

(D) Dispersal of *S. carpocapsae* in the field

Viable infective juveniles of *S. carpocapsae* were recovered from the fumigated field during the 4 wk of the experiment (Table 4). Seven days after application, nematodes were found 25 cm from the point of application, indicating that some nematodes moved as much as 3.55 cm a day (Table 4). Four weeks after application, infective juveniles were recovered 100 cm from the point of application.

Twelve days after application, infective juveniles of *S. carpocapsae* were recovered from 45 cm from the point of application in the mulch-covered plots (Table 5). However, in bare soil, nematodes were not recovered from 45 cm until day 16. The total number of nematodes

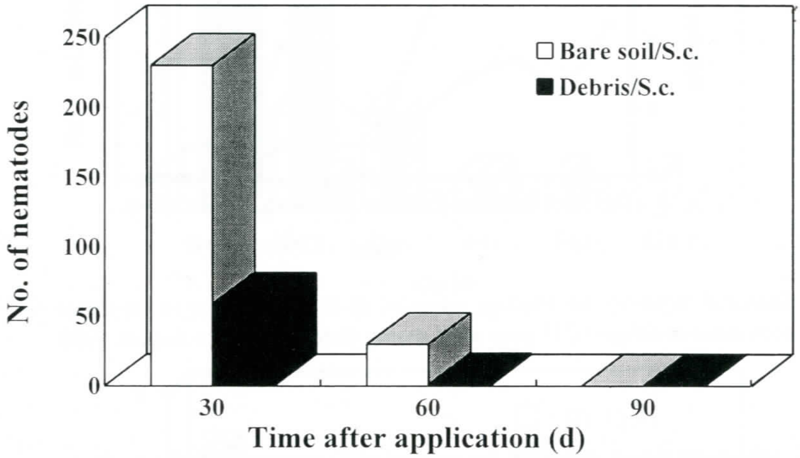

Fig. 4. Effect of corn debris on survival of infective juveniles of *S. carpocapsae* (S. c.) in cups with sandy loam soil and soil covered with corn debris. Means with the same letter in the figure are not significantly different (P<0.05) in Duncan's Multiple Range Test.

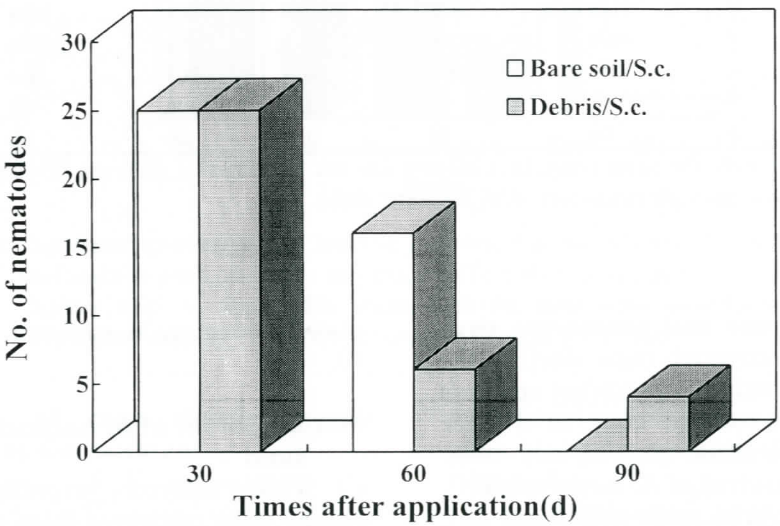

Fig. 5. Effect of sorghum debris on survival of infective juveniles of *S. carpocapsae* (S. c.). Means with the same letter in the figure are not significantly different (P<0.05) in Duncan's Multiple Range Test.

Table 4. Horizontal dispersal of infective juveniles of *S. carpocapsae* in a 1986 field test

Days after appl.	Mean no. nematodes recovered from soil				Moisture(%)[a]
	Distance (cm)				
	25	50	75	100	
7	11	0	0	0	10.3
14	41	29	0	0	8.0
21	46	44	42	0	6.1
28	38	64	38	36	3.0

[a] Moisture measured at the site of 100 cm.

Table 5. Horizontal dispersal of infective juveniles of *S. carpocapsae* in bare and mulch-covered plots[a]

Day after appl.	Mean no. nematodes recovered from soil							
	Bare				Mulch			
	15cm	30cm	45cm	Total	15cm	30cm	45cm	Total
4	1 a	1.5b	0b	2.5A	5.0b	0.5b	0.0b[b]	5.5A
8	2.5a	0.5b	0b	3.0A	1.0b	0.5b	0.0b	1.5A
12	2 a	0.5b	0b	2.5B	13.5a	17.0a	24.5a	55.0A
16	4 a	10.0a	14a	28.0A	4.5b	3.5b	2.5a	10.5B

[a] The numbers of *S. carpocapsae* that were extracted on day 16 remaining at the application site were 76.5 in bare plots and 20.75 in mulch-covere plots.

[b] Means with the same letter in a column are not significantly different ($P<0.05$) in Duncan's multiple range test. Means of total number with the same letter in a row are not significantly different ($P<0.05$) in Duncan's multiple range test (SAS Institute, 1985).

recovered from mulch-covered plots were higher than from bare plots on day 12 after application. However, higher numbers of nematodes were recovered from bare plots than from mulch-covered plots on day 16. The numbers of *S. carpocapsae* that were extracted on day 16 which remained at the application site in bare plots were 76.5 and in mulch-covered plots were 20.75. There was a greater total number of *S. carpocapsae* remaining at the site of application in bare plots than in mulch-covered plots. These results indicate that *S. carpocapsae* has greater tendency for movement in mulch-covered soil. This is probably due to higher moisture and reduced UV light in mulch-covered plots.

Horizontal movement of *S. carpocapsae* in soil was studied by Georgis and Hague (1981) and Moly and Kaya (1981) in the laboratory and by Poinar and Hom (1986) in the field. *S. carpocapsae* moved 1.4 cm / day in sterilized forest soil (Georgis and Hague, 1981) and 7 cm / day in sand (Moyle and Kaya, 1981). Poinar and Hom (1986) observed 4.35 cm / day movement by *S. carpocapsae* in field clay loam soil. Our observations on the dispersal rate of 3.5 cm / day in the 1987 field trial and 7.5 cm / day in mulch-covered plots agree with these values.

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蟲生線蟲 *Steinernema carpocapsae*（線蟲綱：Steinernematidae）在不同作物系統中之自然棲群及在田間之移動

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摘 要

本試驗在三種糧食作物上研究作物耕作系統對蟲生線蟲*Steinernema carpocapsae*自然棲群之影響。在美國喬治亞州休耕及裸露的試驗小區因1988年2月的下雪造成所粹取出的線蟲數極低。自1988年10月至1989年8月試驗期間發現在不耕犛玉米試區的蟲生線蟲數目比傳統耕犛之試區多。蟲生線蟲的棲群在不耕犛或傳統耕犛的玉米、高粱、及大豆試區在1988年10月至1989年4月期間，皆隨試驗時間之進行而下降。在施用後五個月，除了傳統耕犛玉米試區外，大部份的試區都無法粹取出線蟲。施用蟲生線蟲於土表後再以玉米及高粱殘株覆蓋，移往25℃的溫箱下，30天後*Steinernema carpocapsae*線蟲數顯著下降。在田間水平移動試驗中，不覆蓋黑麥草的情形下*S. carpocapsae*可移動3.5公分／天，覆蓋牧草下線蟲可移動7.5公分／天。由此結果指出牧草覆蓋可增進*S. carpocapsae*的移動。

關鍵詞：*Steinernema carpocapsae*，不耕犛，傳統耕犛，玉米，高粱，大豆

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