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Effect of animal manure on the survival and pathogenicity of the entomopathogenic nematode, Steinernema carpocapsae 【Research report】

動物有機肥對蟲生線蟲Steinernema carpocapsae 存活及致病力之影響【研究報告】

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Abstract

The impact of animal manure on the survival and pathogenicity of the entomopathogenic nematode, Steinernema carpocapsae (All strain) was investigated in laboratory and field tests. Poultry, swine, and beef cattle manure were inhibitory to S. carpocapsae as significantly lower numbers of nematodes were extracted from manure-amended soils on 4 sequential sampling dates following application. The total number of nematodes recovered from both coarse sandy loam and silty loam soils began to decline 21 d after application and no nematodes were found in soil samples of either treatment at 45 d. Nematode-induced mortality of manure larvae of the greater wax moth, Galleria mellonella, declined to 92% by day 21 and to 44% on day 45 in the non-amended group, as compared with 86% to 15%, respectively, for sandy loam containing poultry manure. In a test with larvae of the grape root borer, Vitacea polistiformis Harris, nematode-induced mortality was 100% for up to 21 d. In a field trial involving nematode amendment of soil in no-tillage and plow-tillage systems, only 0.3%-4% of the infective juveniles were alive after 1 h, probably due to the deleterious effect of sunlight and high temperature. There were no major differences in the 2 tillage systems.

摘要

本實驗在室內及田間同時進行探討動物有機肥對蟲生線蟲Steinernema carpocapsae 存活及致病之效應。將雞、豬、及肉牛等動物性有機肥添加於砂質壤土及矽質壤土後，由四次連續取樣的結果得知線蟲數顯著下降，故推知動物有機肥對蟲生線蟲有抑制作用。於添加有機肥的兩種土壤粹取線蟲，其總數至第21天皆開始下降。砂質壤土不添加有機肥組至第45天線蟲粹取數為零，添加有機肥組線蟲粹取數為5。砂質壤土不添加有機肥組對臘蛾(Galleria mellonella)的致死率於施用後第21天為92%，第45天則降至44%。添加有機肥組則由86%降至15%。在探討添加動物有機肥後對葡萄根蛀蟲Vitacea polistiformis Harris的致死效應時發現，處理第21天後仍能維持100%死亡率。田間實驗中，耕犛或不耕犛的系統中，線蟲於土中再粹取其回收率介於0.3%~4%之間，這可能是陽光及高溫所導致的結果，在隨後不同取樣時間所粹取的線蟲在處理間並無差異。

Key words: Steinernema carpocapsae, animal manure, no-tillage, plow-tillage, Vitacea polsitiformis

關鍵詞: 蟲生線蟲、動物有機肥、耕犛、不耕犛、葡萄根蛀蟲。

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Effect of Animal Manure on the Survival and Pathogenicity of the Entomopathogenic Nematode, *Steinernema carpocapsae*

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ABSTRACT

The impact of animal manure on the survival and pathogenicity of the entomopathogenic nematode, *Steinernema carpocapsae* (All strain) was investigated in laboratory and field tests. Poultry, swine, and beef cattle manure were inhibitory to *S. carpocapsae* as significantly lower numbers of nematodes were extracted from manure-amended soils on 4 sequential sampling dates following application. The total number of nematodes recovered from both coarse sandy loam and silty loam soils began to decline 21 d after application and no nematodes were found in soil samples of either treatment at 45 d. Nematode-induced mortality of mature larvae of the greater wax moth, *Galleria mellonella*, declined to 92% by day 21 and to 44% on day 45 in the non-amended group, as compared with 86% to 15%, respectively, for sandy loam containing poultry manure. In a test with larvae of the grape root borer, *Vitacea polistiformis* Harris, nematode-induced mortality was 100% for up to 21 d. In a field trial involving nematode amendment of soil in no-tillage and plow-tillage systems, only 0.3% - 4% of the infective juveniles were alive after 1 h, probably due to the deleterious effect of sunlight and high temperature. There were no major differences in the 2 tillage systems.

Key Words: *Steinernema carpocapsae*, animal manure, no-tillage, plow-tillage, *Vitacea polistiformis*

Introduction

The entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) and its bacterial associate, *Xenorhabdus nematophilus*, is highly virulent, kills hosts quickly, can be cultured easily in vitro, has a high reproductive potential, and has a numerical but non-functional response to insect population increases.

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The free-living, non-feeding infective juveniles have a broad host range (Gaugler, 1988; Kaya and Gaugler, 1993), are safe to vertebrates, plants, and other non-target organisms (Akurst, 1990; Poinar, 1989), and are exempt from registration in the United States (Gorsuch, 1982).

Infective juveniles of *S. carpocapsae* are very environmentally sensitive and are functionally active in a relatively narrow range of temperatures, moistures, and ultraviolet light exposures, etc. They can avoid slow desiccation at high relative humidities (Womersley, 1990) and can survive low temperatures (Molyneux, 1985; Schmidt and All, 1979). Nematode survival is enhanced in soil buffered from environmental extremes (Klein, 1990).

The beneficial effects of animal manure ammendments to soil on plant growth have been recognized for centuries. However, there is evidence that manure has deleterious effects on plant-parasitic nematodes (Alam *et al.*, 1980). Relatively low larval mortality occurred on manure-treated *Musca domestica* L. (Geden *et al.*, 1985; Georgis *et al.*, 1987; Mullens *et al.*, 1987a; Renn *et al.*, 1985). Wicht and Rodriguez (1970) applied *S. carpocapsae* against *M. domestica* and *Fannia femoralis* (Stein) in chicken manure, but found no significant reduction. Mullens *et al.* (1987b) applied 8-9 million infective juveniles per square meter on wet manure but found no reduction of *Fannia canicularis* (L.). Geden *et al.* (1985) noted that a few *S. carpocapsae* lived less than 5 d on the manure surface. Renn *et al.* (1985) reported that *S. carpocapsae* survived less than 5 d, but most were dead or inactive within 48 h. Ammonia, salts, or other materials associated with fresh chicken manure were suspected to be toxic and appeared to inactivate nematodes quickly under field conditions.

The purpose of this study was to determine the impact of 3 animal manures, i.e., poultry, swine, and cattle, on the survival and pathogenicity of *S. carpocapsae* (All strain) to *Galleria mellonella* larvae in the laboratory and field.

Materials and Methods

Nematode culturing and inoculum preparation

Infective juveniles of *S. carpocapsae* (All strain) used throughout the experiments were collected from the larvae of the grape root borer, *Vitacea polistiformis* Harris, in 1974 (All *et al.*, 1980) and continuously cultured in larvae of the greater wax moth, *Galleria melonella* L., using the method described by Dutky *et al.* (1964). Forty-eight hours after infection, cadavers of infected larvae were placed in an emergence system composed of a plastic container (17.5 x 8 x 4 cm^3) with a lid, inside of which was a piece of tile (10 x 5 cm^2) wrapped with filter paper (Whatman #1, 15 cm), and submerged in a 0.1% formalin solution. This was designated as stock. Infective juveniles were stored in a 0.1% formalin solution at 5 ℃ after they emerged from the cadavers. New nematode inoculum solution was cultured monthly to maintain a fresh, highly infective stock. The *G. mellonella* colony used in this study was obtained from a colony from a bee hive and reared on a meridic diet (Glycerol 1 l, wheat germ 1 lb, honey solution 1 l, yeast 200 g, dog food (Pedigree) 50 lb) at 27 ℃ . This colony had been maintained in the laboratory for at least 6 years.

Soil preparation and analysis of physical characteristics

Coarse sandy loam was obtained from the The University of Georgia Plant Science Farm near Watkinsville, Georgia, and the silty loam was obtained from the Southeastern Branch Experiment Station near Midville, Georgia. A sample of soil was collected from the upper 15 cm of the A horizon. Texture of these soils was determined by the Soil Testing and Plant Analysis Laboratory of the USDA in Athens. The characteristics of these 2 soils are listed in Table 1.

Amended soil preparation

Fresh manure was collected from the Poultry Research Center, Swine Research Center, and Beef Cattle Research Center of the University of Georgia. The coarse sandy loam and silty loam soils were placed on concrete pavement, covered with a plastic sheet, and methyl bromide was added to fumigate the soils for 7 d. After fumigation, soils were stored in 50-gal plastic barrels. Manure was mixed with fumigated coarse sandy loam soil and silty loam (sieved to 2-mm size) in the proportion of 11 g poultry manure, 13 g swine manure, and 16 g cattle manure per kg of soil. These amounts of manure are equivalent to the levels used for soil fertilization amendments in agriculture (Bitzer and Sims, 1988, Mathers and Goss, 1979).

Laboratory survivorship tests

Four sets of survivorship tests were conducted with *S. carpocapsae*. In the 1st test, 5000 infective juveniles (IJs) were added directly to 5 g fresh manure (poultry, swine, or beef cattle) in 1-l Mason jars® with lids. Each treatment was replicated 4 times. After 21 d, the number of live nematodes was counted directly from the manure slurry using a dissecting microscope.

In the 2nd test, 30 g of unamended soil and 3 manure-amended soils were put into separate petri plates (dia. 9 cm). The moisture content was 10% (W/W) for each treatment. We added 5000 IJs were added to each plate. Each treatment was replicated 5 times. Plates were incubated at 25 ℃ at 12L:12D. After 21 d the nematodes were extracted from the soil with a Baermann funnel. The total number of nematodes recovered from each treatment was counted and used as a criterion to compare the effect of each type of manure on survival of IJs.

In the 3rd test, fresh chicken manure was mixed with either coarse sandy loam or silty loam soil at a rate of 0.025% (W/W). Thirty grams of amended soil was placed into each petri plate (dia. 9cm) and 2500 IJs were added to each treatment. Each treatment was replicated 5 times. At days 7, 14, 21, and 45 after inoculation, nematodes were extracted from the soil using a Barmann funnel. The number extracted and the number on the inside

Table 1. Physical characteristics of Cecil coarse sandy loam and silty loam used in the experiments with *S. carpocapsae*.

Soil types	Proportion (%)			pH	Organic Matter (%)
	Sand	Silt	Clay		
Cecil coarse sandy loam	85	5	10	5.4	0.54
Silty loam	95	2	3	6.2	0.70

of lids were pooled for the total number of nematodes recovered. The moisture content of each treatments was 10% W/W.

Analysis of variance and Duncan's Multiple Range Test were used to analyze data from all tests (SAS Institute 1985).

Laboratory Pathogenicity Tests

Fifth-instar *G. melonella* larvae and neonate *V. polistiformis* (Harris) were used in the 1st pathogenicity test. In a separate test with *G. mellonella* larvae, treatments included amended soil (soil +manure), amended soil with *S. carpocapsae*, unamended soil, and unamended soil with *S. carpocapsae*. Thirty grams of each combination was placed into separate petri plates. Each treatment was replicated 5 times. Soil moisture was maintained at 10 % by gravimetric wetness. We added 5000 IJs to each treatment for *G. melonella* treatments, and 100 IJs for *V. polistiformis* treatments. Ten *G. melonella* larvae were placed into each of 5 plates in the following sequence: immediately after preparation (day 0), and at 7, 14, and 21 d after adding the nematodes. Mortality of *Galleria* larvae was recorded 48 h after placement in the petri plate. Cadavers were dissected to confirm nematode infection.

In the test with *V. polistiformis*, a piece of grape root (*Vitis labruska* L. var. *concord*) was placed in the bottom of a 1-dram vial (14.5 × 45mm) and soil was then added to a depth of 5 cm. We added 1000 IJs of *S. carpocapsae* to each vial followed by 10 neonate *V. polistiformis* larvae. Vials were sealed with a plastic cap, and mortality of the larvae was recorded after 48 h. Cadavers containing nematodes were recorded as nematode-induced mortality.

In the 2nd pathogenicity test, chicken manure was mixed with dry coarse sandy loam or silty loam at a concentration of 0.025 % (W/W). Treatments were : (1) poultry manure amended soil; (2) unamended soil (Control); (3) unamended soil + *S. carpocapsae*; and (4) amended soil + *S. carpocapsae*. Other procedures were the same as in the first pathogenicity test. On each of 7, 14, 21, and 45 d after applying nematodes, 10 *G. mellonella* larvae were placed into each of 5 plates and mortality was recorded after 48 h. Cadavers containing nematodes were recorded as nematode-induced mortality.

Analysis of variance and Duncan's Multiple Range Test were used to analyze data from each test (SAS Institute 1985).

Field trials

A field test was conducted with corn, *Zea mays* L., in a field separated into no-tillage and conventional tillage blocks at the Southeastern Branch Experiment Station near Midville, Georgia. The test was initiated on 24 April 1989 and the 4 treatments were: (1) *S. carpocapsae* (check); (2) poultry manure + *S. carpocapsae*; (3) terbufos 1.03 kg AI/ha (Counter) + *S. carpocapsae*; and (4) *S. carpocapsae* + poultry manure + terbufos 1.03 kg AI/ha.

Another field test was conducted on 12 September 1989, using no-tillage and conventional tillage sorghum, *Sorghum halenpense* L., at the Plant Science Farm near Watkinsville, Georgia. In this test, 4 treatments were used: (1) *S. carpocapsae* (check); (2) poultry manure + *S. carpocapsae*; (3) chlorpyrifos (Lorsban ®) 1.03 kg AI/ha + *S. carpocapsae*; and (4) *S. carpocapsae* + poultry manure + chlorpyrifos.

Three hundred and fifty grams of silty loam and coarse sandy loam soil were placed into 0.3-l plastic cups (12 holes in the bottom) and saturated with

water before transferring to the corn and sorghum fields. Both soils were fumigated with methyl bromide before use. We placed 15,000 IJs of *S. carpocapsae* at a depth of 1 cm in a cup. Five cups of each soils were placed in each replicate.

The experimental design used in both fields was a randomized complete block split-plot design. Tillage treatments were main plots, and nematode treatments were split plots. Tillage blocks were 20 x 10 m^2 in size and were replicated 4 times.

After planting the corn and sorghum with a John Deere, Flex 71 no-tillage planter, cups were placed in holes so that the top edge was at the soil surface. Poultry manure (11 lb/plot) was then distributed evenly by hand over the whole area of each plot. In the no-tillage treatments, plant debris from the field was placed on the cups in a coverage similar to the surrounding area. One cup was collected immediately from each replicate and defined as the 1 h treatment. At 7, 14, 21, and 45 d after planting, 1 cup from each plot was collected and brought to the laboratory to extract nematodes and conduct the mortality bioassay with *G. mellonella* larvae as previously described.

Results

Laboratory survivorship tests

No live infective juveniles of *S. carpocapsae* were found in the 3 manure slurries. All 3 manures became liquefied and had a very strong ammonia odor. Almost twice as many *S. carpocapsae* were recovered from non-amended soil as compared with the soil amended with the three animal manures 21 d after inoculation (Fig. 1). There were no significant differences in nematode survival between the 3 types of manure in soil.

The total number of nematodes recovered from both coarse sandy loam and silty loam soils began to decline on day 21, whether with amended or non-amended soil (Table 2). We observed that many infective juveniles climbed to the inner surface of the lid of the petri plate. This behavioral response may be due to negative geotaxis and absence of a host.

There were greater numbers of nematodes on the lid of plates than were extracted from the soil on both manure-amended and non-amended coarse sandy loam soil (Table 2). The numbers of infective juveniles extracted in non-amended coarse sandy loam soil remained statistically similar ($P<0.05$) from day 7 to 21, but dropped significantly ($P<0.05$) by day 45 (Table 2).

The numbers of infective juveniles extracted from amended coarse sandy loam soil were statistically similar from day 7 to 14, but dropped significantly ($P<0.05$) from 126 on day 14, to 65 on day 21, and to 5 on day 45 (Table 2). The total number of nematodes from amended soil showed a significant decrease in number of infective juveniles from day 7 to 14 and 21, followed by another significant decrease between days 21 and 45.

In the silty loam soil, the total number of nematodes in non-amended soil remained similar from day 7 to 21, but dropped significantly ($P < 0.05$) on day 45 (Table 2). In the manure-amended treatments, the total numbers of nematodes significantly decreased between day 7 and 45.

There were significantly reduced numbers of infective juveniles extracted from both types of soil amended with manure as compared with non-amended soil at each sampling date (Fig. 2).

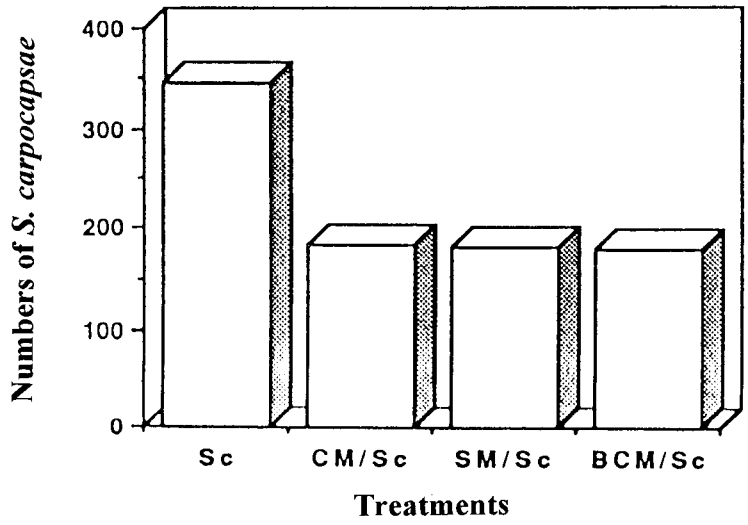

Fig. 1. Survival of *S. carpocapsae* in unamended sandy loam and in sandy loam amended with 3 different manures with a sample size of 5 for each treament. (S.c.=*S. carpocapsae*; CM=poultry manure; SM=swine manure; BCM=beef manure).

Table 2. Effect of poultry manure on the survival of *S. carpocapsae* in coarse sandy loam and silty loam soils

Day	Non-amended soil				Amended soil			
	% Mois.	Lid	Extr.	Total	% Mois.	Lid	Extr.	Total
	Coarse sandy loam (Mean no. of nematodes recovered)							
7	8.74	742a	202 a	944aA	8.98	483 a	152a	635aB[a]
14	6.88	624a	225 a	849aA	6.96	336 b	126a	462bB
21	4.02	658a	174 a	832aA	3.51	356 b	65b	421bB
45	0.97	601a	0 b	601bA	0.15	277 b	5c	282cB
	Silty loam (Mean no. of nematodes recovered)							
7	7.38	332c	710a	1042aA	8.26	241a	528a	769aB
14	6.75	425b	606ab	1031aA	7.24	354a	289ab	643abB
21	6.05	587a	450bc	1036aA	6.92	345a	272b	617abB
45	2.97	432b	323bc	755bA	3.95	302a	232b	534bB

[a] For a given day, means within a column followed by the same lower case letter are not significantly different ($P<0.05$) in Duncan's multiple range test. For a given treatment, means in a row followed by the same upper case letter are not significantly different ($P<0.05$) in Duncan's multiple range test (SAS Institute 1985).

There were significantly ($P<0.05$) more nematodes extracted from both non-amended and amended silty loam than from non-amended and amended coarse sandy loam soil throughout the study (Fig. 2)

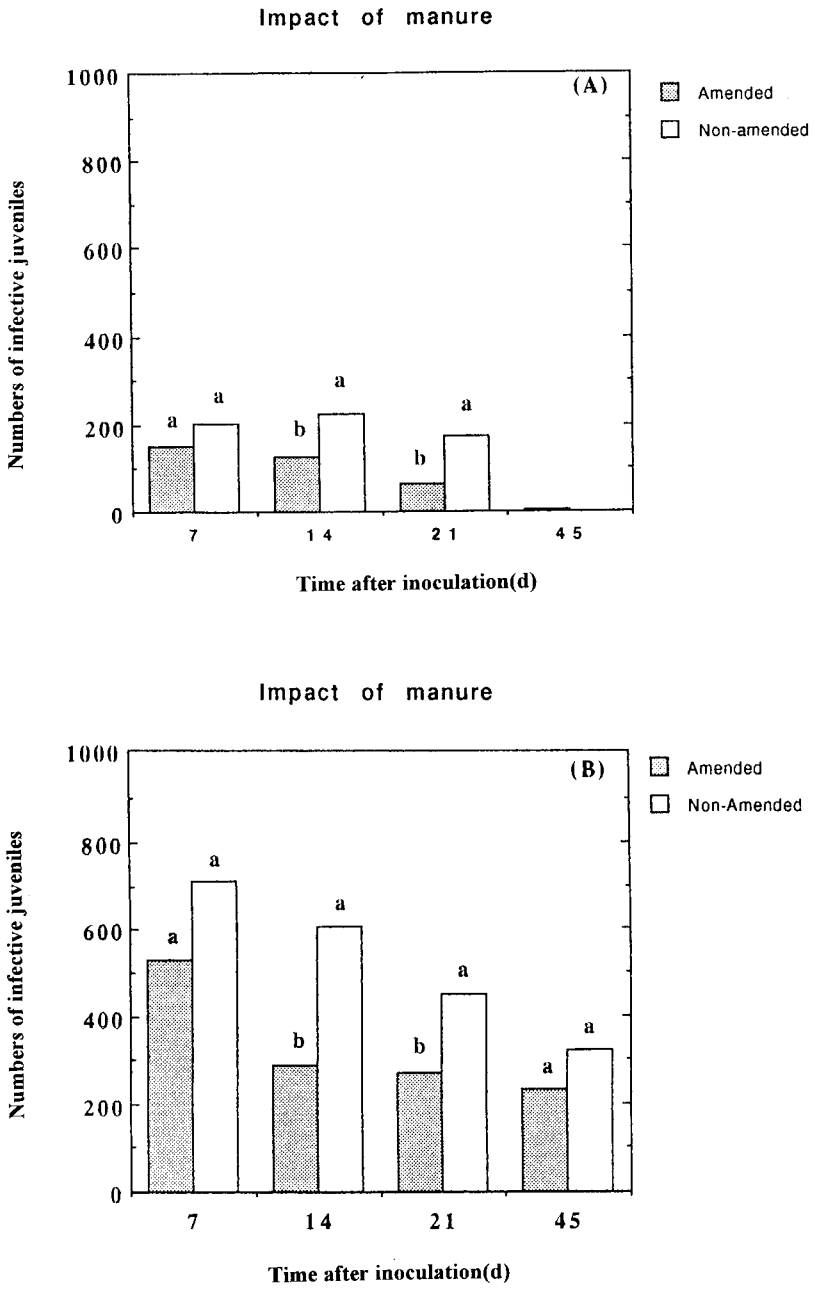

Fig. 2. Comparison of the numbers of *S. carpocapsae* recovered from polultry manure-amended and non-amended coarse sandy loam (A) and silty loam (B) at various sampling intervals.

Laboratory pathogenicity test

Table 3 shows the results of the 1st experiment, namely the effect of 3 animal manures on the pathogenicity of *S. carpocapsae* to mature *Galleria* larvae and to the neonate larvae of the grape root borer. On days 0, 7, and 14, the mortality rate 48 h after introducing both species of insect into the plates was 100% in all treatments. On day 21, the pathogenicity to *Galleria* in soil with *S. carpocapsae* and the 3 manures was less than in soil without manure, but the differences were not statistically significant (Table 3).

For the grape root borer larvae, mortalities were 100% up to 21 d. The closed system of the bioassay permitted the soil in the vials to retain much of its moisture. However, fewer juveniles were required to ensure high mortality of the small grape root borer than were required for the considerably larger *Galleria* larvae (Table 3).

In the 2nd test, the effect of poultry manure on the pathogenicity of *S. carpocapsae* to *Galleria* larvae was evaluated in coarse sandy loam and silty loam soil on days 7, 14, 21, and 45 (Table 4). In coarse sandy loam, *Galleria* larvae mortality dropped from 92% to 44% on day 45 in the non-amended treatments, as compared with 86% to 15% in manure-amended soil, and these differences were significant at $P<0.05$. The moisture in the sandy loam soil decreased from 10% to 0.2%, which probably resulted in nematode mortality and lower toxicity to *Galleria*.

In the silty loam soil, the mortality remained 100% up to day 45 in both amended and non-amended soils. No

Table 3. Effect of animal manures on the pathogenicity of *S. carpocapsae* to 5th instar *Galleria* larvae and neonate grape root borer larvae

Treatment	Mortality (%)			
	Day 0	Day 7	Day 14	Day 21
Galleria larvae				
Chicken manure + nematodes	100a	100a	100a	76a[a]
Swine manure + nematodes	100a	100a	100a	88a
Beef cattle manure+nematodes	100a	100a	100a	88a
Nematodes	100a	100a	100a	92a
Control	0b	0b	0b	0b
Grape root borer				
Chicken manure + nematodes	100a	100a	100a	100a[a]
Swine manure + nematodes	100a	100a	100a	100a
Beef cattle manure+nematodes	100a	100a	100a	100a
Nematodes	100a	100a	100a	100a
Control	0b	0b	0b	0b

[a] For a given insects, means within a column followed by the same letter are not significantly different ($P<0.05$) in Duncan's multiple range test (SAS Institute 1985).

mortality was found in either the manure-amended coarse sandy loam or the silty loam soils (Table 4).

Comparing the pathogenicity of *S. carpocapsae* at different time intervals in the coarse sandy loam soil, there was a significant decrease on day 45 as compared with the other samples (Table 4). In the *S. carpocapsae*-plus-manure treatment, the pathogenicity of the Baerman extracts to *Galleria* larvae decreased at 14, 21, and 45 d. In the silty loam soil, no difference occurred among treatments at the different time intervals.

Field trials

In the field trial, the number of IJs declined quickly after 45 days following application of nematode solutions in the corn field near Midville, Georgia (unpublished data). In the conventional tillage plots, the number of S. carpocapsae declined from 35 to 2 in the nematode plots, 155 to 1 in the manure-amended soil plots, 114 to 0 in chlorpyrifos- treated plots, and 14 to 0 in chlorpyrifos-plus-manure plots.

In the no-tillage plots, after 45 d, the number of infective juveniles dropped from 369 to 0 in nematode plots, 218 to 6 in manure plots, 215 to 2 in the chlorpyrifos-treated plots, and 15 to 3 in the chlorpyrifos-plus-manure plots. A large population of predaceous nematodes, Monochus spp., were found in the manure-amended plots. The relation between those 2 species is unknown. The decline in nematode number to very low levels in all the treatments makes interpretation of

Table 4. Number of nematodes recovered from different cultural treatments involving conventional tillage and no tillage near Midville, Georgia, 1989[ab]

Treatment	Mean no. of infective juveniles				
	1h	Day 7	Day 14	Day 21	Day 45
Conventional tillage					
Nematodes	35ab	6a	1a	1a	2a[c]
Manure+nematodes	155a	5ab	1a	1a	1a
Lorsban+nematodes	114ab	3b	0a	0a	0a
Lorsban+manure nematodes	14b	3b	0a	0a	0a
No tillage					
Nematodes	369a	1a	0a	0a	0a
Manure+nematodes	218ab	9a	1a	0a	0a
Lorsban+nematodes	215ab	3a	0a	0a	0a
Lorsban+manure nematodes	15b	5a	1a	2a	3a

[a] This test was conducted from 25 April 1989 to 5 May 1990.
[b] Soil moisture was 6.48% in conventional tillage and 8.31% in no-tillage plots.
[c] For a given treatment of each soil, means in a column followed by the same letter are not significantly different ($P<0.05$) in Duncan's multiple range test (SAS Institute 1985).

results difficult but the data suggest that there were no major differences among the cultural practices in the 2 tillage systems.

Overall, more nematodes were found in the no-tillage as compared with conventional tillage plots at 1 h after application. The plant residues present on the surface in the no-tillage system decreased the deleterious effect of ultraviolet light on the infective juveniles. The extremely high temperatures (above 35 ℃) during the at day were probably the major factor which resulted in the dramatic decrease in nematode numbers in the field. In addition, the extremely dry conditions in both no-tillage and conventional tillage treatments probably contributed to the reduced field population.

Discussion

Renn *et al.* (1985) indicated that nematodes survived no more than 48 h on chicken manure due to the presence of toxic substances in the manure. Unsuitable pH values also may be inhibitory to nematode development and survival (Renn *et al.* 1985).

Geden *et al.* (1985) reported poor survival, inhibition of searching ability, and reduced infectivity of *S. carpocap* sae in poultry manure. Manure quality, especially moisture content (70%-75%), may influence nematode survival (Renn *et al.*, 1985). This may explain the results presented here.

In tests with *Spodoptera litura* Boisduval, Kondo and Ishibashi (1986) reported that, when insect larvae were not present, *S. carpocapsae* moved to petri dish lids or nictated on the surface of the soil in the dishes. Previous observations of nematode behavior indicated that infective juveniles of the "All strain" of *S. carpocapsae* have a tendency, to move upward (Kondo and Ishibashi, 1986). The nematodes move readily through the condensed water droplets on the petri plates lids. Metabolism of the microorganisms in the fresh manure generated more moisture condensation on the lids of manure-amended sandy loam and silty loam soils as compared with the non-amended treatments. This was probably the reason why more infective juveniles were found on the lids in the amended soil than in the non-amended treatments. Another possibility is that nematodes were repelled by the ammonia produced in manure-amended soil.

Research by Renn *et al.* (1985), Geden *et al.* (1985), and Mullen *et al.* (1987a) indicates that infectivity of *S.* carpocapsae to *Musca domestica* L. is reduced on pure manure. The results of this study show a similar trend, but the effect of the soil/manure mixture may have reduced the degree of inhibition. This test also indicates that the inhibitory effects of manure may require more than 21 d to occur. Longer intervals between inoculation of manure-treated soil and extraction of the nematodes were allowed in subsequent tests.

Moore (1965) indicated that in the field with 50%-60% RH, 5%-10% of the infective juveniles were alive 1 h after the spray evaporated. Gaugler (1988) indicated that brief exposures to short UV radiation produces high nematode mortality and also severely reduces nematode pathogenicity. In the above study, only 0.3%-4% of the extracted infective juveniles were alive 1 h after exposure in the field, probably because of the deleterious effect of sunlight and high temperatures.

In the Midville test, the number of *S. carpocapsae* juveniles recovered from each treatment was also very low in

the no-tillage treatments. This may be due to heavy rain that occurred immediately following application of the nematodes. The total number of other free-living nematodes increased with time and reached a peak on day 21 in conventional tillage and day 45 in no-tillage plots.

Overall, the results of the field tests indicate that the survival rate and pathogenicity of *S. carpocapsae* are dramatically lower when the moisture content falls below 0.5% in both soil types. The application of manure has deleterious effects on the survival of nematodes and on the pathogenicity of infective juveniles of *S. carpocapsae* to *Galleria* larvae. Soil type and manure treatment have a significant effect on the survival of *S. carpocapsae*. In the field test, the low number of nematodes that were recovered following application in selected cropping environments make interpretation of results difficult. The deleterious effect of intense sunlight and dry soil on *S. carpocapsae* during the experiment was probably lethal to most of the nematodes.

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動物有機肥對蟲生線蟲 *Steinernema carpocapsae* 存活及致病力之影響

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摘　要

本實驗在室內及田間同時進行探討動物有機肥對蟲生線蟲 *Steinernema carpocapsae* (All strain) 存活及致病之效應。將雞、豬、及肉牛等動物性有機肥添加於砂質壤土及砂質壤土後，由四次連續取樣的結果得知線蟲數顯著下降，故推知動物有機肥對蟲生線蟲有抑制作用。於添加有機肥的兩種土壤粹取線蟲，其總數至第 21 天皆開始下降。砂質壤土不添加有機肥組至第 45 天線蟲粹取數爲零，添加有機肥組線蟲粹取數爲 5 。砂質壤土不添加有機肥組對大臘蛾 (*Galleria mellonella*) 的致死率於施用後第 21 天爲 92% ，第 45 天則降至 44% 。添加有機肥組則由 86% 降至 15% 。在探討添加動物有機肥後對葡萄根蛀蟲 *Vitacea polistiformis* Harris 的致死效應時發現，處理第 21 天後仍能維持 100% 死亡率。田間實驗中，耕耨或不耕耨的系統中，線蟲施於土中再粹取其回收率介於 0.3%-4% 之間，這可能是陽光及高溫所導致的結果，在隨後不同取樣時間所粹取的線蟲在處理間並無差異。

關鍵詞：蟲生線蟲、動物有機肥、耕耨、不耕耨、葡萄根蛀蟲

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