A NEW NEMATODE PARASITE OF MOLE CRICKETS: ITS TAXONOMY, BIOLOGY AND POTENTIAL FOR BIOLOGICAL CONTROL

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1988

ACKNOWLEDGEMENTS

The author is deeply grateful to the chairman of his committee, Dr. G. C. Smart, Jr., who has given considerable time and immeasurable assistance during the term of this study. His suggestions made during the writing of this dissertation are greatly appreciated.

Gratitude is expressed also to Drs. R. P. Esser, S. R. Farrah, J. L. Nation, and A. C. Tarjan for their invaluable assistance while serving as members of his supervisory committee.

Thanks are given also to Dr. T. J. Walker for information related to mole crickets, and to Dr. H. L. Cromroy for identifying the mite *Rhizoglyphus* sp.

Finally, the author is very grateful to his wife and children for their encouragement and understanding which were essential for the completion of this work.

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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By

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December, 1988

Chairman: G. C. Smart, Jr.
Major Department: Entomology and Nematology

A new steinernematid nematode parasite of mole crickets was collected from Uruguay, South America. The nematode does not fit any nominal species of the genus Steinernema and is herein described as a new species.

The life cycle and sex ratio of the nematode is influenced by temperature. At 10 to 15 C the life cycle is not completed, at 20 C the cycle takes 12 days to complete, at 24 C, 10 days and at 30 C, 8 days. At 15-24 C the number of females in the population is greater than the number of males, but at 30 C the reverse occurs.

The new species does not reproduce well, if at all, in larvae of the wax moth, Galleria mellonella, which is a universal host for all other species. Its host range appears to be much narrower than that of other species, with a penchant for mole crickets. When released in the field in

North Florida, the nematode became established, has spread out from the original release sites, and continues to kill mole crickets after 3 1/2 years. When released on the soil surface, the nematode moved down and killed mole crickets placed 10 cm below the soil surface. The nematode survived in the soil for 10 weeks and retained its ability to kill mole crickets. These attributes make it a very good candidate for the biological control of mole crickets imported accidentally to Florida.

In a survey of nematodes associated with mole crickets, the following genera were found: *Binema*, *Cameronia*, *Cruznema*, *Diplogaster*, *Mesorhabditis*, *Pulchrocephala*, *Steinernema*, and *Talpicola*.

CHAPTER 1
INTRODUCTION

According to Walker (1984), the most important pests of turf and pasture grasses in Florida are mole crickets in the genus *Scapteriscus*, which cause damage also to crops such as vegetables, ornamentals, tobacco, etc. The cost due to mole cricket activity, including damage and cost of control, to Floridians is about $45 million annually (Hudson and Short, 1988).

Management of the mole cricket problem in Florida began in 1940 when tons of calcium arsenate bait were used to control mole crickets on truck crops in central Florida. The introduction of synthetic insecticides, especially DDT and Chlordane, in 1945, temporarily solved the mole cricket problem. When these insecticides were banned, however, and when bahiagrass was used increasingly as pasture grasses, the mole cricket problem became more serious (Walker, 1986). In response to the concerns of cattlemen, farmers, turf grass managers, and home owners, a research project on mole crickets was begun at the University of Florida in 1978. The objectives of this project were to eliminate or reduce the damage caused by mole crickets in Florida, and at the same time to study the fundamental biology of mole crickets. One

of the major directions of the project was to search for natural enemies of mole crickets.

When searching for natural enemies of an organism, in principle one should begin the search in areas indigenous to the organism. The non-native mole crickets in Florida, those in the genus Scapteriscus, most likely were introduced to the southeastern United States from South America (Walker and Nickle, 1981) where they are of little economic importance. Evidently, there are factors in South America which keep population densities below economic threshold levels. A search for natural enemies of mole crickets in that area showed that a steinernematid nematode may be the limiting factor. This nematode was found in Brazil and Uruguay and different isolates from Uruguay were hand-carried to the United States by Dr. G. C. Smart, Jr. and myself in 1985. We have worked with this nematode since.

The major objective of the research reported herein was to study the biology and ecology of the nematode to determine its effectiveness as a biological control agent of mole crickets in Florida. A secondary objective was to determine if nematodes are associated with mole crickets in Florida.

CHAPTER 2
STEINERNEMA SCAPTERISCI N. SP. (STEINERNEMATIDAE : NEMATODA) FROM URUGUAY, SOUTH AMERICA

While searching for natural enemies of mole crickets in South America, a steinernematid nematode infecting mole crickets was found in Brazil and Uruguay. The nematode was thought to be *Steinernema carpocapsae* (=*Neoaplectana carpocapsae*), and several isolates from Uruguay were brought to the quarantine laboratory in Gainesville, Florida by Dr. G. C. Smart, Jr. and me in 1985. Its morphology and biology showed that it is different from that species and it is herein described as *Steinernema scapterisci* n. sp. and named after its host, *Scapteriscus*, a genus containing mole crickets.

Materials and Methods

Nematodes collected in Uruguay were inoculated into mole crickets which were hand-carried to Florida. In Florida, populations of the nematode were increased in the mole crickets, *Scapteriscus vicinus* and *S. acletus*, and later in the house cricket, *Acheta domestica*. These nematodes, or their progeny were used for all studies.

Morphology

The first generation adults were collected from infected mole crickets 2-3 days after the crickets died, the second generation adults were collected 5-7 days after the crickets died and the third-stage infective juveniles were collected 7-15 days after the crickets died. The nematodes were killed in warm water at 40 C, and mounted in water on glass slides with coverglass supports. Many live nematodes or nematodes stained with acid fushin were observed to confirm the presence and/or nature of some anatomic structures.

Scanning Electron Microscopy (SEM)

Nematodes prepared for SEM were placed live in lactophenol at 43 C for 30 minutes, transferred to a desiccator for two days, removed,rinsed with water, and then prepared by the method of Stone and Green (1971). Specimens were examined in a Hitachi S450 SEM.

To prepare spicules and gubernacula for SEM, male nematodes of the first generation were placed in a petri dish containing water, killed by low heat and stored at room temperature. After 2-3 days when the bodies had softened due to decay, they were transferred to clean water, and, with two small needles, the rear portion of each nematode was torn open, the spicules and gubernaculum dissected out and washed free of debris by sloshing them about in water. Then the spicules and gubernaculum were picked up with a

needle and placed on a previously-prepared SEM stub close to a hair used as a marker.

Cross Hybridization

These studies were conducted using two different techniques. In one technique, a drop of blood (hemolymph) from a mole cricket was placed in a 35 x 10 mm sterile petri dish, and one third-stage juvenile of *S. scapterisci* and one of *S. carpocapsae* strain Breton added. The dish was placed in a plastic bag containing a paper towel saturated with water. The plastic bag was closed, tied and stored in the dark. The treatment was replicated 25 times.

In the second technique, two drops of blood were prepared as above, and 10 third-stage juveniles of *S. scapterisci* were placed in one drop and 10 third-stage juveniles of *S. carpocapsae* strain Breton were placed in the other drop. They then were handled as above. The treatment was replicated 10 times for each nematode. The nematodes were observed daily and when the sexes could be distinguished but before they became adults, all males in the dishes of *S. scapterisci* were removed and placed in a separate drop of blood. Similarly, the males of *S. carpocapsae* strain Breton were removed and placed in a separate drop of blood. Then the males of *S. scapterisci* were transferred to the drop of blood containing females of *S. carpocapsae* strain Breton, and males of *S. carpocapsae* strain Breton were transferred to the drop of blood containing females of *S. scapterisci*.

The nematodes were observed frequently to see if they mated and produced offspring. Nematodes of each species were retained in drops of blood in two dishes as controls.

Biology

Four insects, fall army worm (Spodoptera frugiperda), velvet bean caterpillar (Anticarsia gemmatalis), granulate cut-worm (Feltia subterrania), and wax-moth larva (Galleria mellonella) were used to compare the rate of kill by S. scapterisci to that of some other species and strains of Steinernema.

Two pieces of Whatman No. 2 filter paper were placed in a 100 x 15 mm petri dish and 8,000 third-stage juveniles in 2 ml water, and 10 insects were added. Controls were prepared similarly but without nematodes. Treatments were replicated 4 times. After 2 days the number of dead insects was determined.

Results

Measurements: Measurements for first and second generation females are presented in Table 1, those for first and second generation males in Table 2 and those for third stage juveniles in Table 3.

Description

Females, first generation (Fig. 1D,1F)

The body cuticle is smooth, the lateral fields and phasmids were not observed. The head is rounded, continuous

Table 1: Measurements (in um) of first and second generation females of Steinernema scapterisci n. sp. (n=10).

	First generation		Second generation	
Character	Mean (SD)	Range	Mean (SD)	Range
Body length	4162 (540)	3531-5156	2209 (223)	1841-2530
Greatest width	179 (13)	159-203	123 (14)	94-141
Stoma length	7.5 (1)	6-9	6.7 (1.4)	5-9
Stoma width	10 (3)	9-12	8.9 (0.9)	8-11
EP	89 (5)	78-94	78 (6.8)	66-88
NR	174 (13)	153-194	169 (12)	147-184
ES	242 (17)	219-269	241 (15)	222-266
Tail length	46 (8)	34-59	58 (4)	48-64
Anal body width	58 (9)	41-72	47 (2.8)	43-52
Percentage vulva	53 (2)	50-54	52 (2)	52-60
EP:ES	0.37 (0.03)	0.32-0.41	0.32 (0.3)	0.28-0.36

EP = Distance from anterior end to excretory pore

NR = distance from anterior end to nerve ring

ES = distance from anterior end to end of esophagus

Table 2: Measurements (in um) of first and second generation males of <u>Steinernema</u> <u>scapterisci</u> n. sp. (n=10).

	First generation		Second generation	
Character	Mean (SD)	Range	Mean (SD)	Range
Body length	1728 (358)	1319-2271	1147 (95)	1031-1342
Greatest width	156 (49)	97-231	73 (8)	62-84
Stoma length	4.4 (1)	3-5	4.3 (1)	3-6
Stoma width	6.1 (1)	5-8	6.0 (1.2)	5-8
EP	71 (11)	63-98	68 (7)	50-75
NR	136 (11)	120-152	121 (10)	103-131
ES	187 (21)	164-216	168 (13)	138-181
Testis flexure	374 (52)	306-447	205 (19)	176-234
Anal body width	33 (5)	31-45	33 (4)	28-41
Tail length	25 (3)	21-30	25 (3)	22-30
Spicule length	83 (5)	72-92	78 (3)	75-83
Spicule width	13 (4)	13-14	12 (1)	11-14
Gubernac. length	65 (5)	59-75	54 (3)	47-59
Gubernac. width	8 (0.5)	8-9	6 (0.7)	5-8
EP:EF	0.36 (0.02)	0.32-0.39	0.40 (0.06)	0.29-0.52
Mucro length	4.3 (0.6)	3.1-4.7	3.9 (0.6)	3.1-4.6

EP = Distance from anterior end to excretory pore

EN = Distance from anterior end to nerve ring

ES = Distance from anterior end to end of esophagus

Table 3: Measurements (in um) of the third-stage juvenile of *Steinernema scapterisci* n. sp. (n=20).

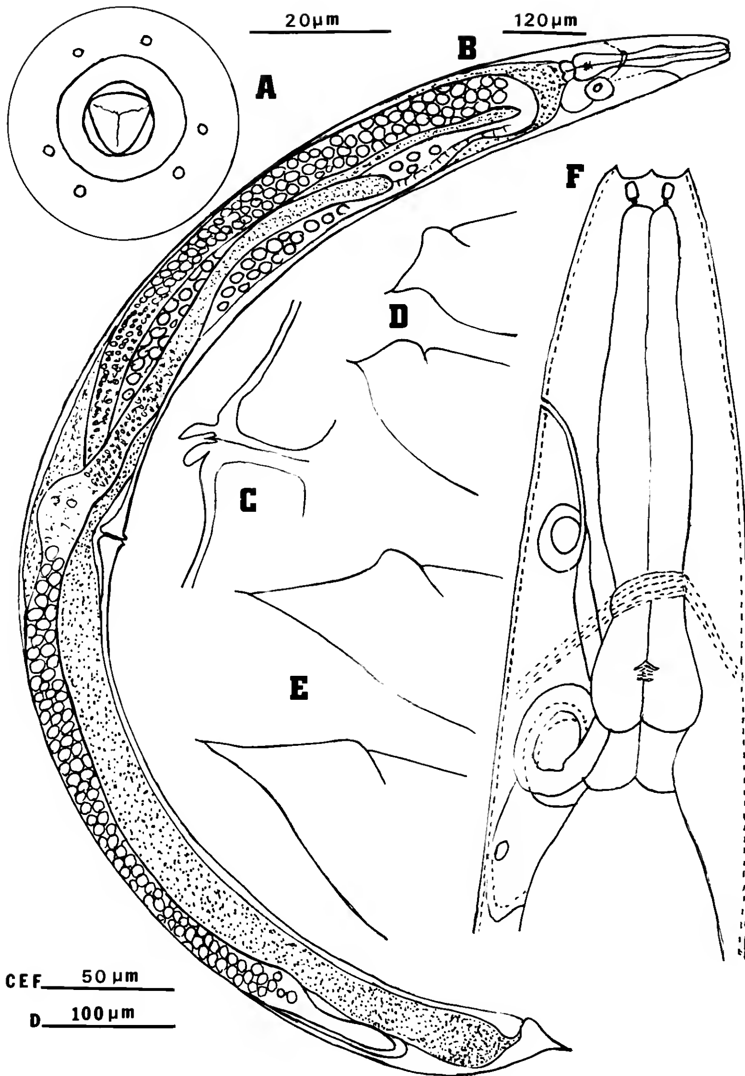
Character	Mean	SD	Range
Body length	572	27	517 - 609
Greatest width	24	4	18 - 30
EP	39	4	36 - 48
NR	97	1.1	83 - 106
ES	127	6	113 - 134
Tail length	54	3	48 - 60
EP:ES	0.31	0.03	0.27 - 0.40
EP:Tail length	0.73	0.06	0.60 - 0.80

EP = Distance from anterior end to excretory pore

NR = Distance from anterior end to nerve ring

ES = Distance from anterior end to the end of esophagus

Fig. 1. *Steinernema scapterisci* n. sp.. A) Face view showing unevenly distributed papillae. B) Female of the second generation, entire body. C) Double-flapped epiptygma. D) Variation in tail of the first generation females. E) Variation in tails of the second generation female. F) Anterior part of the first generation female showing large cheilorhabdion and excretory duct with doughnut-shaped structure.


20μm
120μm
A
B
C
D
E
F
CEF 50 μm
D 100 μm

with the body, and bears both labial and cephalic papillae. There are six lips which are united at the base with each terminating in a labial papilla. The six labial papillae (Fig. 1A, 2A, 2B) are not evenly distributed when viewed en face, while the 2 subventral and 2 subdorsal papillae are located as expected, the 2 lateral papillae are located lateroventrally making the ventral and lateral papillae closer together than are the lateral and dorsal papillae (Fig. 2A, C). The apex of each papilla is usually covered with a thin layer of whitish material, (electron lucent) (Fig. 2A, C). Cephalic papillae are present, appear to be 4 in number, but they are not distinct; therefore, the exact number has not been determined. The amphids were not observed. The stoma is very shallow, circular anteriorly, then becomes subtriangular. The cheilorhabdions are strongly sclerotized, unusually thickened, appearing as a circular or hexagonal ring en face (Fig. 1F, 2C, 3C). The prorhabdions just posterior to the cheilorhabdions, also are well-sclerotized. Posterior to this ring, no other sclerotized structure was observed. The esophagus is typical of the Steinernematidae, i.e. muscular throughout with the procorpus followed by a slightly swollen, nonvalvate metacorpus, isthmus, and basal bulb with a small, but quite visible, valve. The nerve ring is distinct, located in the region of the isthmus. The esophago-intestinal valve is long and prominent (Fig. 1F). The

Fig. 2. *Steinernema scapterisci* n. sp.. A) Anterior part of the nematode showing the mouth, lips and excretory pore. B) Head with 6 labial papillae. C) Face view showing unevenly distributed labial papillae; the mouth circular at the opening, becomes subtriangular. Note the white material covering the papillae. D) Face view showing the mouth and labial papillae.

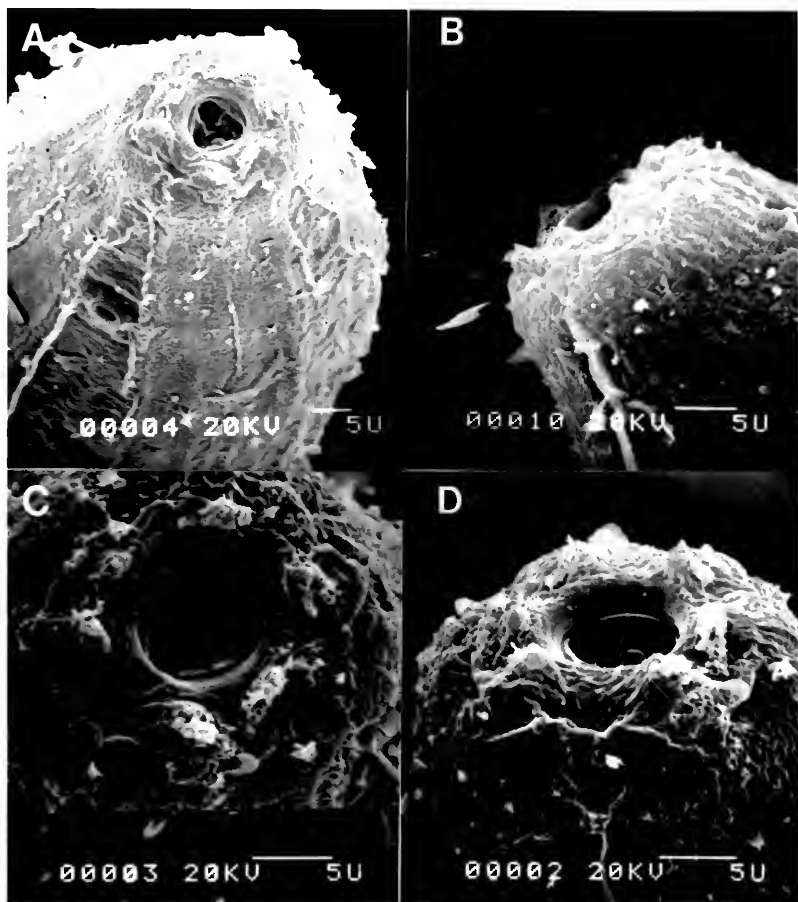
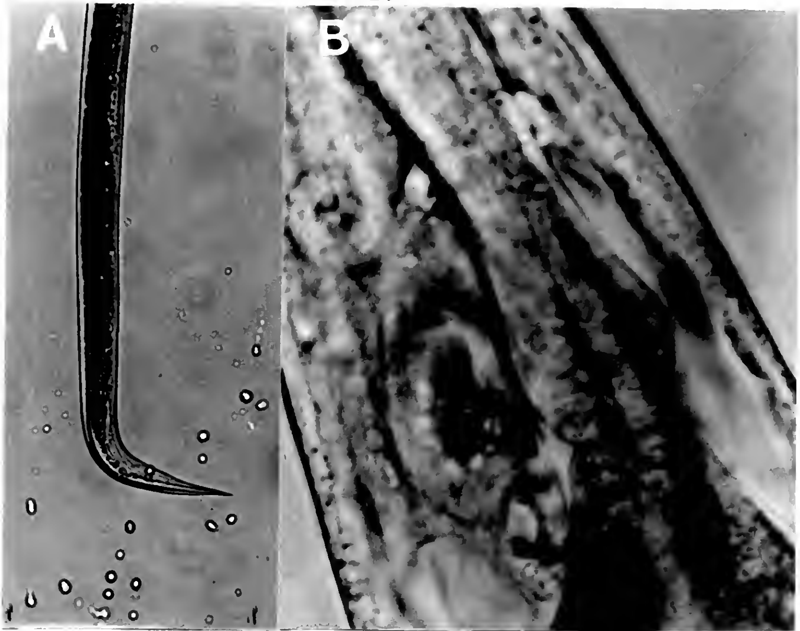
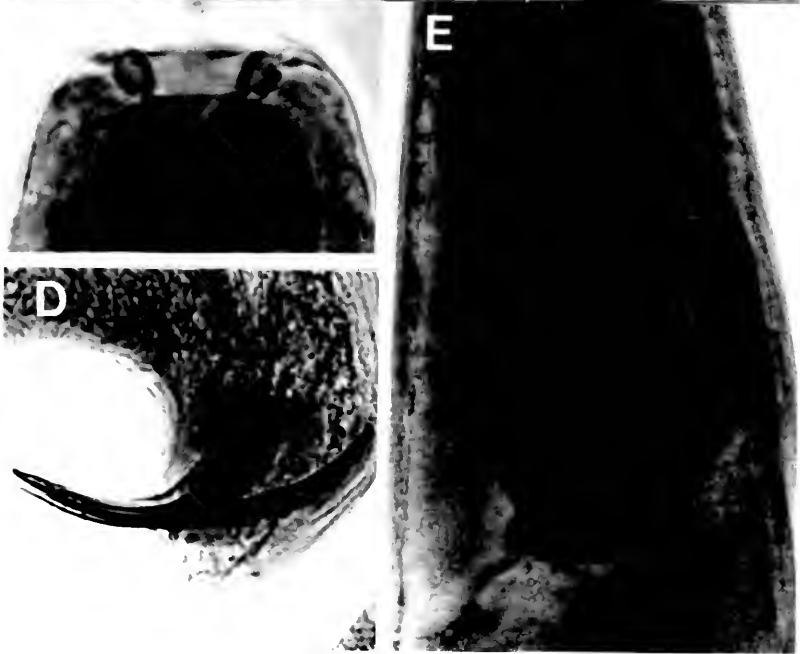

A
B
C
D
00004 20KV 5U
00010 20KV 5U
00003 20KV 5U
00002 20KV 5U

Fig. 3. Steinernema scapterisci n. sp.. A) Posterior region of the third-stage infective juvenile showing the curvature. B) Live nematode showing doughnut-shaped structure. C) Head of the nematode showing thick cheilorhabdions. D) Spicules and gubernaculum of male. E) Fixed nematode showing doughnut-shaped tructure.


A
B


E
D

excretory pore is located anterior to the middle of the metacorpus, usually in the procorpus region. The excretory duct is unusually prominent: it forms a small loop midway between the excretory pore and the base of the esophagus, then turns to the right side of the esophagus, or sometimes extends to the anterior part of the intestine then returns on the ventral side of the intestine at its junction with the esophagus; here it coils upon itself 2 or more times forming a doughnut-shaped structure (Fig. 1F) complete with a hole at the center. A uninucleate gland is located posteriorly to the doughnut-shaped structure but a junction of the excretory duct with the gland has not been observed. This doughnut-shaped structure has been seen in almost every first generation female and is visible even with a dissecting microscope. The gonads are didelphic, opposed; the ovaries are reflexed (Fig. 8B, E). The vulva appears as a transverse slit with a prominent double-flapped epiptygma (Fig. 1C, 6C) The vagina is sclerotized; it's length is about 1/3 of the body width at the vulva, and it leads to paired uteri. The body width of the nematode anterior to the vulva is always greater than that posterior to the vulva. The tail is somewhat variable in shape, but usually has a post-anal swelling ventrally and a mucron (Fig. 1D); the length of the tail is less than the width of the body at the anus. The pigmy form referred to for other species (Poinar, 1979) was not observed.

Female, second generation (Fig. 1B, 1E)

The second generation female is similar to the female of the first generation, but differs in that the second generation female is much smaller, the valve in the basal bulb of the esophagus is more prominent, the doughnut-shaped structure is not as prominent, the tail, which tapers to a point bearing a mucron, is longer than the body is wide at the anus (Fig. 1E).

Male, first generation (Fig. 4A, B, E)

The first generation male (Fig. 4E) is much smaller than the first generation female, but anatomically the two are similar anteriorly. The body is usually plump. The nerve ring is located in the isthmus region but its exact position is variable. The excretory duct does not form the doughnut-shaped structure which occurs in females. The posterior part of the nematode is curved ventrally. The nematode body is spiral in shape when killed by minimal heat. There is one gonad with a reflexed testis. The spicules are paired, uniformly curved, dark brown in color with the head large and somewhat angular (Fig. 4A, 4B, 3D, 5B). The angle formed by the shaft and blade of the spicules averages 110 degrees (range 100-120). The shaft of the spicules is long when compared to that of other species and appears to be encased in a sheath (Fig. 5B, 6B). The blade tapers smoothly to the end with the posterior portion thinner than that in other species of Steinernema

Fig. 4. *Steinernema scapterisci* n. sp. A) First generation male tail with mucron; spicules with angular head and ribs, gubernaculum with anterior portion bent upward. B) Variation in tail shape of the first generation male. C) Tail of the second generation male showing elongate spicule head. D) Variation in tail shape of the second generation male. E) Entire body of the first generation male.

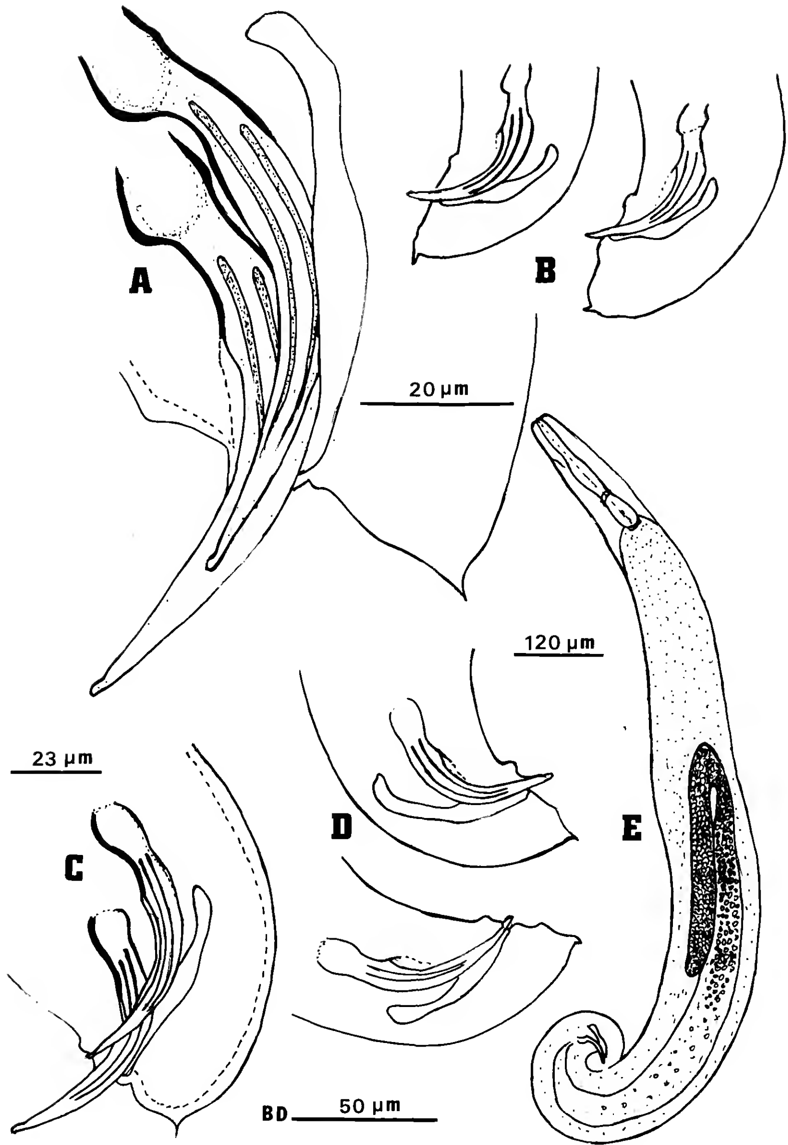

A
B
20 µm
120 µm
23 µm
C
D
E
BD 50 µm

Fig. 5. A) *Steinernema scapterisci* n. sp.. Spicule blade showing the thin posterior part with a small aperture. B) The spicule shaft showing the angular head, and a sheath around the shaft. C) Cross section of the spicule showing 2 lumens in the spicule blade. D) Gubernaculum showing the long anterior part which bends upward.

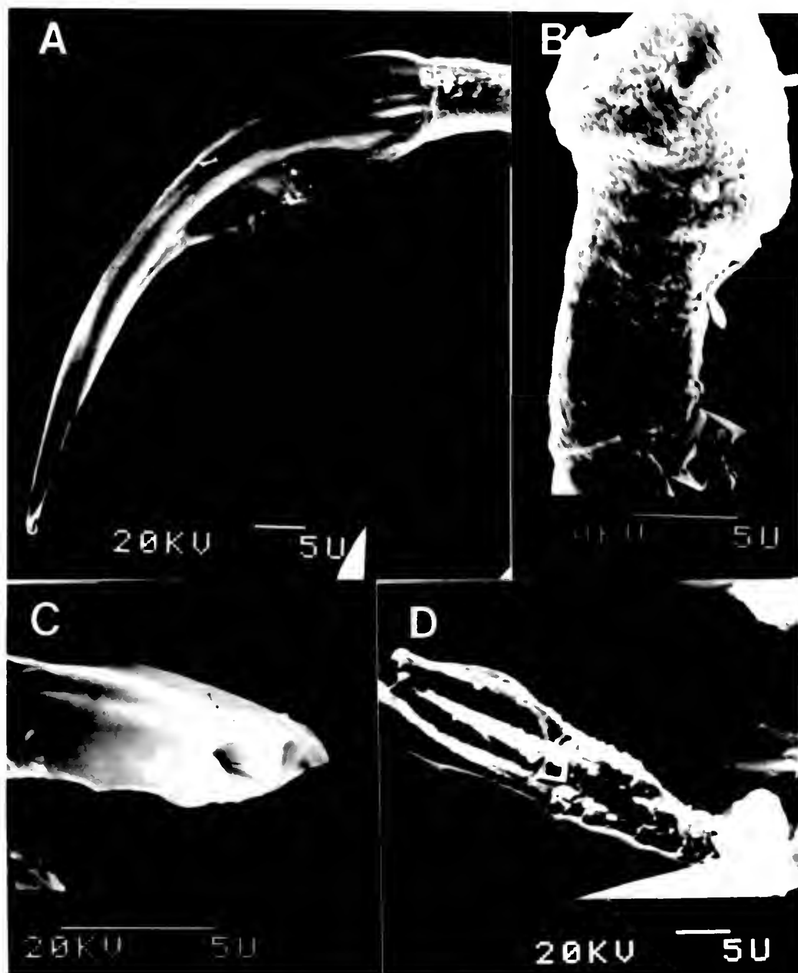

A
20KV
5U
B
5U
C
20KV
5U
D
20KV
5U

Fig. 6. A, B): *Steinernema carpocapsae* strain Breton. A) Spicule showing the short shaft without a sheath and with rounded head (compare with Fig. 5). B) Gubernaculum showing short anterior part. C, D): *Steinernema scapterisci* sp. C) Double-flapped epiptygma. D) The body of the third-stage infective juvenile showing annulation and lateral field with 6 incisures.

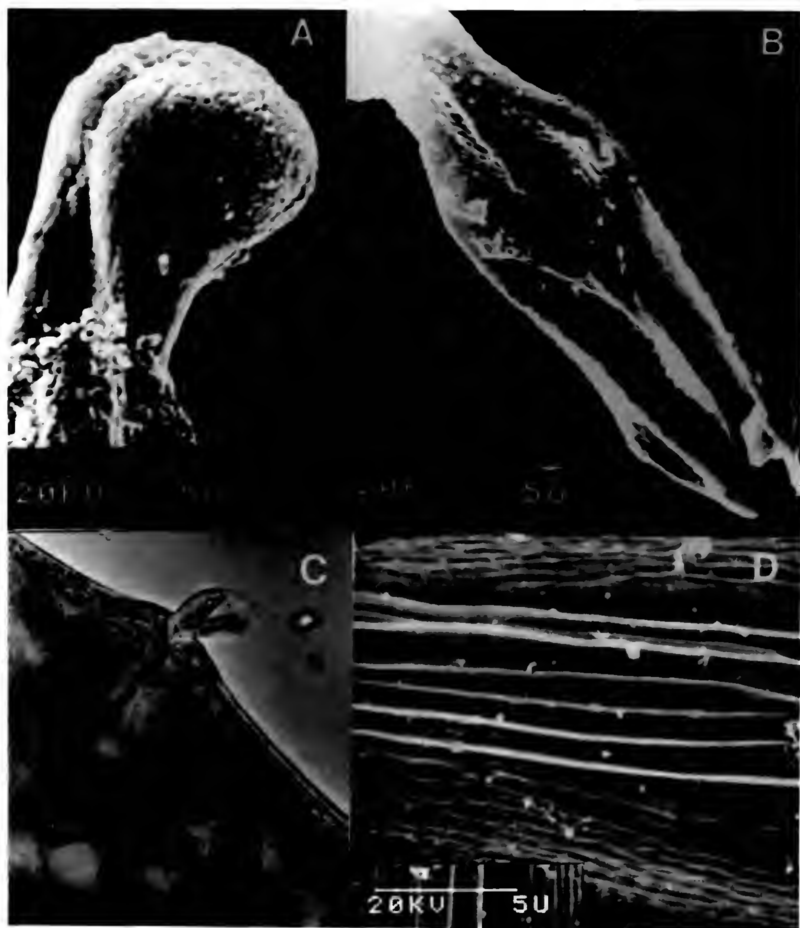
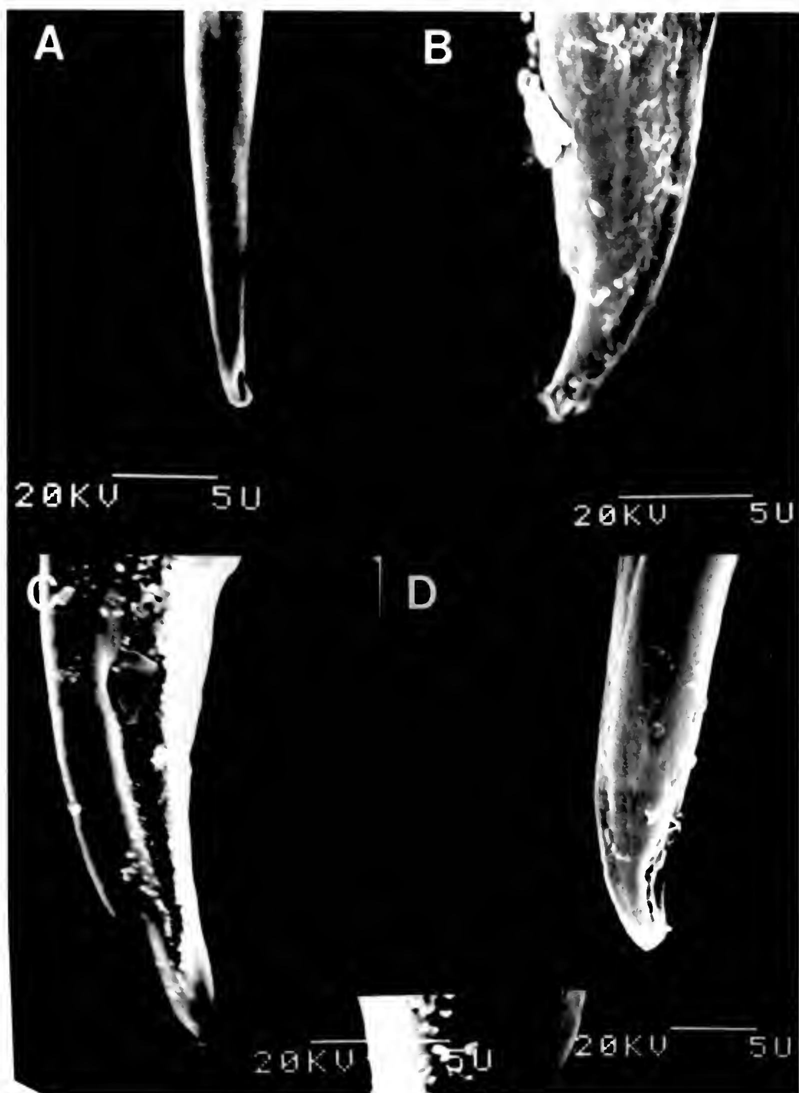

A
B
C
D
20KV
5U

Fig. 7. A) *Steinernema scapterisci* n. sp.. Posterior part of the spicule, thin, with small aperture.
B, C, D) Posterior part of the spicule of *S. glaseri*, *S. carpocapsae*, and *S. bibionis*, respectively, showing the difference in shape, thickness and size of the aperture.


A
B
C
D
20KV 5U
20KV 5U
20KV 5U
20KV 5U

(Fig. 4A, 4B, 7). In the cross section the blade of the spicule contains two lumens (Fig. 5C), but only one aperture was seen on the ventral side close to the tip (Fig. 7A). This aperture is smaller than that on the spicule in other species (Fig. 7). Under the compound microscope, each spicule shows two internal ribs (Fig. 4A, 4B). The point where the two ribs terminate proximally is variable. These ribs are strengthening thickenings of the upper and lower walls between the two lumens in the blade (Fig. 8F). The gubernaculum is boat-shaped, with a thin, long and ventrally curved anterior part. Compared to S. carpocapsae strain Breton, the anterior part of the gubernaculum of S. scapterisci is much longer (Fig. 5D, 6A). Its posterior end is bifurcate (Fig. 5D). The spicules glide along the gubernaculum in two grooves separated by a ridge (Fig. 5D). The cloaca is on a raised area and bears an anterior flap, seen easily when the spicules are protracted or retracted. Ten pairs and one single genital papillae were observed (Fig. 8A) with pairs 1 and 6 difficult to see. The single papilla is located ventrally and between pairs 4 and 5; pairs 1-9 are located ventro-laterally and pair 10 subdorsally. The tail bears a mucron, and the posterior region is always curved ventrally (Fig. 4A, B, E).

Male, second generation

The second generation male is similar to that of the first generation but is smaller, especially in width, and

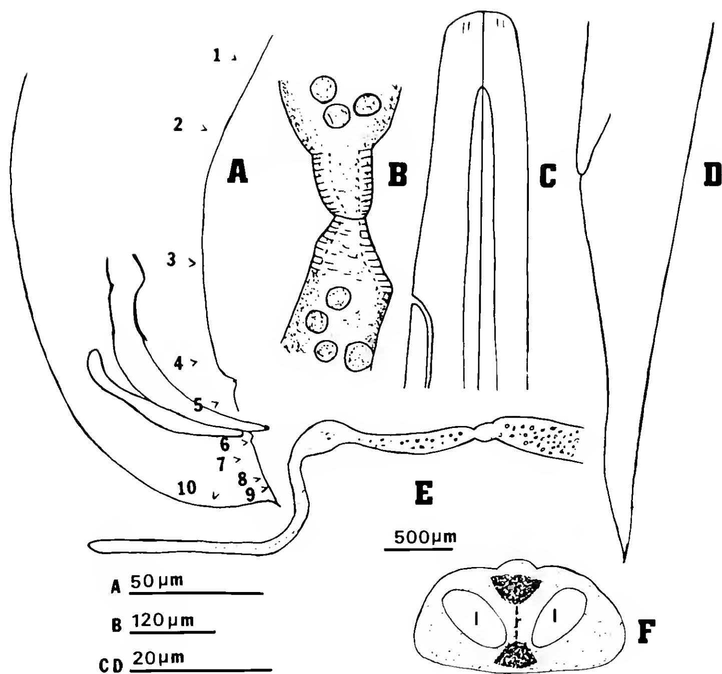


Fig. 8. _Steinernema_ _scapterisci_ n. sp.. A) Male tail of the first generation showed 10 pairs of genital papillae. B) Flexure of the ovary (reflexed in the body). C, D) Head and tail of third stage infective juvenile. E) Ovary with spermatheca (reflexed in the body). F) Cross section of spicule blade showed two lumens (l) and 2 internal ribs (r).

the spicules have an elongate head (Fig. 4C, 4D).

Juveniles, third stage (Fig. 8C, 8D)

Measurements are given in Table 3. The third stage juvenile, when newly formed, is always enclosed in the cuticle of the second-stage juvenile as a sheath. However, the sheath is lost rather easily, even in storage, and thus may not always be present. The body is thin. The lip region is not offset; the oral aperture (mouth) was not observed; the esophagus is degenerate and thus not seen clearly, but its basal bulb is elongate and has a valve. The lateral fields have 6 incisures (Fig. 6D). The tail tapers gradually dorsally but abruptly ventrally (Fig. 8D).

Cross hybridization

In cross hybridization experiments, males and females never mated and thus no offspring were present. In the controls, males and females mated and offspring were present after 10 days.

Biology

All species of *Steinernema*, except *S. scapterisci*, and all strains of *S. carpocapsae*, killed from 20 - 100% of the insects tested. *S. scapterisci* killed no more than 10 % (Table 4). The large difference in the percentage of wax moth larvae killed by all nematodes except *S. scapterisci* is significant since the wax moth larva has been used as a universal host for *in vivo* culture of species and strains of *Steinernema*. This difference indicates that wax moth larvae

Table 4: Effectiveness of different species of *Steinernema* and strains of *S. carpocapsae* in killing four species of lepidopterous insects, two days after inoculation.

Nematode	Percentage of insects killed*			
	FAW	VBC	GCW	WML
S. glaseri	100	90	50	100
S. bibionis	100	90	55	100
S. carpocapsae				
Breton	100	100	--	100
Italian	100	100	--	100
Mexican	100	100	80	100
Agriotos	100	100	20	100
All	100	100	--	100
S. scapterisci	8	3	10	9
Control	0	0	0	0

* Average of four trials

FAW = fall army worm; VBC = velvet bean caterpillar; GCW = granulate cut worm; WML = wax moth larva.

can be used as test insects to differentiate between S. scapterisci and all other species and strains of Steinernema.

Diagnosis

Steinernema scapterisci n. sp. can be distinguished from other species of Steinernema as follows: from S. glaseri, by the presence of a mucron on the tail of the male of S. scapterisci, and by the shorter third-stage juvenile of S. scapterisci (517-609 um) than of S. glaseri (860-1500 um); from S. bibionis and S. intermedia by the shorter third stage juvenile (700-1000 um for S. bibionis and 608-800 um for S. intermedia); from S. carpocapsae by the ratio of head to excretory pore divided by tail length, this ratio is 0.73 (0.60-0.80) in S. scapterisci compared to 0.60 (0.54-0.66) in S. carpocapsae; and by the shape of the tail of the third stage juvenile; when relaxed, the tail of S. scapterisci usually curves ventrally forming an angle about 110 degree with the body. The ratio of the head to excretory pore/head to end of esophagus is 0.38 compared to 0.90 in S. glaseri, 0.59 in S. bibionis, 0.72 in S. intermedia, 0.43 in the Czechoslovakian and DD-136 strains of S. carpocapsae.

S. scapterisci n. sp. also can be separated from all other species by the following characters: The presence of thick cheilorhabdions (about 4.8 um thick by 5.8 um long in lateral view), the doughnut-shaped structure in the excretory canal, and a prominent double-flapped epiptygma in

the 1st generation female. Spicules of the male are brown, pointed, and taper smoothly to the end; end of the blade narrow; shaft long and bearing a sheath; gubernaculum with long and upward-bent anterior part.

S. scapterisci n. sp. cannot be cultured on wax moth larvae (*Galleria mellonella*), but sometimes a few wax moth larvae will be killed by the nematode. When this occurs, the bodies of the wax moth larvae turn black while those killed by other species of *Steinernema* turn whitish or yellowish but never black. Also, other species of *Steinernema* develop very well in wax moth larvae. Finally, this nematode can be distinguished from other species by bioassay on 3 insects: fall army worm, velvet bean caterpillar, and wax moth larvae. In two days, other species of *Steinernema* will kill 100% of the test insects, but *S. scapterisci* will kill at most only a small percentage of them (Table 4).

CHAPTER 3
MODE OF ENTRY OF STEINERNEMA SCAPTERISCI N. SP INTO MOLE CRICKETS

Animal parasitic nematodes, in general, enter their hosts in one of three ways: (1) by direct penetration through the cuticle, (2) through natural openings or wounds or (3) by vectors. While the first two apply to insect-parasitic nematodes, transmission by vectors has not been reported. Most of the insect-parasitic nematodes in the orders Tylenchida and Mermithida are reported to enter the hosts by direct penetration, while those in the order Rhabditida enter the hosts through natural openings or sometimes by direct penetration through the cuticle. Most of the information on the rhabditid genus _Steinernema_ has been collected over a long period of time on the species _S. carpocapsae_. Since _S. scapterisci_ is described herein as a new species, the following experiments were conducted to determine its mode of entry into its primary hosts, mole crickets in the genus _Scapteriscus_.

Literature Review

Bronskill (1962), and Welch and Bronskill (1962) reported that the infective stage of _S. carpocapsae_, strain

DD-136, entered the body of mosquitoes passively through the mouth. Weiser (1966) reported that *S. carpocapsae* entered the body of some insects through the spiracles and the tracheal system. Poinar and Himsworth (1967) observed steinernematids in the crop and mid gut of wax moth larvae (*Galleria mellonella*) and concluded that the nematode entered through the mouth of the insect. Friggiani and Poinar (1976) showed that *S. carpocapsae* entered the body of some adult insects in the order Lepidoptera through the spiracles.

Materials and Methods

The mole cricket, *Scapteriscus acletus*, was used in the following experiments.

Experiment 1

The purpose of this experiment was to determine whether *S. scapterisci* entered mole crickets through either the mouth or anus. Three hours after 5 mole crickets were exposed to 8,000 infective-stage nematodes in a petri dish, the mole crickets were killed, the digestive system dissected out and cut into 3 parts. The first part included the mouth through the crop; the second part began just posterior to the crop and continued to the junction of the Malpighian tubules with the gut; the third part was from this junction to the anus. Each part was dissected in a separate petri dish and examined for nematodes.

Experiment 2

The purpose of this experiment was to determine whether the nematode can kill mole crickets and develop in their bodies when the crickets were inoculated through the mouth or through the anus. A small-diameter hypodermic needle attached to a 1 ml syringe was used as the inoculation vehicle (Fig. 9). The angled tip of the needle was removed, the end filed smooth and the syringe mounted on a stand to facilitate the inoculation process. Twenty mole crickets were deprived of food and water over night, and then ten of them were inoculated through the mouth and 10 through the anus with about 80 third-stage juveniles in 1/10 ml of water. After they died, the cadavers were incubated, each separately, to determine if the nematodes reproduced in them.

Experiment 3

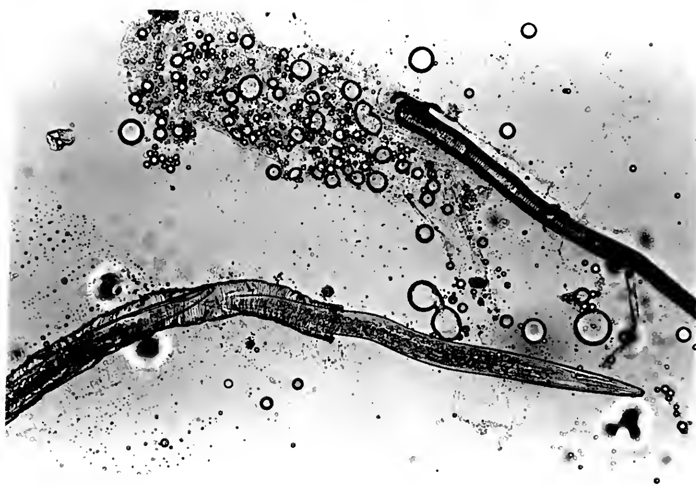
The purpose of this experiment was to determine if the nematode can enter the mole cricket through the spiracles. Several hours after the mole crickets were inoculated with nematodes, as in Experiment 1, the tracheal system (as much as possible) was dissected out carefully from about 20 mole crickets and observed under a compound microscope.

Experiment 4

The purpose of this experiment was to determine if the nematode would kill mole crickets and develop in their bodies when inoculated through the spiracles. The first and

Fig. 9. Inoculating a mole cricket through the mouth with third-stage Steinernema scapterisci.

Fig. 10. Third-stage nematodes Steinernema scapterisci in tracheal tube of mole cricket. The part of the tracheal tube above the nematode was obvious to be broken by the nematodes from the main tube (nematodes inside).



second thoracic spiracles of mole crickets are large enough to be used as inoculation ports when using a very small needle. Thus the same needle and syringe mounted on a stand that was used in Experiment 2 was used here. Fifteen mole crickets were inoculated through the first thoracic spiracle and 4 were inoculated through the second thoracic spiracle as follows: About 50 nematodes were collected in a droplet of water at the end of the hypodermic needle. Then, while viewing through a stereomicroscope, the mole cricket was squeezed slightly to expel the air in the tracheal system, and the droplet of water containing the nematodes was applied to the spiracle and the pressure on the body of the mole cricket released. The water was sucked through the spiracle and into the tracheae.

Three days after the mole crickets died, their bodies were dissected to see if developing nematodes were present.

Experiment 5

The purpose of this experiment was to see if the nematodes would enter the tracheal system when inoculated through the spiracle. Ten mole crickets were inoculated through the first and second thoracic spiracles as in Experiment 4; all of the crickets were dissected 30 minutes later and the tracheal system examined for nematodes.

Results and Discussion

Experiment 1

When the three parts of the digestive system were dissected and examined for nematodes, with an average of five digestive systems examined, 66 nematodes were found in the anterior part, none in the middle part, and one in the posterior part.

Since by far the greatest number of nematodes were found in the anterior part of the digestive system, it seems that the majority of nematodes enter mole crickets through the mouth. This agrees with the mode of entry reported by Poinar and Himsworth (1967) for wax moth larvae. Since one nematode was found in the posterior part of the digestive system it is possible that some of the nematodes enter through the anus.

Experiment 2

Nine of the ten mole crickets inoculated through the mouth produced large numbers of nematodes one week after the mole crickets died while none of those inoculated through the anus produced nematodes after the crickets died. This suggests that nematodes that enter mole crickets through the mouth penetrate the gut wall and entered the thoracic cavity where they reproduce. Any that enter through the anus may die in the rectum or they may penetrate the gut wall and entered the abdominal cavity. Previous experiments have

shown that the nematode does not develop in the abdominal cavity.

Experiment 3

Nematodes were found several times in the tracheal tubes of mole crickets, mostly in the thoracic area, and especially in the pronotal region. A few of the nematodes were observed to break through the wall of the tracheal tubes (Fig. 10).

Experiment 4

Three of the 4 mole crickets inoculated with nematodes through the second thoracic spiracle, and 12 of the 15 inoculated through the first thoracic spiracle were found with developing nematodes in the head and thorax. Since *in vivo* culture (See Chapter 5 on culture) showed that most of the nematodes were produced in the pronotal region, and since the first thoracic spiracle is located underneath the pronotum, the first thoracic spiracle may be a very important entry route for the nematode.

Experiment 5

Nematodes were found in the tracheal system of all mole crickets inoculated. The nematodes found in very small tubes with a diameter barely greater than that of the nematode, thrashed about vigorously, but did not break through the tracheal tubes during the period of observation.

Experiments 3, 4 and 5 demonstrated that the nematode can kill mole crickets by entering the spiracles, breaking through the tracheal wall and invading the body cavity. Thus the spiracles may represent a very important port of entry for the nematode.

CHAPTER 4
LIFE CYCLE OF STEINERNEMA SCAPTERISCI N. SP.

Information about the life cycle of a parasitic nematode is always helpful, but especially so when one wants to use the nematode as a biological control agent. Such information is essential to know what life stage to apply in the field and often gives some indication of the best method of application. It is useful also in conducting research to increase the effectiveness of the nematode against the target pest or group of pests and in developing or improving efficient and low-cost mass-rearing techniques. The life cycle of the nematodes *Steinernema bibionis*, *S. carpocapsae*, and *S. glaseri* were studied by Bovien, 1937, Wouts, 1980 and Poinar, 1979. In this chapter, I report the results of my studies on the life cycle of *S. scapterisci.*

Materials And Methods

Mole crickets were not available for these studies so house crickets, which react similar to mole crickets in pathogenicity tests, were used in all experiments. The house crickets were purchased from a local bait and tackle shop.

Influence of Temperature on the Life Cycle

Fifty house crickets were anesthetized with carbon dioxide, placed in each of 10 petri dishes (100 x 15 mm) and exposed to about 8,000 third-stage juvenile nematodes. Two of the petri dishes were placed in incubation chambers at 10, 15, 20, 24 and 30 C. Starting two days later, up to 3 crickets were dissected daily and the life cycle stage of the nematode determined.

Intermediate Cycle

At 24 C, male and female nematodes were detected in the bodies of house crickets. In one experiment with 3 replicates, a small piece of tissue from the thorax (1/2 of the pronotum) of the cricket was placed on a small piece of filter paper (1 cm2) saturated with water in a 60 x 15 mm petri dish. Two gravid nematode females were transferred to each piece of tissue. Four drops of water were placed in the petri dish surrounding the filter paper to insure adequate moisture in the dish. Covers were applied and the dishes placed in the dark and examined daily. The experiment was repeated twice. In a second similar experiment larger pieces of insect tissue (the entire pronotum) were used.

The reason for using a small piece of tissue was to determine whether the nematodes, when provided with a limited food supply, would cease development at the third stage, engulf and store a pellet of bacteria in the foregut

and become third-stage infective juveniles. The reason for the larger piece of tissue was to determine whether the nematode, when provided with a sufficient food supply, would continue to develop to adults instead of becoming third-stage infective juveniles.

Sex Ratio

Ten to twelve house crickets were placed in a petri dish and exposed to about 8,000 third-stage juveniles. The dishes then were placed in an incubator at either 15, 24 or 30 C. After 3 days, all crickets were dissected and the number of male and female nematodes in each cricket were counted.

Results and Discussion

Influence of Temperature on the Life Cycle

At 10 C: The third-stage infective juveniles never reached the adult stage. A few began to develop to the 4th stage but had poorly-developed, shortened, plump bodies, and all died within 5 days.

At 15 C: The third-stage infective juveniles developed into males and females of the first generation after 10 days; they were in the thoracic cavity. The second generation juveniles appeared about 15 days after inoculation. Most, but not all, moved to the abdominal cavity and embedded themselves in the fat tissue lining the abdominal wall. These nematodes moved very slowly, many appearing immobile.

Until the 18th day, the second generation juveniles had not increased in size and they became completely immobile. No third-stage infective juveniles were produced.

At 20 C: First generation males and females were observed after 7 days, but many of them died, and those that were alive were not very active. The second generation adults appeared after 8-9 days; after 10-11 days, some infective-stage juveniles were observed with increasing numbers appearing a day later.

At 24 C: First generation adults appeared in less then 3 days. Most of them were in the anterior part of the cricket cadaver After 4 days, most of the first generation adults had decayed but a few females containing second generation juveniles remained. After emerging from the body of the first generation females, the first-stage juveniles of the second generation migrated to all parts of the cricket bodies. During the 5th and 6th days, the second generation juveniles grew rapidly, and after 7 days, infective third-stage juveniles, preinfective second-stage juveniles and second generation adults were seen inside and outside the cricket bodies. After 8-9 days preinfective second-stage juveniles and infective third-stage juveniles appeared increasingly abundant. In the preinfective second-stage juvenile the esophagus and stoma are not well-developed and the chamber containing a pellet of bacteria is forming. When it molts to the third-stage infective juvenile, the

retained as a sheath. At day 10, infective third- stage juveniles were present throughout the inside and outside of the cadavers.

At 30 C: First generation adults were observed in 2 days, and second generation adults in 5 days. Third stage infective juveniles first appeared at 6 days, the number increased at 8 days and large numbers appearing at 10 days.

Intermediate Cycle: On the isolated small piece of tissue, some third stage infective juveniles were observed after 6 days, and many of them appeared after 7 days. None continued to develop to second generation adults. In the dish with a larger piece of tissue, some of the juveniles stopped development and became third stage infective juveniles but others continued to grow and became adults.

These observations show that third-stage infective juveniles may be produced by the first generation adults or by the second generation adults. In this study, those that were produced by the first generation adults appeared in 6-7 days. With the small piece of tissue, apparently an insufficient supply of food prevented the juveniles from becoming second generation adults, so they ceased development and became third-stage infective juveniles presumably as a survival mechanism.

Table 5: Influence of temperature on the life cycle of *Steinernema scapterisci*.

Life cycle stage	10 C	15 C	20 C	24 C	30 C
	No. of days to 1st appearance				
1st generation adults	NC	10	7	3	2
2nd generation adults	NC	NC	8	7	5
Intermediate life cycle	NC	NC	10	6-7	6
Regular life cycle	NC	NC	12	10	8

NC = Not completed

Even when adequate food was available, some of the juveniles wandered away from the food supply and became third-stage infective juveniles from the first generation adults, but others developed to second generation adults and then produced third-stage infective juveniles in 9-10 days. The regular cycle took 9-10 days at 24 C.

Whether or not *S. scapterisci* completed the life cycle, and the length of time required for that cycle when it was completed, was influenced by temperature. Temperatures colder than 20 C are not suitable for development (Table 5).

Life Cycle of S. scapterisci n. sp.

The life cycle of *S. scapterisci* is schematically presented in Fig. 11 and described as follows: The third-stage infective juveniles invade the host through the mouth or spiracles; some may enter through the anus but they fail to develop (on mole crickets). Immediately after entering the body cavity of the host, the juveniles accumulate in the thorax and head and start to develop. The nematode enlarges in width, the stoma opens and its walls thicken, the esophagus becomes prominent, the bacterial chamber enlarges and moves posteriorly releasing the bacteria into the intestinal lumen, and then into the blood stream or tissues of the host. This process takes less than 24 hours after the nematode enters the host. The nematode grows rapidly and feeds on bacterial cells which develop in the host and becomes the fourth stage. First the body width increases

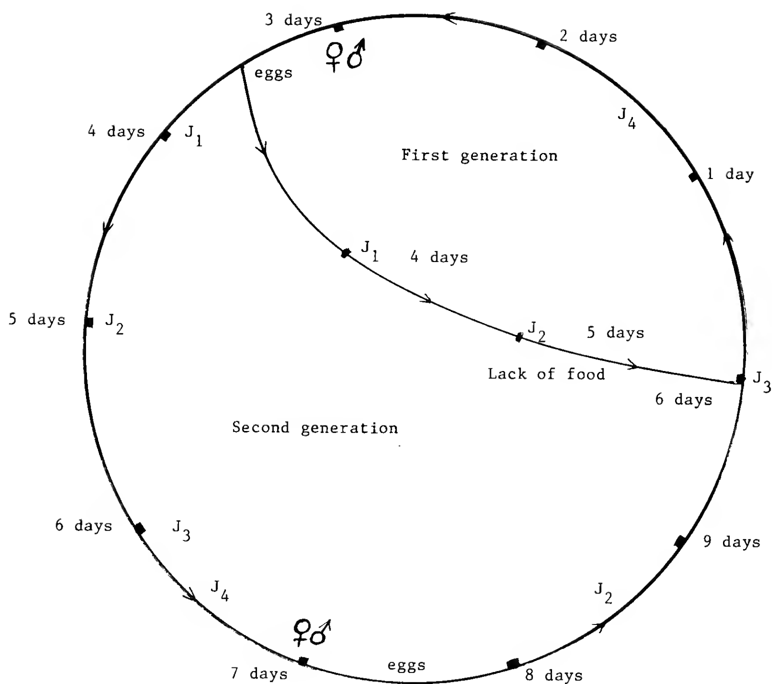


Fig. 11. Diagram of the life cycle of <u>Steinernema scapterisci</u> n. sp..

much faster than does the body length until its width reaches almost the width of the adult; the excretory duct becomes larger and complicated; then the nematode increases in length until the adult size is reached. During this time the reproductive system is also formed. It takes 60 to 72 hours to reach the first generation adult stage at 24 C and just 48 hours at 30 C, but up to 240 hours at 15 C and 168 hours at 20 C. The female becomes about 7 times longer and 7 times wider than the third stage infective juvenile. The sex ratio is dependent upon temperature and density of nematodes in the host. At 15-24 C the number of females is greater but at 30 C the number of males is greater. Males and females mate and the females initially lay eggs, but later the eggs are retained and hatch in the female's body. The nematodes reproduce bisexually. About 24 hours after all eggs hatch in the body of the female, the second generation first-stage juveniles break out of the female body and move into the body cavity of the insect. At that point, an individual may develop by one of two cycles depending upon the availability of nutrients and space. If nutrients are insufficient and/or space is limited (overcrowding), a number of the second generation second stage juveniles become third-stage infective juveniles in about 6-7 days after the nematodes enter the body of the host. The remaining second generation second-stage juveniles continue to develop to become second generation females and males

which are much smaller than females and males of the first generation. These nematodes appear at about 7 days, almost the same length of time required to become infective-stage juveniles in the intermediate cycle. These males and females mate and the females lay some eggs which hatch as first stage juveniles. A number of eggs are not laid, however, but hatch in the female's body. These first-stage juveniles break out of the female's body after she dies to enter the body cavity of the host. The juveniles develop to the second stage and then to the third stage infective juveniles in 9-10 days after inoculation. They emerge from the host in large numbers and enter the environment to seek out a new host.

Sex Ratio: The results of the sex ratio experiments are presented in Tables 6, 7 and 8.

The greatest average number of 1st generation females and males, 257, (Table 6) were produced in the house crickets at 15 C. This number was approximately 2 and 4 times as great as the numbers produced at 24 and 30 C (Tables 7 and 8). This was surprising because the life cycle was not completed at 15 C (Table 5).

One reason for this seemingly unusual situation could be that at the lower temperature, a greater number of third stage infective juveniles entered the house crickets because of reduced activity of the crickets which had been living at ambient temperatures. Conversely, the activity of the

Table 6: Number and percentage of first generation females and males of *Steinernema scapterisci* which developed at 15 C.

Rep	No. Females	% Females	No. Males	% Males	Total
1	171	60	114	40	285
2	204	50	202	50	406
3	231	59	159	41	390
4	102	48	109	52	211
5	113	55	93	45	206
6	126	55	104	45	230
7	61	56	47	44	108
8	136	53	123	47	259
9	182	59	125	41	307
10	66	40	100	60	166
Means	139	54a	118	46b	257

Numbers with different letters in the same row are significanttly different at the 5% level according to Duncan's multiple range test.

Table 7: Number and percentage of first generation females and males of Steinernema scapterisci which developed at 24 C.

Rep	No. Females	% Females	No Males	% Males	Total
1	122	54	105	46	227
2	56	70	24	30	80
3	42	70	18	30	60
4	93	52	86	48	179
5	51	66	26	34	77
6	26	70	11	30	37
7	19	66	10	34	29
8	63	76	20	24	83
9	45	63	26	37	71
10	43	65	23	35	66
11	125	54	107	46	232
12	54	56	42	44	96
Means	62	60a	42	40b	104

Numbers with different letters in the same row are significantly different at 5% level according to Duncan's multiple range test.

Table 8: Number and percentage of first generation females and males of Steinernema scapterisci which developed at 30 C.

Rep	No Females	% Females	No. Males	% Males	Total
1	10	56	8	44	18
2	6	55	5	45	11
3	23	53	20	47	43
4	7	44	9	56	16
5	47	52	43	48	90
6	65	49	69	51	134
7	63	51	60	49	123
8	23	51	22	49	45
9	40	47	46	53	86
10	44	47	50	53	94
11	23	72	9	28	32
12	41	27	102	73	143
Means	32	47a	36	53a	68

Numbers with the same letters in the same row are not significantly different at 5% level according Duncan's multiple range test.

nematodes would have increased at the warmer temperature because those used for these experiments had been stored in a refrigerator at 6-10 C.

If this is the case, this information may be useful for in vivo culture for producing greater populations of third-stage infective juveniles. First, infective-stage juveniles stored at 6-10 C and the host crickets kept at ambient temperatures would be placed in an inoculation chamber at 15 C. After two days when the house crickets were dead, they would be transferred to an incubation chamber at 24 C. This technique should allow total populations of 1st generation adult females to develop at the cooler temperature and then more third-stage infective juveniles to develop at the warmer temperature.

When the experimental temperature was increased to 24 C and to 30 C, the number of first generation adults which developed in house crickets was reduced. This might be explained by the higher temperature causing increased activity of the house crickets which might have caused fewer infective-stage juveniles to enter the house crickets. It is possible also, that at the higher temperatures, especially at 30 C, water in the incubation chamber evaporated more quickly and after some length of time, the nematodes may be inactivated because of the lack of humidity.

At 15 C: The first generation female:male ratio was 54:46. The nematodes did not develop beyond this stage.

At 24 C: the first generation female:male ratio was 60:40. When the numbers of adult females and males in a house cricket were less than or equal to 83 the average female:male ratio was 69:31 (calculated from Table 7). In some individual crickets, the female:male ratio was as high as 76:24 (one cricket), and 70:30 (3 crickets). Regression analysis suggested that the hypothesis, percentage of males is a function of the total number of nematodes in house crickets, is highly significant, P = 0.0003 (Fig. 12). This implies that nutrients influence the sex ratio.

At 30 C: the first generation female:male ratio was 47:53. The linear relationship between the number of males and the total number of nematodes in house crickets was highly significant, P = 0.0001 (Fig. 13).

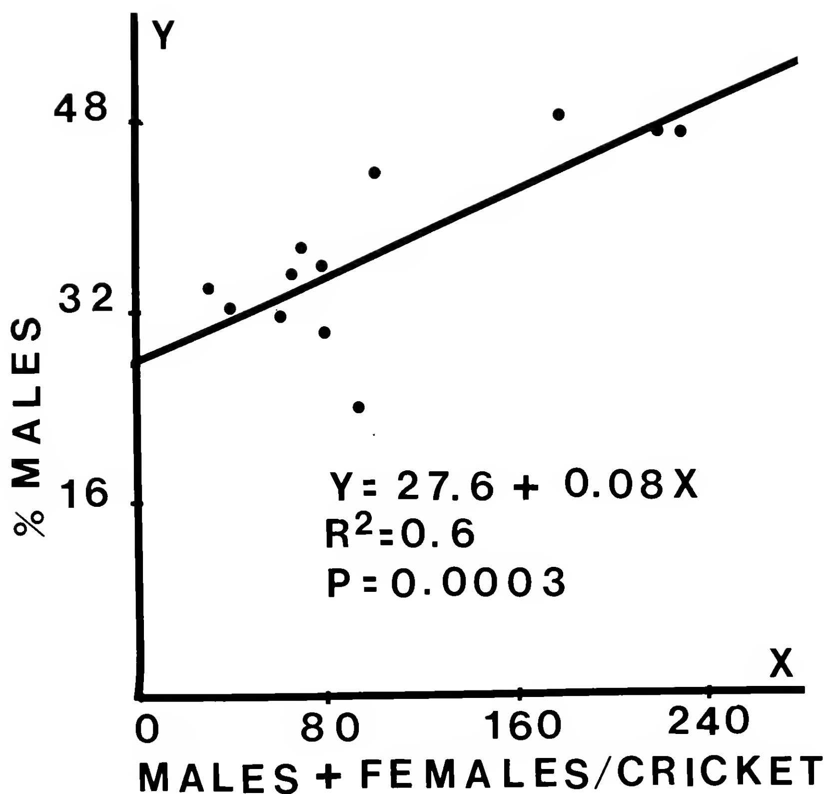


Fig. 12. The relationship between the percentage of males and the total number of nematodes in each house cricket at 24 C.

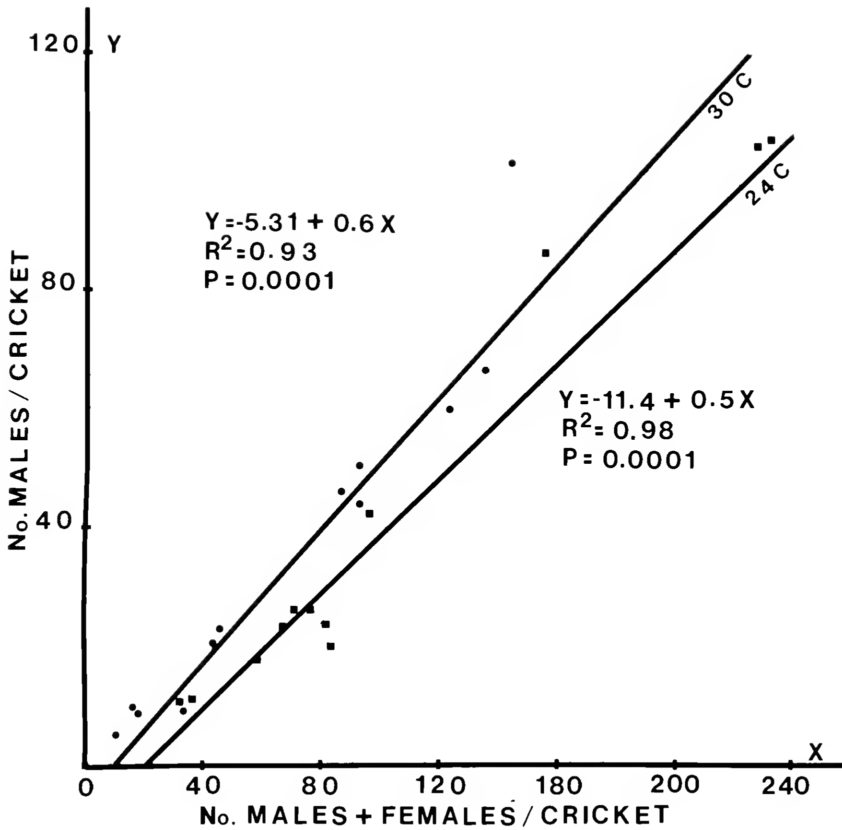


Fig. 13. The relationship between the number of males and the total number of nematodes in each house cricket.

CHAPTER 5

CULTURE OF STEINERNEMA SCAPTERISCI N. SP. IN VIVO

Maintaining a continuous supply of nematodes for research purposes is one of the most important considerations in studying the effects of nematodes on mole crickets. This need led to the development of different methods for producing quantities of the nematodes sufficient for research purposes. Depending upon the number of nematodes needed, and other considerations, they were cultured either *in vivo* or *in vitro*. This chapter covers only *in vivo* culture.

In vivo culture in the wax moth larva, *Galleria mellonella*, has been used by researchers for many years. Since *S. scapterisci* reproduces very poorly or not at all in wax moth larvae, mole crickets have been used as the *in vivo* host to maintain populations and strains of the nematode, to insure that the nematode retains the ability to kill mole crickets, and to produce numbers sufficient for small-scale experiments. The purpose of this chapter is to present the methods used to culture *S. scapterisci* in mole crickets and to evaluate other techniques in order to improve production.

Literature Review

The culture of nematodes *in vivo* was first carried out by Dutky and Hough (1955) when they grew what is now the DD-136 strain of *Steinernema carpocapsae* in larvae of the greater wax moth, *Galleria mellonella*. Dutky et al. (1964) reported in detail their culture techniques in which wax moth larvae were inoculated with infective stage juveniles of the nematode in petri dishes, then, when the wax moth larvae died, incubated the cadavers at 24 C. The nematodes which emerged from the cadavers were trapped in a tray containing a solution of 1 part formaldehyde and 1,000 parts water (1/1,000). Each wax moth larva produced up to 200,000 infective-stage juveniles. The juveniles harvested were stored in a flask at 7.1 C in oxygenated 1/1,000 formaldehyde.

Materials and Methods

In Vivo Culture in Mole Crickets

Two pieces of filter paper, Whatman No. 2, are placed in the bottom of a petri dish (15 x 100 mm) and 5,000 - 20,000 infective-stage juveniles in water added. Mole crickets are anesthetized by carbon dioxide, and then placed in the dish (Fig. 14). The number of mole crickets per dish varies depending upon their availability and the purpose of the inoculation. The lids are applied and the petri dishes

placed in the dark at room temperature (25 C). After 2-3 days, the dead mole crickets are washed with water to remove any phoretic nematodes and placed on a filter paper in incubation chambers (Fig. 14). The incubation chambers consist of a large petri dish (20 x 150 mm) containing an inverted lid of a small petri dish (15 x 60 mm) on which a piece of filter paper (90 mm diameter) is placed. Water is added to the large petri dish to reach and wet the filter paper. The mole cricket cadavers are arranged on the filter paper with the heads directed outward. The dishes are incubated at 24 C. After 5-7 days, third generation third stage infective juveniles migrate from the cadavers to the filter paper and into the water (Fig. 15). The nematodes are harvested by collecting them on a fine mesh sieve, openings 20-24 um, washing them onto a filter (dust mask) and allowing the active nematodes to migrate through the filter into clean water. The nematodes are used immediately or stored under aeration from an aquarium pump at 6-12 C for several weeks. This method of production takes 7-10 days to produce infective stage juveniles, and yields 20,000 - 80,000 per mole cricket.

Experiment 1

The purpose of this experiment was to determine whether rates of inoculum greater than 20,000 infective stage juveniles would yield greater numbers of infective stage

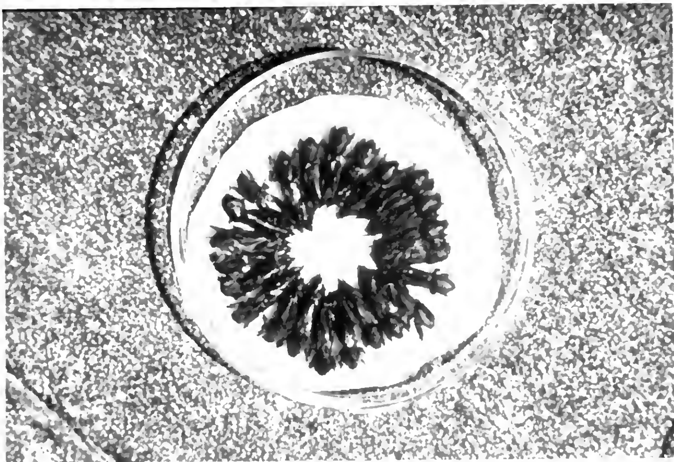


Fig. 14. *In vivo* culture of the nematode *Steinernema scapterisci* n. sp. Top: Live mole crickets placed in an inoculation chamber with third-stage juvenile nematodes. Bottom: Dead mole cricket arranged in an incubation chamber.

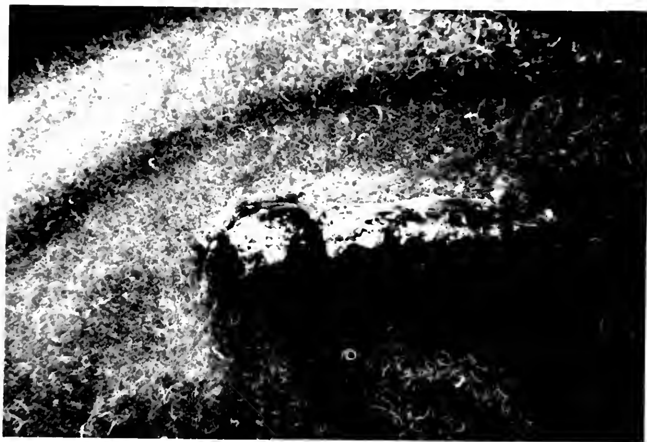


Fig. 15. Top: Nematodes which developed within the bodies of mole crickets have migrated to the exterior. Bottom: Third-stage juveniles have migrated from the mole crickets into the water in the incubation chamber.

juveniles than would 20,000 or less. Inoculum levels of 5, 10, 20, 40, 60, 80, 100 and 120 thousand infective-stage juveniles were released into each of 8 petri dishes prepared as reported above. Five anesthetized mole crickets were placed in each dish and stored in the dark at room temperature (25 C). After the mole crickets died, they were washed and placed in incubation chambers. The dishes were examined daily.

Experiment 2

The purpose of this experiment was to determine whether the infective-stage juveniles reproduced equally well in all parts of the mole cricket. Ten mole crickets were inoculated with nematodes as above. Two days after the mole crickets died, their bodies were separated into head, thorax, and abdomen, and each part dissected and examined for developing nematodes.

In Vivo Culture in House Crickets

Since mole crickets are not available during the winter months, another good host was sought. An alternate host, to be effective, must produce a large number of infective stage juveniles and the nematodes obtained must have similar virulence to the target host as those produced in mole crickets. Other experiments (Chapter 9) showed that *S. scapterisci* killed a high percentage only of mole crickets and house crickets, *Acheta domestica*. House crickets were used in the experiments reported below to determine whether

they would be a satisfactory host in which to produce the nematode.

Experiment 3

Six house crickets were placed in each of two 15 x 100 mm petri dishes prepared as reported above. After 4 days, 11 of the 12 house crickets died. The cadavers were washed by shaking them vigorously in a container of water, and then incubated as in the rearing method for mole crickets. Third stage juveniles which emerged from the house cricket cadavers were referred to as the H1 population. The H1 population was cycled through house crickets again to yield the H2 population and the H2 population in turn yielded the H3 population. The number of infective stage juveniles produced per house cricket were counted.

Experiment 4

In order to compare the effectiveness of nematodes produced through three cycles in house crickets, population H3, with nematodes produced in mole crickets, population MC, the following experiment was carried out.

Ten petri dishes were prepared as in Experiment 1; 8,000 MC infective-stage juveniles were released in each of 5 dishes, and 8,000 H3 infective-stage juveniles were released in each of the other 5 dishes. Mole crickets were anesthetized and 5 placed in each dish. The lids were applied to the dishes and taped to the bottoms. All dishes

were kept in the dark at room temperature (25 C). The number of dead crickets was reported after 2 and 4 days.

Results and Discussion

In Vivo Culture in Mole Crickets

Experiment 1

At inoculum rates of 5,000-20,000 nematodes per dish of 5 mole crickets, yields of infective-stage juveniles ranged from 20,000-80,000. At inoculum rates of 40,000-120,000 nematodes per dish of 5 mole crickets, a large number of the first generation females, due to overcrowding apparently, emerged from the mole cricket cadavers 4 days after inoculation. The majority of these females moved down into the water in the incubation chamber and many of them died without reproducing. The bodies decayed very quickly and their decay, plus the decay of the mole cricket cadavers, gave off a strong odor of ammonia from the incubation chambers. These decaying bodies rendered the water toxic to the infective stage-juveniles when they moved into it. Other females which contained active juveniles in their bodies, moved down into the water. These females produced uninfective second stage juveniles which died very quickly in storage.

When the situation described above occurs during rearing, the mole cricket cadavers should be rinsed with water and all of the nematodes and water in the dish must be

discarded and clean water added. This process must be repeated until no, or very few, adult nematodes move into the water.

Experience showed that only those females on the filter paper of the incubation chamber, or on or in the cadavers of the mole crickets produced infective-stage juveniles.

Considering the above problems encountered when 40,000 or more infective stage juveniles per 5 mole crickets were used as inoculum, it is obvious that those rates were too high for optimum rearing. One may want to use such high rates, however, when the first generation females are needed to obtain juveniles for axenic culture.

Experiment 2

When the head, thorax and abdominal sections of the mole cricket were examined, developing nematodes were found mainly in the head and the thorax, especially in the pronotum; very few were found in the abdomen. These results indicate that the abdominal cavity is not very suitable for development of the nematode.

Producing the nematodes on mole crickets has some disadvantages. Mole crickets, being soil-dwelling insects, carry many different microorganisms on and in the body; these develop quickly after the mole crickets die. Also, the decaying mole cricket cadavers, the dead bacteria and decaying nematode bodies in the dishes cause a high death rate of the juveniles. The nematodes harvested usually were

contaminated with other bacteriophagous nematodes such as diplogasterids and rhabditids, sometimes in very high numbers. These disadvantages can be ameliorated, but not eliminated, by forcing the mole crickets to swim in a container of water for 2 minutes before inoculating them. Immediately after the inoculated mole crickets die, their wings must be removed, and the mole crickets washed by shaking them in a container of warm water (40 C) for 10 seconds. Then the posterior 2/3 of the abdomen must be removed to reduce the amount of body fluids in the incubation chamber (the abdomen does not support the development of nematodes as mentioned above). The nematodes then may be placed in the incubation chamber.

In Vivo Culture Using House Crickets

Experiment 3

It took the same length of time, 7-10 days, to produce infective-stage juveniles in house crickets as in mole crickets. Each house cricket can produce from 20,000-60,000 infective-stage juveniles. Dissection of cadavers of house crickets showed that the nematodes were present in all parts of their bodies. Because of this distribution, reproduction was not much less than that in mole crickets even though the house cricket is considerably smaller.

Experiment 4

The nematode, H3, produced from three cycles through house crickets, killed 22 of 25 mole crickets in 2 days and

24 of 25 in 4 days. The nematode from mole crickets, MC, killed 23 of 25 mole crickets in 2 days and 25 of 25 in 4 days (Table 9). These results indicate that nematodes cycled through house crickets 3 times do not lose their pathogenicity, but kill mole crickets as readily as do those produced in mole crickets.

Both house crickets and mole crickets have been used for *in vivo* culture of *S. scapterisci* at the Nematology Laboratory, University of Florida. The use of house crickets has the advantage that they are readily available throughout the year, but the disadvantage that somewhat fewer infective stage juveniles are produced per house cricket than per mole cricket. The house cricket also may serve as a good test insect in lieu of the mole cricket because of its availability, and because it is killed by *S. scapterisci* as readily as is the mole cricket. It may serve, also, as an excellent host to test the LD50 and LC50 of the nematode.

Table 9: Numbers of mole crickets that died after 2 and 4 days when using 8,000 Steinernema scapterisci strains H3 and 8,000 strain MC Steinernema scapterisci in petri dishes containing mole crickets.

Rep	No. mole crickets killed/total			
	2 days		4 days	
	H3	MC	H3	MC
1	5/5	5/5	5/5	5/5
2	5/5	5/5	5/5	5/5
3	4/5	5/5	5/5	5/5
4	4/5	3/5	5/5	5/5
5	4/5	5/5	4/5	5/5
Total	22/25	23/25	24/25	25/25

H3 = Nematodes produced from house crickets after 3 cycles.
MC = Nematodes produced from mole crickets.

CHAPTER 6
CULTURE OF STEINERNEMA SCAPTERISCI N. SP. *IN VITRO*

In vivo culture of entomogenous nematodes is satisfactory for maintaining strains or species, and for producing moderate numbers for small-scale experiments, but when large numbers are needed, as for field experiments, *in vitro* culture must be used. *In vitro* culture has been used since 1932 with several different methods reported. In this chapter are presented the different methods used to produce *Steinernema scapterisci*.

Literature Review

Glaser (1932) was the first to culture an entomogenous nematode (*Neoaplectana glaseri*) *in vitro*. He used veal infusion agar, dextrose and baker's yeast. According to his report, yeast furnished some nutritional elements found in the insect host. While his method produced a moderate number of nematodes, it did not yield quantities sufficient for field release. McCoy and Glaser (1936) reported an improved technique by using fermented potato mash with a mixture of two thirds Irish potato and one third sweet potato. They believed that sweet potato enhanced the growth of yeast. The medium was mixed with a pure culture of yeast

grown on sweet potato gruel. Then a 2-cm layer of the mixture was spread on a tray. After 20-40 hours of fermentation, the nematodes were added on top. This method produced about 4 million nematodes per 2,000 cm2 of surface. A further improvement was described by McCoy and Girth (1938) who used ground, extracted veal pulp. This method consisted of grinding fresh veal through a food chopper, then infusing it with water. After 2 days the infusion was poured onto a flannel cloth, drained, and the pulp squeezed as dry as possible. After adding water and a preservative (0.06% formaldehyde and 0.05% of the sodium derivative of methyl-hydroxybenzoate) the medium was spread in a thin layer and inoculated with nematodes. The authors said that this diet produced larger and more robust nematodes and the yield was 9,000-12,000 nematodes per cm2 of culture area.

All of the above methods can be used to produce nematodes but they are either too expensive or do not produce sufficient quantities for field release.

The symbiotic relationship between the nematode and the associated bacterium was not understood when the above culture methods were developed. However, when _N. carpocapsae_ was described in 1955, the symbiotic relationship was known and that knowledge led to a considerable improvement of _in vitro_ culture. The associated bacterium serves as a food source for the nematode but apparently does not supply all the nutrients

needed (Poinar, 1979). After conducting several different experiments, Poinar stated that he had been able to obtain only up to two and a half generations of the nematode on any kind of bacteriological agar even when the nematodes were transferred to a new plate seeded with the bacterium. But, when a medium containing additional nutrients was used, continuous culture could be achieved. The medium on which the bacterium is cultured apparently supplies other nutrients for the nematodes. The first medium used for nematode production contained commercial dog food as a base (House et al., 1965). Hara et al. (1981) improved production of the nematode by growing it monoxenically on a dog food agar medium. Dutky et al. (1967a, 1967b), proved that sterols are necessary for growth and development of the nematode. That information, as well as the knowledge that the bacterium, Xenorhabdus nematophilus, has a primary and a secondary form (Akhurst, 1980, see also, section on "Associated bacterium" below), was very important in increasing the in vitro production of the nematode. A major break-through in mass production of the nematode was accomplished by Bedding (1981) who provided a large surface area by using a sterilized, polyether-polyurethane sponge thinly coated with a homogenate of 70% pig's kidney, 10% beef fat and 20% water. Before adding infective-stage nematodes, the primary form of the bacterium was added to the sterilized medium. Bedding (1984) improved his mass-

production technique by coating the sponge with a homogenate of chicken offal and by using autoclavable plastic bags as containers.

The cost of producing the nematode using the method of House et al. (1965) was estimated to be $1.00 per million infective-stage nematodes, that of Hara et al. (1981), 28 cents per million, and that of Bedding (1981), 2 cents per million.

Associated bacterium:

The bacterium associated with steinernematids was described by Poinar and Thomas (1965) as Achromobacter nematophilus. The bacterium was isolated from Steinernema carpocapsae strain DD-136. Hendrie et al. (1974) rejected the genus Achromobacter and transferred all but one species in the genus to other genera. Since A. nematophilus could not be accommodated by other genera, Thomas and Poinar created a new genus for it, Xenorhabdus nematophilus. Another bacterium resembling X. nematophilus, X. luminescens, was described by Poinar and Thomas (1979) from Heterorhabditis bacteriophora. The authors placed the genus Xenorhabdus in the family Enterobiaceae, but pointed out that it differs from the currently accepted genera in the family by its large cell size, its immunological properties, its intimate association with entomogenous nematodes, and its pathogenesis to insects.

Akhurst (1980) demonstrated that the bacterium was morphologically and functionally dimorphic having a primary and a secondary form. The primary form, which is carried into a new host by the infective-stage nematode, apparently remains stable in the host (Akhurst, 1980), but the primary form may revert to the secondary form in in vitro culture. These two forms can be distinguished by biochemical tests and by color and size of the colonies. Either form is equally pathogenic when inoculated into larvae of the greater wax moth, Galleria mellonella, but the primary form is much more effective than the secondary form for production of nematodes. Akhurst (1980) showed that yield of infective stage juveniles obtained from axenic nematodes plus the primary form of the bacterium was seven times greater than that from axenic nematodes plus the secondary form. He noted that the reason the bacterium changes to the secondary form is not known, but that no bacteriophage, or plasmid is involved in mediating the change.

A wide range of microorganisms was found to be inhibited by the primary form of Xenorhabdus spp. but not by the secondary form. This inhibition suggests that Xenorhabdus spp. produce antibiotic substances.

Akhurst (1983) studied the taxonomy of the bacteria associated with various species of the nematode and, based on the guanine and cystosine content of DNA, the color of the colonies, and the production of acid from carbohydrates

of the bacterium, suggested the following subspecies: X. nematophilus subspecies nematophilus for bacteria symbiotic with S. carpocapsae; X. nematophilus subspecies bovienii for bacteria symbiotic with S. bibionis; and X. nematophilus subspecies poinarii for bacteria symbiotic with S. glaseri.

Materials and Methods

Isolation of Bacteria

The bacteria associated with Steinernema scapterisci were isolated by one of three methods.

Method 1: This method was a modification of the hanging drop technique suggested by Poinar (1966). A drop of hemolymph taken aseptically from a mole cricket was placed in a 35 x 10 mm petri dish. Five infective-stage juvenile nematodes, sterilized previously by placing them for 2 hours in 0.1% merthiolate and washing them 3 times in sterilized deionized water, were transferred into the drop of hemolymph. The petri dish was placed in a plastic bag containing a folded facial tissue saturated with water, the bag was tied and kept in the dark at 25 C. After 24 hours, the bacteria had developed sufficiently and were transferred to nutrient agar or tergitol-7 agar with triphenyltetrazolium chloride and bromothymol blue (T-7 agar) (Poinar and Thomas, 1965) in order to identify the colonies as those of the associated bacterium.

Method 2: This was a modification of Akhurst's method (1980). Infective-stage juveniles were surface-sterilized by immersion in 0.1% merthiolate for 2 hours, washed 3 times in sterile water, then about 50 of them in water were pipetted into a 0.25 cc tissue grinder (Akhurst used yeast and salt broth instead of water) and macerated. The macerate was spread on nutrient agar or T-7 agar. The bacterial colonies appeared after 36-48 hours at 25 C.

Method 3: In this method, bacteria were collected from the hemolymph of mole crickets as follows: Mole crickets were inoculated with a suspension of infective-stage juveniles in an inoculation chamber as explained for in vivo culture. After 24 hours, a mole cricket that slowed in movement and had trembling legs (a sure sign of infection) was selected. Its abdomen was squeezed gently to press out any fecal matter in the rectum to prevent the release of such matter into culture media when the hemolymph was collected. The entire mole cricket was washed in tap water followed by a 2-3 minute dip of its rear end into 30% hydrogen peroxide. Then the tip of the cercus was cut off with sterilized scissors. The hemolymph which oozed from the circus was collected in a dish containing T-7 agar and spread over the surface with a loop. After 24 hours bacterial colonies developed in the culture medium and were transferred to new T-7 agar plates.

Preparation of Bacteria for Culturing the Nematode

To produce sufficient quantities of the bacteria on which to grow the nematode *in vitro*, a bacterial colony was transferred aseptically to a flask containing brain-heart infusion broth which had been autoclaved previously. The flask was attached to a shaker arm and shaken continuously. After 48 hours, the bacteria were ready to inoculate onto the solid culture medium.

Experiment 1

To determine the cultural and fermentation characteristics of the bacterium, tests on various culture media and test substances (Table 10) were carried out. Bacterial colonies 24 hour old were inoculated onto previously prepared media in petri dishes. Different substances, prepared as instructed by their producers were placed in separate test tubes with bromcresol purple as a pH indicator when needed. A drop of 24-hour-old bacteria produced in liquid medium was placed in one of two tubes of the same substance, with the other tube serving as a control. The results were obtained after 24 hours.

Experiment 2

The purpose of this experiment was to determine the suitability of eight culture media for rearing the nematode. The 8 culture media listed below were prepared as indicated for each medium. Where agar was used, the agar was melted in warm water. Where crickets and animal parts were used,

they were macerated in a blender in the amount of water indicated for each medium. Where sponge was used, the sponge was chopped into small pieces. All media were placed in 500 ml flasks, each plugged with a two-hole stopper fitted with a glass tube in each hole and a short piece of autoclavable hose attached to each glass tube. The end of the two hoses were covered with aluminum foil and all flasks were autoclaved for 30 minutes at 121 C and 15 psi pressure.

Medium 1: 3 g nutrient agar, 120 ml H2O, 1 ml corn oil, and 10 g sponge. The combination was mixed well in a 500 ml Erlenmeyer flask.

Medium 2: Similar to Medium 1 but without sponge. Also, after autoclaving and before the agar became firm, the flask was rotated to distribute agar on the flask wall. The purpose of this maneuver was to increase the rearing surface.

Medium 3: 3 g nutrient agar, 120 ml H2O, 2 mole crickets.

Medium 4: 3 g nutrient agar, 1 g brain-heart infusion, 120 ml H2O, and 1 ml corn oil.

Medium 5: 20 g pork kidney, 50 ml H2O, 1 ml corn oil, and 10 g sponge.

Medium 6: 20 g pork kidney, 5 g pork brain, 50 ml H2O, and 10 g sponge.

Medium 7: 20 g pork liver, 1 ml corn oil, 50 ml H2O, and 10 g sponge.

Medium 8: 20 g pork liver, 5 g pork brain, 50 ml H2O, and 10 g sponge.

One day after the media were prepared and autoclaved, they were inoculated with bacteria by adding 5 ml of bacteria-brain-heart infusion broth. Two days later, about 20,000 surface-sterilized infective-stage juveniles were added to each flask. At that time a bacteriological filter was fitted to each hose emanating from the stopper in each flask. One hose served as an inlet for air from an aquarium pump and the other as an outlet. The air entering the flask was passed through water to increase its relative humidity and thus reduce drying of the medium (Fig. 16). The system was maintained at 25 C. After 14 days the nematodes were harvested by flooding the flasks with water and emptying the contents onto a filter in a pan (modified Baermann funnel). Nematodes that moved out of the media, through the filter and into the water in the pan were collected after 24 hours.

Experiment 3

This experiment was conducted in order to determine whether nematodes produced *in vitro* would kill mole crickets. Five mole crickets were exposed in a petri dish to 8,000 infective-stage juvenile nematodes cultured *in vitro*. The experiment was replicated four times. After 3 days, the number of dead crickets was recorded.

Fig. 16. Culture flasks inoculated with bacteria and nematodes receive humidified and sterilized air filtered through a bacteriological filter.

Experiment 4

This experiment was conducted to determine whether there was any difference in the kill rate of mole cricket nymphs by nematodes produced *in vitro* or *in vivo*. The mole cricket nymphs used in this experiment had a pronotum length of 4-9 mm. There were three treatments: (1) the control without nematodes, (2) infective stage juveniles produced *in vivo* and (3) infective stage juveniles produced *in vitro*. The inoculation technique was similar to that for *in vivo* culture, but the number of nematodes used was 20,000. The results were determined after 4 days.

Results and Discussion

Isolation of Bacterium

All three methods used to isolate the bacterium are satisfactory. However, Methods 1 and 3, which involve isolating the bacterium from mole crickets, were less satisfactory than Method 2, which involved isolating the bacterium from the nematode. The reasons are that in isolating the bacterium from mole crickets, which are associated with soil, the chance of contamination is high, and the methods are more complicated. Thus, Method 2 is simpler and results in less contamination than Method 1 and 3.

Description of the Bacterial Colony

After two days on T-7 agar plates, small round colonies 1/2-1 mm in diameter were formed. Initially the colonies were grey in color, but gradually became darker until after 2 days a small reddish-brown spot was formed at the center of the colonies. The area surrounding the reddish spot was clear to slightly blue. The spot in the center of the colonies increased in size and changed color gradually to become dark purple. The outer margin of the colonies were undulated, some more so than others.

Experiment 1

Results of cultural and biological studies are summarized in Table 10. This bacterium developed on all media tested. The fermentation test showed that the bacterium is fermentative in its metabolism of the tested substances. The negative result in Voges-Proskauer test suggested that in the fermentation of glucose, the bacterium cannot produce the neutral end product, acetylmethylcarbinol (acetoin). The positive results of amino acid tests show that the bacterium can decarboxylate these acids to form amines. This bacterium is not capable of utilizing citrate as the sole source of carbon for its metabolism.

Table 10: Cultural and biological characteristics of the bacterium associated with Steinernema scapterisci.

Materials	Reaction	
Culture Media		
Brain-heart infusion agar	+	
MacConkey agar	+	
Nutrient agar	+	
TSA blood agar base	+	
Tryptic soy agar	+	
Test Substances	Acid	Gas
Adonitol	+	*
Arabinose	+	*
Arginine	+	*
BCP + glucose	+	-
BCP + lactose	+w	*
BCP + manitol	+	-
BCP + rhamnose	+w	*
BCP + sucrose	+	-
BCP + trehalose	+	-
Bile esculin	+	
Citrate	-	
Hippurate broth	+	-
Inulin broth	+w	
Indole	-	
Lysine	+	*
Nitrate	+	*
OF glucose	+	*
ONPG broth	+	
Ornithine	+	*
Growth-Peptone + .6% NaCl	-	
Simmon slant	+	
Sorbose	+	*
TSB broth	+	
Urease	+	
Voges-Proskauer	-	

BCP = Bromcresol purple
\+ = reaction
\- = no reaction
w = weak
* = not determined

Experiment 2

By 6 days, nematodes developed rapidly on Culture Media 2, 4, 5, and 7 and were present on the flask walls away from the media. On day 8, the nematode populations were greater on Medium 4 than on the other media. On day 12, nematodes covered the flask walls of Media 4 and 7. On a few other media, a few nematodes were seen on the flask walls.

Nematodes were harvested from all flasks 15 days after inoculation. Medium 7, which contained pork liver, oil, sponge and water, produced the greatest number of nematodes of all media tested (Table 11). While Media 3 and 4 also supported fair growth and reproduction of the nematode, the juveniles were extremely difficult to extract from the agar in the medium to the extent that the numbers reported in Table 11 undoubtedly are low.

Experiment 3

Nematodes from *in vitro* culture killed 100% of the mole crickets tested while 4 died in the controls (Table 12). The results show that nematodes produced *in vitro* are just as effective in killing mole crickets as are those produced *in vivo*.

Table 11: Number of third-stage infective juvenile nematodes harvested from each culture medium.

Medium		No. nematodes
1	Agar, oil, sponge	500,000
2	Agar, oil	5,000
3	Agar, mole crickets	1,500,000
4	Agar, oil, brain-heart	3,500,000
5	Kidney, oil, sponge	1,500,000
6	Kidney, brain, sponge	1,700,000
7	Liver, oil, sponge	40,000,000
8	Liver, brain, sponge	1,000,000

Table 12: Number of mole crickets killed by nematodes produced by *in vitro* culture.

	No. mole crickets that died	
Replicate	Treatment	Control
1	5/5	0/5
2	5/5	1/5
3	5/5	1/5
4	5/5	2/5
Total	20/20	4/20

Experiment 4

Fifteen of 25 mole crickets were killed by nematodes produced by *in vitro* culture compared to 14 of 25 killed by those produced by *in vivo* culture (Table 13). Thus nematodes produced by *in vitro* culture were just as effective in killing mole crickets as were those produced by *in vivo* culture.

Comparing the data in Table 12 with that in Table 13, the rate of kill of adult mole crickets was higher than that of nymphs. This indicates that the nematode does not kill nymphs as effectively as it kills adults. Preliminary tests showed that nymphs with a pronotal length equal to or less than 2 mm were not killed by the nematodes. One of the reasons for this may be that the natural openings used by the nematodes to enter the host are smaller in nymphs than in adults.

Table 13: Comparison of nematodes produced *in vitro* vs. *in vivo* for kill of mole cricket nymphs.

	No. mole cricket nymphs that died		
Replicate	In vivo	In vitro	Control
1	3/5	4/5	1/5
2	2/5	3/5	0/5
3	2/5	3/5	1/5
4	3/5	3/5	1/5
5	4/5	2/5	1/5
Total	14/25	15/25	4/25

CHAPTER 7
VERTICAL MIGRATION OF STEINERNEMA SCAPTERISCI N.SP. IN SOIL

Both laboratory tests and field tests (Chapter 9) have shown that *Steinernema scapterisci* is very effective in controlling mole crickets in Florida. Some preliminary experiments in the laboratory indicated that the nematode moved very little from the site of application. Since knowledge about movement of the nematode through the soil is very important in determining when and how to apply the nematode in the field, additional experiments were conducted. This chapter reports the results of those experiments.

Literature Review

Moyle and Kaya (1981) found that when they released *Steinernema carpocapsae* in sandy soil, at 15 cm below the soil surface, 77% of the nematodes were recovered above the point of release after 48 hours. When they placed them on the soil surface, 90% of them remaining within 1 cm of the surface. When they placed nematodes at depths of 2.5 and 5 cm below the soil surface, almost all of them remained at the release site. Georgis and Poinar (1983) concluded that an increase in the percentage of clay and silt decreased the

movement of the nematode in the soil. They concluded also that nematodes showed a tendency to disperse upward from the point of application and that migration was greatest when pupae of the wax moth (*Galleria mellonella*) were present in the soil. Schroeder and Beaver (1987) reported that nematodes in the families Heterorhabditidae and Steinernematidae dispersed more upward than downward, except for *S. glaseri* which moved both upward and downward.

Materials and Methods

Experiment 1

The purpose of this experiment was to determine whether the infective-stage juveniles of *S. scapterisci* would move downward 6 cm through soil and kill mole crickets in petri dishes below.

PVC (polyvinyl chloride) pipe 8 cm in diameter was cut into rings 2 cm wide (Fig. 17). Three of these rings were taped together with strapping tape and then taped to the top of a plastic petri dish previously perforated numerous times by a hot needle. The rings were filled with sterilized sandy soil (97.86% sand, 1.85% clay, 0.25% silt) with a moisture content of 14%. The soil was packed firmly by hand, and for two of the treatments, approximately 5,000 nematodes (equivalent to 100/cm2) in 2 ml of water were distributed rather evenly over the soil surface, except that none was placed closer than 1 cm from the edge of the ring

none was placed closer than 1 cm from the edge of the ring to prevent any possibility of the nematodes being carried downward by the application water at the interface of the soil and the PVC ring. Then, one more ring was taped to the top ring and filled with soil to prevent the nematodes from becoming dessicated, and to simulate the field application of nematodes 2 cm deep by chisel (Fig. 17).

Two pieces of No. 2 Whatman filter paper were placed in the bottoms of petri dishes, moistened, and the following 3 treatments applied: (1) 2 mole crickets per petri dish, (2) no mole crickets per petri dish, (3) 2 mole crickets per petri dish but without nematodes in the soil above. Then the upper units were placed on the bottoms of the petri dishes and the units stored in the dark at room temperature (25 C). No food was supplied to the mole crickets. The dishes were checked daily, and any dead mole crickets were removed, washed by shaking them in a container of water, and placed individually, without food, in plastic vials. Two days after the mole crickets died, they were dissected and examined for developing nematodes. At the end of the experiment (after 5 days), all mole crickets were placed individually, without food, in vials until they died. Two days after they died (however long that took), the mole crickets were dissected and examined for nematodes.

After 5 days, the tape was removed from the rings and the soil in each ring removed and processed by Baermann pan

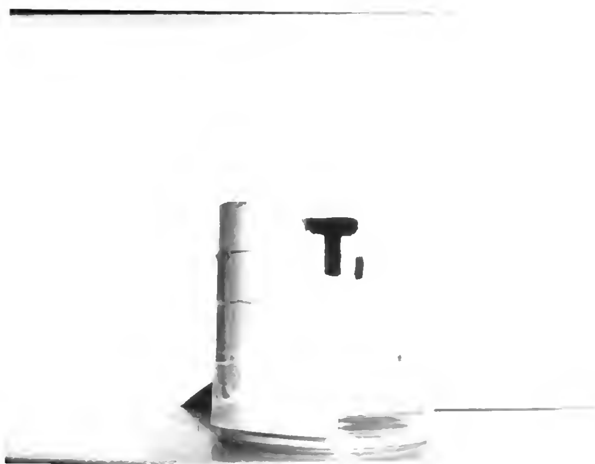


Fig. 17. PVC pipe used in migration test. Two-cm PVC pipe ring were taped together and filled with soil. A perforated petri dish containing 2 mole crickets was attached at the bottom.

to extract the nematodes. At the end of the experiment each petri dish and the filter paper in it was washed by agitation in a container of water to determine if nematodes were present.

The experiment was repeated once using approximately 10,000 nematodes (equivalent to 200/cm2) in the soil above.

Experiment 2

The purpose of this experiment was to determine if the infective stage juveniles would travel 10 cm downward through soil and kill mole crickets. This experiment was similar to the first but with the following differences: 5 experimental PVC rings plus one ring on top were used instead of 3 experimental rings plus 1 ring on top; 3 mole crickets were used instead of 2; the treatment without mole crickets was omitted; and approximately 10,000 nematodes were used. The treatment was replicated 5 times and the control 2 times.

Experiment 3

The purpose of this experiment was to determine whether the nematodes, when placed in a larger container allowing greater freedom of movement laterally, would move downward through 6 cm of soil and kill mole crickets. The bottoms of four plastic storage boxes (31 cm x 23 cm x 10 cm) were perforated numerous times by a hot needle near the center in an area 15 cm in diameter. A 6-cm layer of sandy soil (see Experiment 1) was placed in the boxes and approximately

10,000 infective-stage juveniles in 4 ml of water were released on the soil surface in an area 15 cm in diameter immediately over the perforated area. The top of a glass petri dish 15 cm in diameter containing soil and 5 mole crickets was placed underneath the perforated area of each box. The experiment was checked daily and any dead mole crickets were removed, washed as in Experiment 1 and placed in separate vials. Two days after the mole crickets died, they were dissected and inspected for nematodes.

Experiment 4

The purpose of this experiment was to determine, when given a choice, whether the nematodes would move upward, downward, or both in soil. Four PVC rings 2 cm thick, were taped together and filled with sandy soil as in Experiment 1. About 10,000 infective stage juveniles in 2ml water were distributed on the soil surface. Another set of four rings was placed on top of this set, taped in place and filled with soil. The experiment was replicated 4 times and the experimental units were stored in the dark at room temperature (25 C). After 5 days, the soil in each ring was removed and processed by Baermann pan to extract the nematodes.

Experiment 5

This experiment had two purposes. The first was to determine whether the nematode would move downward in the soil and kill mole crickets as readily in the field as in

the laboratory. The second was to determine whether entomogenous nematodes which attack mole crickets occurred naturally in the experimental site.

Petri dishes perforated numerous times by a hot needle through the tops and bottoms were filled up to one half with soil, four mole crickets, and some alfalfa seeds, which would germinate and serve as a food source for the mole crickets, were added to each dish. The dishes were buried in the field at depths of 4-, 6-, 8- and 10-cm in an 1 m2 area. The treatments were replicated 4 times. Infective stage juvenile S. scapterisci were sprayed on the soil surface just before dark at a rate of 200,000 per square meter (equivalent to 20/cm2). Eight control sets, two at each depth, were buried some 2 meters distant from the treatments; no nematodes were sprayed on the soil surface of the controls. After 5 days, the petri dishes were removed from the soil, the number of dead mole crickets determined and the cadavers dissected and examined for nematodes.

Results and Discussion

Experiment 1

In the experiment using 5,000 nematodes with mole crickets in petri dishes 6 cm below the soil surface, 2 mole crickets died after 5 days and all 10 after 8 days. When the cadavers were dissected, 5 of the 10 contained developing nematodes. Only one of 10 mole crickets died in

the controls and no nematodes were found in the cadaver. In the experiment with 10,000 nematodes 6 crickets died after 5 days and all 10 died after 8 days. Cadavers of 7 of the 10 contained developing nematodes. No mole crickets died in the controls.

These results show that the nematodes can move downward 6 cm through the soil and kill mole crickets. The number of mole crickets killed was greater when 10,000 nematodes were used than when 5,000 were used. Similar numbers of nematodes moved downward in treatments and controls at the 5,000 level, but two times as many or more moved downward in the controls as in the treatments at the 10,000 level (Table 14). Those data indicate that the nematodes may not have been attracted to the mole crickets. However, when the petri dishes and filter papers in those dishes were washed and the wash water examined for nematodes, those petri dishes containing mole crickets contained an average of 24 nematodes while those not containing mole crickets contained an average of 4 nematodes. That indicates that the nematodes may have been attracted to the mole crickets. Obviously, additional research is needed to determine whether or not attraction occurs. It was obvious, also, that after 5 days, most of the nematodes remained near the release site.

Table 14: Distribution of third stage juvenile nematodes in 2-cm layers of soil when 5,000 and 10,000 third-stage juveniles were applied to the soil surface.

Depth	Number of nematodes*			
	5,000		10,000	
(cm)	Treatment	Control	Treatment	Control
0-2	3150	2070	8089	7890
2-4	159	160	1356	2856
4-6	47	42	420	1068
6-8	44	20	47	156
Dish + paper	24	4	--	---

* Average of 5 replicates.

-- No data available

Experiment 2

After 5 days, 8 of 15 mole crickets died in petri dishes 10 cm below the soil surface, and all 8 of them contained developing nematodes. No mole crickets died in the controls. These results show that the nematodes can move downward through soil at least 10 cm and kill mole crickets.

Experiment 3

After 5 days, 5 of 20 mole crickets placed underneath perforated storage boxes had died and 3 of those 5 contained developing nematodes. The experiment shows that when the nematodes are given a choice to move freely laterally in the soil, they also move downward 6 cm and kill mole crickets. Whether this movement is random or directional has not been determined.

Experiment 4

When placed at the center of a soil column, the nematodes moved both upward and downward (Table 15), with some 3 times more found below the release site than above it. This is different than the primarily upward movement reported for S. carpocapsae by Georgis and Poinar (1983) and Schroeder and Beaver (1987). Since greater numbers of S. scapterisci were recovered in the top and bottom 2 cm of soil than in the 2 cm layer adjacent to them, it is likely that the nematode can move much farther than the 8 cm traveled in 5 days. This would be advantageous since nematodes distributed over a greater area would more likely

encounter mole crickets than if concentrated in one area. The number of nematodes collected from each 2 cm band of soil 5 days after the experiment began are shown in Table 15.

Experiment 5

When mole crickets in petri dishes were buried in the soil and nematodes sprayed on the soil surface, all 14 mole crickets that died at the 4-, 8-, and 10-cm depths contained nematodes (Table 16). At the 6 cm depth 14 of the 15 mole crickets that died contained nematodes. None of the 3 mole crickets that died in the controls contained nematodes which indicates that the experimental area did not contain naturally-occurring entomogenous nematodes which attack mole crickets.

More significantly, the results showed that when nematodes are applied to the soil surface in the field they can move downward at least 10 cm to kill mole crickets. Moreover, the nematodes found in the mole cricket cadavers were first generation adults. Since it takes 3 days for the nematode to develop from third-stage infectives to 1st generation adults, the third-stage infectives must have moved the 10 cm and infected the mole crickets in only 2 days.

Table 15: The number of infective-stage juvenile nematodes recovered from each 2-cm layer of soil 5 days after they were released at point 0.

Depth (cm)	No. nematodes
+6 - 8	108 c
+4 - 6	69 c
+2 - 4	151 c
+0 - 2	781 b
-0 - 2	1475 a
-2 - 4	843 b
-4 - 6	246 c
-6 - 8	901 b

+ and - in front of the first number in the first column refers to the number of cm above (+) or below (-) the 0 release site.

Numbers with different letters in the second column are significantly different at the 5% level according to Duncan's multiple range test.

Comparing the combined numbers of mole crickets killed (6/20) after 5 days in the laboratory tests in Experiment 1 at a 6-cm depth using an average of 100 infective-stage juveniles per cm2 and 200/cm2, with the numbers killed (15/16) after 5 days in the field test in Experiment 5 at a 6-cm depth using an average of 20 infective stage juveniles/cm2, the nematode killed much more effectively in the field than in the laboratory.

Table 16: Number of mole crickets that died when buried at each depth listed below when 200,000 infective-stage juvenile nematodes/square meter were released on the soil surface.

Depth*	No. mole crickets died/total	
cm	Total	Control
4	14/16	1/8
6	15/16	0/8
8	14/16	1/8
10	14/16	1/8

* Distance from the soil surface

CHAPTER 8
SURVIVAL OF STEINERNEMA SCAPTERISCI N.SP.

It is well-established that steinernematid nematodes are effective in controlling several soil-inhabiting insects (Simons and Poinar,1973). However, not all experiments have been effective because of such variables as different species or strains of entomogenous nematodes, weather, natural enemies, soil conditions, etc. Studies by Poinar and Hom (1986) on the survival of a related nematode, *Steinernema carpocapsae*, showed that one strain of that nematode can survive in the soil for up to 7 weeks. In this paper we report on the survival and infectivity of *S. scapterisci*.

Materials and Methods

Experiment 1

The purpose of this experiment was to determine whether infective-stage juveniles in sealed containers buried in the soil up to 8 weeks would survive and infect mole crickets. Approximately 2,000 infective-stage juveniles were released in each of thirty-two 757-ml plastic containers filled previously with sterilized sandy soil, the tops applied, and all containers buried in the field about 2.5 cm below the

soil surface and covered with grass sod. Each week for 8 weeks, four containers were removed from the field and the nematodes extracted from the soil in the containers by Baermann pan and counted.

Experiment 2

The purpose of this experiment was to determine whether infective-stage juveniles, when buried up to 10 weeks in containers with the ends covered with fine mesh Nitex cloth to permit the exchange of moisture and gases with the surrounding soil, would survive and infect mole crickets. Segments of PVC (polyvinyl chloride) pipe 40 cm long X 5.5 cm in diameter were used as soil containers in this experiment. One end of each container was covered with Nitex cloth (10um openings) and the container filled with 250 g of sterilized sandy soil at a moisture content of 13%. About 2,500 infective-stage juveniles were released in the containers and the other end covered with Nitex cloth. The containers were buried vertically in the field with the uppermost end about 2.5 cm below the soil surface, and covered with sod. All other steps were similar to those described in Experiment 1 except that this experiment continued for 10 weeks.

Experiment 3

The purpose of this experiment was to determine whether infective-stage juveniles would survive and infect mole crickets when stored for 6-14 weeks in non-sterile soil.

This experiment was similar to Experiment 1, except that the soil was not sterilized and the first four containers were left buried for six weeks.

In all experiments, all nematodes collected from the 6th week and later were concentrated in 2 ml of water and placed on a filter paper in a petri dish containing 3-5 mole crickets or house crickets. When the crickets died the cadavers were washed and each placed in a separate vial for two days after which they were dissected and examined for developing nematodes.

Results and Discussion

Experiments 1 and 2

When infective stage juveniles were buried in sealed containers and recovered weekly, the number of nematodes recovered at weeks 2-8 in Experiment 1 and at weeks 2-10 in Experiment 2 were significantly less ($P=0.05$) than the number recovered at week 1 in each experiment (Table 17). Thus the number of nematodes recovered declined rapidly during the first two weeks after their release but gradually during the remaining weeks.

In Experiment 1 linear regression analysis of the data proved that the hypothesis "the number of nematodes recovered each week was a function of time", was highly significant ($P=0.0001$). The equation is $Y = 1270 - 109 X$ and $R2 = 0.85$. That indicates that time was the factor

responsible for the decrease in numbers of nematodes recovered.

In Experiment 2, however, linear regression analysis of the data proved that the hypothesis that the number of nematodes recovered each week was a function of time was weakly significant ($P=0.10$). The equation is $Y = 1294 - 136 X$ and $R2 = 0.37$. That indicates that the reduction in the numbers of nematodes recovered was not only a function of time but also a function of other factors. Some of the other factors may have been lack of uniformity in size of the openings of the Nitex cloth, with some being large enough to allow the nematodes to escape from the containers; the Nitex cloth may not have been affixed to the containers securely enough to prevent the nematodes from escaping; and insects or plant roots may have damaged the cloth allowing the nematodes to escape.

The soil moisture at the 8th week in Experiment 2 was 7%. At this moisture level, the nematodes survived well for at least two additional weeks (Table 17). Both experiments showed that many of the nematodes survived 8 or 10 weeks in the soil and retained the ability to kill mole crickets and house crickets.

Experiment 3

When infective stage juveniles were buried in non-sterile soil, the number of nematodes recovered at the sixth week was comparable to that of Experiments 1 and 2

Table 17: Numbers and percentages of infective-stage juvenile Steinernema scapterisci recovered weekly after releasing 2,000 (Experiment 1) and 2,500 (Experiment 2) nematodes in containers of sterilized soil buried in the field.

	No. and % of nematodes recovered			
Week	Expt. 1	%	Expt. 2	%
1	1229 a	61	1997 a	80
2	1161 b	58	658 b	26
3	858 b	43	431 bc	17
4	716 b	36	452 bc	18
5	633 bc	32	619 bc	25
6	701 b	35	377 c	15
7	392 c	20	352 c	14
8	553 bc	28	588 bc	24
9	---	--	578 bc	23
10	---	--	412 bc	16

% = percentage of released number recovered.
-- = Experiment set up for 8 weeks.
Numbers followed by different letters in the same column are significant at the 5% level according to Duncan's multiple range test.

(Table 18), and the nematodes retained the ability to kill house crickets.

The number of nematodes recovered declined rapidly after the sixth week, with much lower recovery than at the sixth week in Experiments 1 and 2. The difference may have been due to the use of sterilized soil in Experiments 1 and 2 and non-sterilized soil in Experiment 3. Microorganisms in the soil, including natural enemies of nematodes, may have been the cause of the reduced number of nematodes.

Table 18 : Number and percentage of third-stage *Steinernema scapterisci* recovered 6 to 14 weeks after releasing 2,000 infective-stage juveniles in containers of unsterilized soil and burying them in the field.

WeeK	No. nematodes	%
6	584	29
7	144	7
8	76	4
9	57	3
10	22	1
11	21	1
12	14	0.7
13	11	0.5
14	9	0.4

% = percentage of released number

CHAPTER 9
STEINERNEMA SCAPTERISCI N.SP.
AS A BIOLOGICAL CONTROL AGENT OF MOLE CRICKETS

Mole crickets are the most important insect pests of turf-grasses in Florida (Walker,1984). They also cause damage to seedlings such as vegetable crops, tobacco and ornamentals. Losses, including costs to control mole crickets in Florida, are about 45 million dollars annually (Hudson and Short, 1988). To respond to the concerns of ranchers, farmers, turfgrass managers of all types, and home owners, a mole cricket research project was begun at the University of Florida in 1978. A special emphasis of this project has been the search for natural enemies. The search extended to South America which appears to be the native homeland for some of the exotic mole crickets which have become established in Florida. In Brazil and Uruguay, mole crickets infected with a steinernematid nematode were found, and the nematode appears to act as a biological control agent limiting development of large populations of mole crickets. In 1985, several isolates of the nematode from Uruguay were brought to the quarantine laboratory in Gainesville, Florida. Pathogenicity tests conducted on the mole crickets, _Scapteriscus vicinus_ and _S. acletus_, showed

that the isolate that was desinated No. 5 at a kill rate of 38 % was the most virulent. The virulence was improved to 100% kill (U. S. Patent Application No. 895,385 as "Modified Uruguayan Strain"). These nematodes were used in all experiments reported herein.

Materials and Methods

Experiment I: Pathogenicity Test in Petri Dishes on Four Species of Mole Crickets

Mole crickets used were the tawny mole cricket, *Scapteriscus vicinus*, the southern mole cricket, *S. acletus*, the short-wing mole cricket, *S. abbreviatus*, and the northern mole cricket, *Neocurtilla hexadactylla*.

Two pieces of No. 2 Whatman filter paper, 90 mm in diameter, were placed in a 100 x 15 mm petri dish, and 8,000 infective-stage juveniles in 2 ml water added. Five mole crickets, previously anesthetized by carbon dioxide (CO2), were placed in the dish. Dishes without nematodes served as controls. Each treatment was replicated 5 times, except for the short-wing mole cricket, for which only 4 crickets per petri dish and 4 replicates were used because of the limited number of specimens available, and the Northern mole crickets for which only seven specimens were available. These were placed in two petri dishes.

Each day, any dead mole crickets collected from the experiment were placed in vials, one per vial. After 2 days

the cadavers were dissected; if they contained developing nematodes, the mole crickets were considered to have been killed by the nematodes.

For all except the northern mole cricket, dead mole crickets in each petri dish were counted after 3 days, and Abbott's formula was used to calculate the corrected percentage of kill.

Experiment 2: Pathogenicity Tests in Buckets of Soil on two Species of Mole Crickets

The tests were conducted in buckets (11.4 liters) that were filled with soil to within 5 cm of the top. Mole crickets used were *Scapteriscus vicinus* and *S. acletus*. There were 4 treatments and 14 replicates for *S. vicinus* and 4 treatments and 10 replicates for *S. acletus*. The four treatments were:

1) One nematode-infected cricket in each bucket.

2) Two nematode-infected crickets in each bucket.

3) 12,000 infective-stage juveniles sprayed on the soil surface of each bucket.

4) A control containing mole crickets but no nematodes.

After the treatments were applied, 10 uninfected mole crickets were released into each bucket. After 10 days the number of dead and living mole crickets was determined. The living mole crickets were removed from the buckets, but the dead mole crickets were returned to the original buckets.

Again 10 uninfected mole crickets were released into each bucket, with the process repeated four times.

Experiment 3: Pathogenicity Test on Earth Worms

Earth worms are said to be beneficial and play a role in soil tilth and fertility. Since beneficial organisms should be preserved whenever possible, these experiments were conducted to determine whether the nematode has any detrimental effects on earthworms.

Eight thousand third-stage infective nematodes were mixed with organic soil contained in 237-ml (8 ounce) cups, and 10 earth worms were added. The controls contained earthworms but no nematodes. Treatments were replicated 5 times. The experiment was checked after 5 and 10 days.

To determine whether the nematode can develop in dead earth worms, five earth worms were cut in half and placed in 237 ml (8 ounce) cups filled 1/3 with organic soil. Eight thousand third-stage infective nematodes were released in each cup and the cup shaken well to mix the nematodes with the soil. The treatment was replicated 5 times. After 5 days, the dead bodies of the earth worms were collected, dissected in water and examined for nematodes.

A second experiment was conducted to confirm the results of the first, included two earth worms that were cut in half and placed in a 60 x 15 mm petri dish with organic soil with 8,000 third-stage infective nematodes added. The

treatment was replicated 5 times. The earth worm cadavers were dissected after 5 days and examined for nematodes.

Experiment 4: Host Range

A partial host range was determined by testing American cockroach (*Periplaneta americana*), fall army worm (*Spodoptera frugiperda*), field cricket (*Gryllus* sp.), granulate cut worm (*Feltia subterranea*) honey bee (*Apis mellifera*), house cricket (*Acheta domestica*), wax moth larva (*Galleria mellonella*), velvet bean caterpillar (*Anticarsia gemmatalis*), and two insect predators of mole crickets, *Megacephala virginica*, and *Pacimacus sublaevis*.

The tests were conducted in 100 x 15 mm petri dishes except for the honey bee. Two pieces of Whatman No. 2 filter paper were placed in a petri dish and 5,000 to 8,000 infective juveniles in 2 ml of water added. Five to ten insects, previously anesthetized by CO2, were placed in each dish, and the dishes stored in the dark. Controls contained the insects but no nematodes. Treatments were replicated 5 to 10 times. The number of dead insects was determined after three days.

Only 10 each of the mole cricket predators were available. These were placed in a 150 x 25 mm petri dish prepared as above. After 3 days the dead predatory insects were dissected to see if the nematodes were developing within the body.

The honey bee test was conducted in small cages containing 20 honey bees of different ages. In each cage was placed a petri dish containing 5,000 to 8,000 infective juveniles in a water-saturated cotton ball. The saturated cotton served as a source of water for the bees and assured that the bees came in contact with the nematodes when they visited the cotton to drink. The controls were prepared in the same way but without nematodes. All cages were placed in the dark to simulate the inside of a beehive. The treatment was replicated five times. The experiment was checked every day for 3 days to collect dead honey bees.

RESULTS AND DISCUSSION

Experiment 1: Pathogenicity Tests in Petri Dishes to Four Species of Mole Crickets

These experiments showed that *S. scapterisci* killed 100% of the tawny and southern mole crickets, 75% of the short-wing mole crickets and 71% of the northern mole crickets (Table 19).

Experiment 2: Pathogenicity Tests in Buckets of Soil to Mole Crickets

Kill of *S. vicinus* for all three treatments was significantly greater than that of the control for the first two releases (Table 20). Whether nematode-infected mole crickets or nematodes sprayed on the soil surface were used as the source of inoculum, the rate of kill was almost the same in the first release, but in the second release,

Table 19 : Effect of the nematode *Steinernema scapterisci* on different species of mole crickets.

Mole cricket	% kill
S.vicinus	100
S. acletus	100
S. abbreviatus	75
N. hexadactylla	71

Table 20 : Percentage of the mole crickets, *Scapteriscus vicinus* and *S. acletus*, killed by *Steinernema scapterisci*.

Treatment	Percentage of kill at each of 4 releases			
S. vicinus	1	2	3	4
1 infected cricket	37 b	53 b	32 ab	40 a
2 infected crickets	45 b	56 b	27 ab	41 a
12,000 juveniles	41 b	75 c	39 b	39 a
Control	11 a	17 a	17 a	33 a
S. acletus				
1 infected cricket	18 ab	30 b	18 a	46 a
2 infected crickets	10 a	27 b	11 a	34 a
12000 juveniles	25 b	51 c	18 a	26 a
Control	7 a	6 a	7 a	35 a

Numbers within a column followed by different letters are significant (P=0.05) based on T-test.

nematodes sprayed on the soil surface provided the greatest rate of kill.

The difference between the use of crickets and the use of nematodes as inoculum may be due to the fact that when infected mole crickets were used as inoculum the nematodes emerging from the cadavers were concentrated instead of being distributed throughout the volume of soil. The test showed that after the first treatment, the nematodes remained alive in the soil, either from the first inocula or from the nematodes produced in the mole cricket cadavers, and continued to kill mole crickets in subsequent releases.

These results show that spraying nematodes on the soil surface is an effective way of applying them if soil moisture and relative humidity is high enough to insure survival long enough for them to find a host. The treatments with nematode-infected mole crickets showed that when those mole crickets die, their bodies serve as a source of infective-stage juveniles which infect other mole crickets.

In the third and fourth releases of mole crickets into the same buckets of soil, the percentage of dead crickets in the controls increased to much higher levels than in the first and second releases, and the percentage of kill of mole crickets in nematode-treated buckets was reduced. Experiences in storing mole crickets in buckets of soil for future use showed that after prolonged storage many mole crickets were killed by the fungus Sorosporella sp. which

causes the mole cricket body to turn brick-red (Boucias, 1984). Also, recent studies showed that the mite *Rhizoglyphus* sp. is present on mole crickets. When the mites were placed in a petri dish containing *S. scapterisci*, the mites devoured large numbers of the nematode. It is probable that the mites were present in the experimental buckets and reduced the number of nematodes. Thus the fungus and the mite may have been contributing factors to the large number of dead mole crickets in the controls and to fewer deaths in the nematode treatments.

Kill of *S. acletus*, was less than of *S. vicinus* but the pattern was almost the same in that the percentage of kill was low in the first release, increased in the second, then decreased in the third and fourth releases. The percentage of mole crickets that died in both treatment and control increased, and reasons may be similar to those discussed for *S. vicinus*.

Experiment 3: Pathogenicity Test on Earth Worms

No earth worms died in the nematode treatments and three died in the same replicate in the controls after 10 days (Table 21). Thus *S. scapterisci* appears to cause no harm to earth worms.

In the experiment to see if the nematode develops in dead earth worms, 4 females and one male nematode were dissected from the earth worm segments in the first experiment. In the second experiment none was recovered. In

Table 21 : Effect of *Steinernema scapterisci* on earth worms.

Test	Number dead/total number After	
	5 days	10 days
Treatment	0/50	0/50
Control	1/50	3/50

both experiments large number of the bacterial-feeding diplogasterids and rhabditids were present. These results show that the nematode cannot reproduce in the dead bodies of earth worms.

Experiment 4: Host Range

While the house cricket was as good a host for *S. scapterisci* as was the mole cricket, the field cricket was not a very good host (Table 22).

The two predatory beetles tested were not hosts, and the other insects tested were poor hosts with no higher kill rate than 10%. This indicates that *S. scapterisci* is relatively host-specific, and thus poses little danger to non-target organisms.

Field Release

In addition to the experiments reported above, I have been part of a team of several researchers involved in the field release of *S. scapterisci*. The purpose of the release was to determine whether or not the nematode would survive in North Central Florida, and if so, what affect it would have on populations of mole crickets. Since others are involved in the research, only a summary of the study is presented here. The nematode was released in three field plots (50 m2 each) in Alachua County, Florida. Two of the releases were made in June 1985 and one in October 1985. The survival of the nematode and affect on populations of the mole crickets have been monitored since. Survival is

Table 22 : Percentage of different insects killed by *Steinernema scapterisci*.

Insect	% kill*
Mole crickets (*Scapteriscus* spp.)	100
House cricket (*Acheta domestica*)	100
Field crickets (*Gryllus* spp.)	22
Granulate cut worm (*Feltia subterranea*)	10
Wax moth larva (*Galleria mellonella*)	9
Fall army worm (*Spodoptera frugiperda*)	8
Cockroach (*Periplaneta americana*)	4
Honey bee (*Apis mellifera*)	3
Velvet bean caterpillar (*Anticarsia gemmatalis*)	3
Predatory beetle (*Megacephala virginica*)	0
Predatory beetle (*Pasimachus sublaevis*)	0

*The percentages in the table are corrected using Abbott's formula $(X-Y)/X \times 100$ where X = % insects alive in the controls; Y = % insects alive in the nematode treatment.

determined by finding the nematode in mole crickets trapped in pitfall traps or caught in sound traps during the flight season. Affects on the population of mole crickets is determined by continuously monitoring populations as measured by those captured in both pitfall traps and sound traps. Results are summarized as follows:

Both adults and nymphs of Scapteriscus acletus and S. vicinus are infected by the nematode. A summary of the data for 1985-1986 showed that of the adults and nymphs collected, 17.8% of the adults and 3.9% of the nymphs of S. acletus, and 4.9% of the adults and 1.3% of the nymphs of S. vicinus were infected. In general for both species of mole crickets, rates of infection were highest from February to August (5-24%) and lowest in January (0%).

Populations of mole crickets trapped in the release sites have declined about 95% in three years.

In addition to surviving and continuing to kill mole crickets more than three years after release, the nematode has been recovered outside the release areas. After one year, nematode-infected mole crickets were captured some 200 meters from the nearest release site, after two years, 2,500 meters, and after 3 years, 24,000 meters. Thus, we have demonstrated that S. scapterisci survives when released in the field in North Central Florida, that it kills mole crickets, reducing their populations, and that it is disseminated from a point of release.

CHAPTER 10
FACTORS INFLUENCING THE CONTROL OF MOLE CRICKETS BY STEINERNEMA SCAPTERISCI N.SP.

In host-parasite relationships, the host usually has some kind of mechanism to resist invasion by natural enemies. Contrarily, natural enemies have factors that help them overcome the resistance mechanisms of their hosts. One mechanism of resistance is that foreign bodies are encapsulated by the host they invade. When working with mole crickets and _Steinernema_ _scapterisci_, I found mites present on the mole crickets, and since mites have been shown to eat nematodes in the soil, I wondered if these mites may help reduce the number of nematodes attached to mole crickets and thus help protect the mole cricket from invasion. Whether nematode-infected mole crickets die in the soil or on the soil surface is another factor which is important in the biological control of mole crickets. The above considerations led to the studies reported herein.

Materials and Methods

Scapteriscus _acletus_ was used in all experiments.

Experiment 1

The purpose of this experiment was to see if nematodes would be encapsulated by the blood cells of mole crickets

when placed in a drop of their blood. Mole crickets were washed free of soil by letting them swim for several minutes in a container of tap water. Then the posterior portion of a mole cricket was dipped in 30% hydrogen peroxide for 2 minutes, the cercus cut at the tip with sterilized scissors, and a drop of blood which oozed from it was placed in a 35 x 10 mm petri dish. One third-stage infective juvenile nematode, which had been surface-sterilized in merthiolate for 2 hours then rinsed three times in deionized sterilized water, was transferred to the drop of blood. The nematode was observed continuously for about 10 minutes and photographs were made of each changing event. After the 10 minute observation period, the petri dish containing the nematode in a blood drop was placed in a larger (50 x 15 mm) petri dish containing a small amount of water and kept at 25 C. The experiment was replicated 10 times. Each replicate was observed every 15 minutes for 10 hours and again after 20 hours.

Experiment 2

The purpose of this experiment was to see if nematodes would be encapsulated when placed in the blood stream of mole crickets. A mole cricket, previously anesthetized by carbon dioxide, was dissected partially to remove the ventral cuticle and the body organs. Then an incision was made in the dorsal diaphragm and 5 nematodes were released at the site of the incision with some of them inside and

some outside the heart (the heart continued to beat during the experiment). After 10 minutes, the nematodes were removed and transferred to a drop of fresh blood and observed immediately under a compound microscope (immediate observation is important to insure that any changes observed occurred when the nematodes were in the blood stream of the mole cricket instead of in the drop of blood). The experiment was replicated 3 times.

Experiment 3

The purpose of this experiment was to see if a mite found on mole crickets would feed on infective-stage juveniles of *Steinernema scapterisci*. Mites were collected from mole crickets, and 10 of them placed in a petri dish containing nutrient agar. The agar plate was examined every day to see if the mites survived and reproduced. Infective-stage juveniles of the nematode were released in the agar dish containing mites and observed at intervals to see if the mites ate the nematodes.

Experiment 4

The purpose of this experiment was to observe the movement of mole crickets in soil and to determine if mole crickets infected with *S. scapterisci* would die underground or on the soil surface. Observation chambers were made from two pieces of plexiglass (1 m2) held 1 cm apart by a wooden frame which enclosed all four sides. The space between the two pieces of plexiglass was filled with a sandy soil and 8

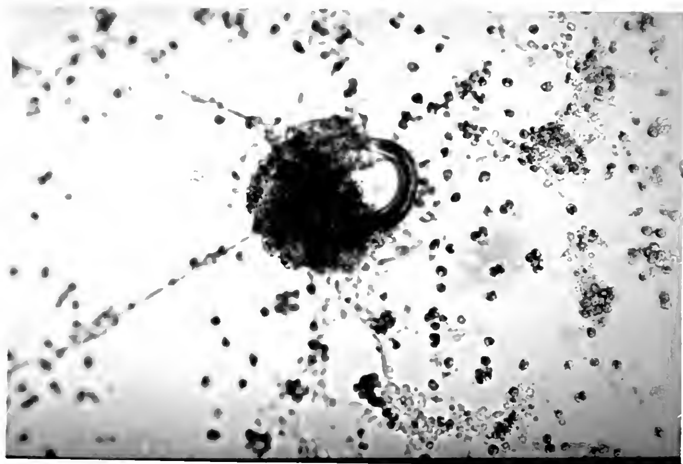
mole crickets infected with *S. scapterisci* were released in each chamber at the uppermost side of the upright chambers. The experiment was replicated 4 times. Movement of the mole crickets was observed, and the place where they died noted. After all mole crickets died, another set of uninfected mole crickets was released. The reason for releasing the second set of mole crickets was to see if they made new tunnels or used the tunnels already made by other mole crickets.

Results and Discussion

Experiment 1

Almost immediately after a third-stage juvenile nematode was placed in a drop of blood, the blood cells began to adhere to the head and tail regions. The more the nematode moved, the more cells attached until the nematode became totally encapsulated by blood cells (Fig. 18-20). The cells around the nematode appeared to be held together by a thread-like substance which formed. The process of encapsulation took no more than 10 minutes, and the capsule remained around the nematode for the 10-hour period of observation. However, at the 20-hour observation period, 6 of the 10 nematodes had escaped from the capsule and moved freely in the drop of blood. In one of the capsules from which a nematode escaped, its shed cuticular sheath remained.

Fig. 18. Encapsulation of the nematode by blood cells of the mole cricket. Top: Blood cells attached together as a band along the body of the nematode. Bottom: Blood cells attached on one end of the nematode.



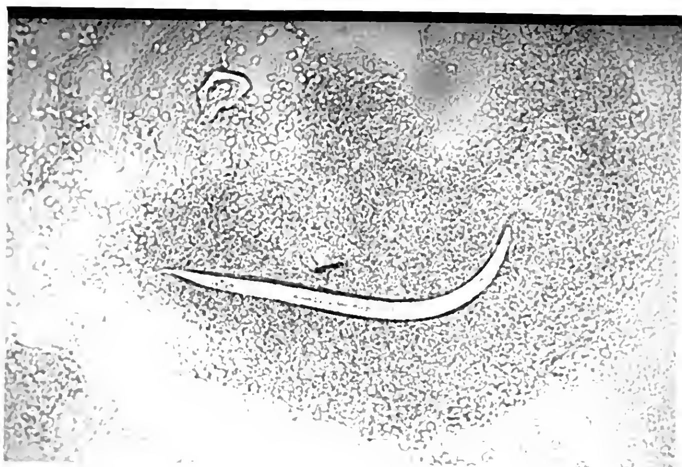


Fig. 19. Encapsulation of the nematode by blood cells of the mole cricket. Top: Head and tail of nematode encapsulated. Note that thread-like structures connect the cells and capsule. Bottom: Entire nematode in capsule (flattened by a cover slip on a glass slide).

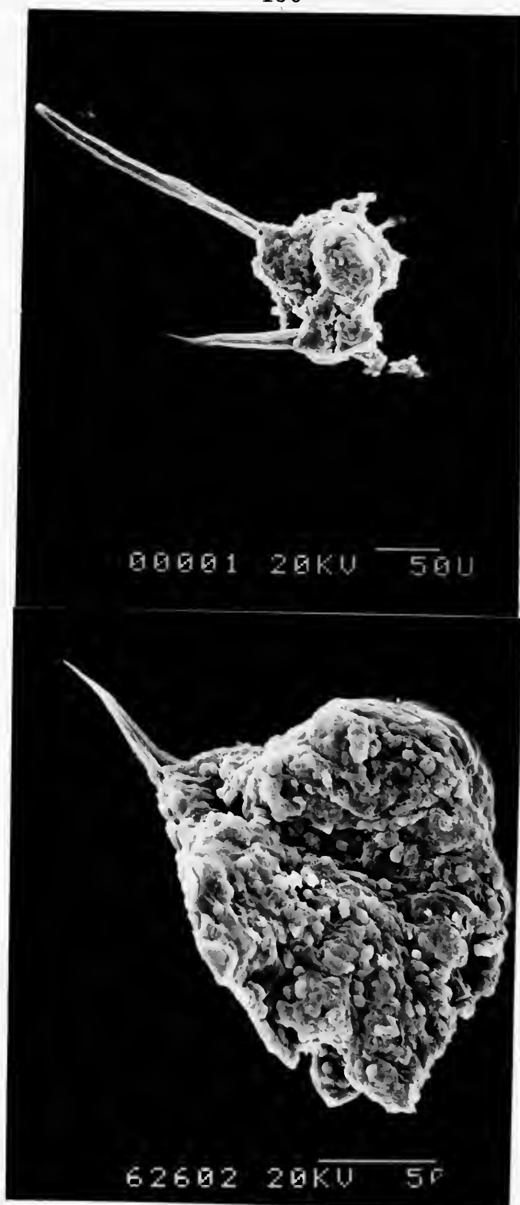


Fig. 20. SEM photographs of nematodes encapsulated by blood cells of mole cricket.

The nematodes may have been able to escape from the capsules, because, as the blood cells died, the cells lost the property that caused them to congregate around a nematode and encapsulate it. In the case of the exsheathed cuticle found encapsulated, it is likely that as the nematode exsheathed, it escaped rather easily from the relatively few cells around the anterior end of the old cuticle. Even though this may have happened in the drop of blood, it is unlikely that it would happen in the body cavity of a mole cricket, because, according to Poinar (1979), nematodes exsheath before or in the process of entering the body cavity of an insect.

At the termination of the above experiment, it was performed many more times to collect capsules for SEM observations. The capsules were collected, transferred to a small glass petri dish containing water, and then prepared for SEM observations according to the method of Stone and Green (1971). The SEM photographs (Fig. 20) confirmed observations made through the light microscope.

Experiment 2

The nematodes removed from the blood inside and outside the heart of a partially dissected mole cricket were partially or totally encapsulated by blood cells. The cell layer (capsule) was neither as thick nor as uniform as occurred in the drop of blood. The capsule was thicker at the head and tail regions than along the body. Many of the

nematodes were encapsulated only over a part of the body, but the experiment showed that encapsulation occurs in the blood stream of a mole cricket as well as in an isolated drop of blood.

Experiment 3

After 5 days in an agar dish, mites grew quickly and different life stages were observed. When third-stage juvenile nematodes were placed in the agar dish containing mites, the mites consumed the nematodes readily. Mites were observed to capture and consume a nematode in about 15 seconds (Fig. 21). About 50 mites in one petri dish consumed about 2,000 nematodes overnight - an average of 40 nematodes each. The mite was identified as Rhizoglyphus sp., family Acarididae, suborder Astigmata by Dr. Harvey L. Cromroy, Acarologist, Entomology and Nematology Department, University of Florida, Gainesville.

Experiment 4

Mole crickets observed in the plexiglass chambers moved as deep as 70 cm below the soil surface. All of the infected mole crickets died in their tunnels from 3 to 50 cm below the soil surface. When uninfected mole crickets were placed in the chambers after the nematode-infected ones died, they began to burrow into the soil, but when they ran across a tunnel formed by the previous inhabitants, they tended to move in the old tunnels instead of making new ones.

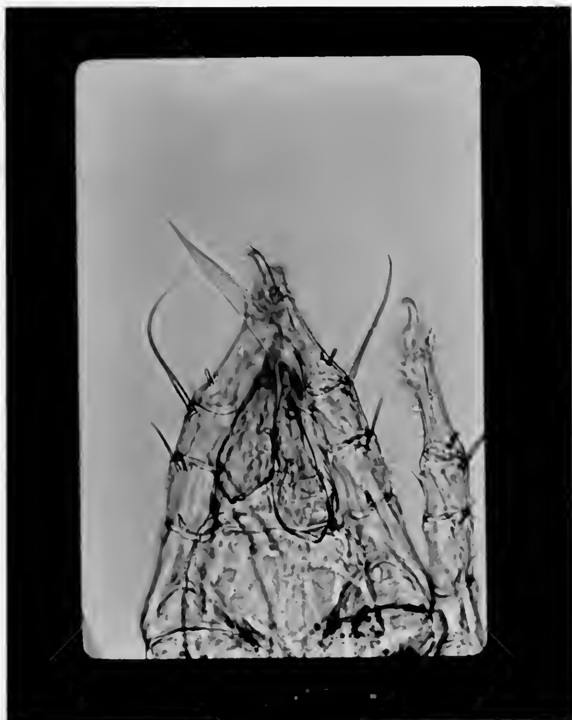


Fig. 21. Anterior part of the mite Rhizoglyphus sp. eating a nematode.

The above experiments indicate that there are several factors which influence the effectiveness of the nematode when used as a biological control agent of mole crickets. The fact that mole crickets can encapsulate invading nematodes increases their resistance to the nematode, but the number of nematodes that a mole cricket can encapsulate is not known. Neither do we know whether or not the bacterium is released from an encapsulated nematode.

The presence of nematode-eating mites on the body of mole crickets is another factor that can reduce the effectiveness of the nematode. More studies are needed to determine the frequency of occurrence of mites and their population density on mole crickets. Neither do we know whether nematodes constitute a significant part of the diet of the mites.

The fact that mole crickets died in the soil instead of on the surface, and that they used the tunnels made previously by other mole crickets are positive factors in biological control. Nematodes which emerge underground from mole cricket cadavers have a much better chance of surviving long enough to find a host than do those which emerge on the soil surface where they are subject to desiccation and ultraviolet radiation. Since infected mole crickets die in their tunnels and since other mole crickets use old tunnels, the chance of nematodes and mole crickets encountering each other are enhanced.

CHAPTER 11
NEMATODE PARASITES AND ASSOCIATES OF MOLE CRICKETS

Reports have been published on nematodes associated with mole crickets in Africa, Brazil, India and the U.S.S.R., but not in the United States. The purpose of the study reported in this chapter was to collect nematodes associated with mole crickets in Florida, USA and identify them to genus.

Literature Review

Nematode parasites of mole crickets were first reported by Sergiev (1923) in Russia when he described two species, *Thelastoma skrjabini* and *Oxyuris korsakowi* (later changed to *Gryllophyla skrjabini* and *Binema korsakowi* by Basir (1956). Travassos (1925a, 1925b) in Brazil described two new genera from mole crickets, the genus *Binema* with two species *Binema binema* and *Binema ornata* and the genus *Pulchrocephala* with the species *Pulchrocephala pulchrocephala*.

Seventeen years after Travassos' paper, Basir (1942) in India described three new genera of nematodes from mole crickets, *Gryllophyla*, *Mirzaiella* and *Talpicola*. Chakravarty (1943) described the genus *Indiana*. Basir (1948) described two more genera *Chitwoodiella* and

Cameronia. Rao (1958) reviewed all nematodes described from mole crickets and described 4 new genera, Isobinema, Pteronemella, Psilocephalla and Singhiella, all of them were from India. Kloss (1959) in Brazil, presented a classification scheme to the families, subfamilies and genera of nematodes associated with mole crickets. He redescribed all genera and type species and described a new genus, Schubartnema.

Bain (1965) found 6 species of nematodes associated with mole crickets in Madagascar. He identified them as Gryllophyla skrjabini Basir, 1942, Pteronemella macropapillata Rao, 1958, Indiana gryllotalpae Chakravarty 1943, Binema korsakovi Basir, 1942, Binema ornata Travassos, 1925 and Binema mirzaia Basir, 1942. After studying them further, he felt that they were different enough from the above species to merit subspecies rank. However, since he had only a limited number of specimens from different regions to compare, he preferred to refer to them as geographical subspecies.

Parveen (1982, 1984, 1985) described the following new species from mole crickets in India: Isobinema dimorphicauda Chitwoodiella neoformis, Cameronia klossi and Psilocephala nisari.

Materials And Methods

A casual survey was made in Florida from 1985 through

1987 to search for nematodes associated with mole crickets. Nematodes were collected from mole crickets in one of three ways. One method was to anesthetize live mole crickets with carbon dioxide, decapitate them, dissect out the digestive system, open it up in 2% sodium chloride and examine it for nematodes. Another method was to drown mole crickets in water in a vial and collect any nematodes that left the cadaver. A third method was to collect live mole crickets from the field, place them separately in vials until they died, and collect any nematodes which left the cadavers. Any newly dead mole crickets found in the field, also were stored in vials and observed for any nematodes which left the cadavers. Any nematodes found by any of these methods were counted, identified and preserved in fixative (propionic acid:formaldehyde, 1:4).

Mole crickets examined for nematodes were the imported species, *Scapteriscus abbreviatus*, *S. acletus* and *S. vicinus* and the native species, *Neocurtilla hexadactylla.*

Results And Discussion

Parasitic Nematodes

Only one genus, *Steinernema*, known to contain parasitic nematodes was found in mole crickets. Numerous specimens of one undescribed species were found in three specimens of *S. vicinus* of 1,256 specimens examined. Another undescribed species was found in 24 of 128 specimens of *N. hexadactylla*.

Eight hundred thirty one specimens of _S. acletus_ were examined and no parasitic nematodes were found.

Associated Nematodes

Nematodes reported in this category were found by dissecting and examining the hind gut and rectum of mole crickets. It is not known whether the genera found have any detrimental effects on their hosts, but it is generally accepted that they are commensals feeding on the gut contents rather than the tissues of the host. Listed below are the genera and approximate number of nematodes found in each species of mole cricket, and the number of mole crickets examined.

Scapteriscus abbreviatus

Only 10 of these mole crickets were examined.

Binema sp. (Fig. 22). An average of 15 specimens were found in each of the 10 mole crickets examined.

Scapteriscus acletus

One hundred and thirty of these mole crickets were examined.

Cameronia sp. (Fig. 23). A range of 1-15 specimens were found in each of 93 of the 130 mole crickets examined.

Cruznema sp. Only 1 male and 1 female were found in 1 of the 130 mole crickets examined.

Talpicola sp. (Fig. 24). Only 2 females were found in 1 of the 130 mole crickets examined.

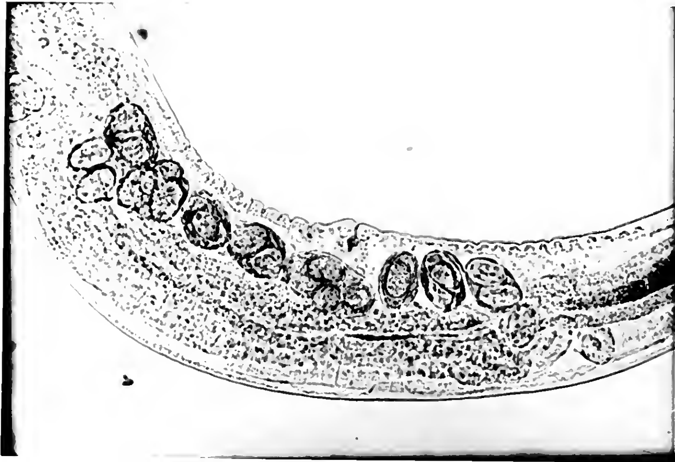


Fig. 22. The nematode Binema sp. found in the mole crickets Scapteriscus abreviatus and Neocurtilla hexadactylla. Top: Entire body of a female. Bottom: Mid-section of a female showing vulva and eggs.

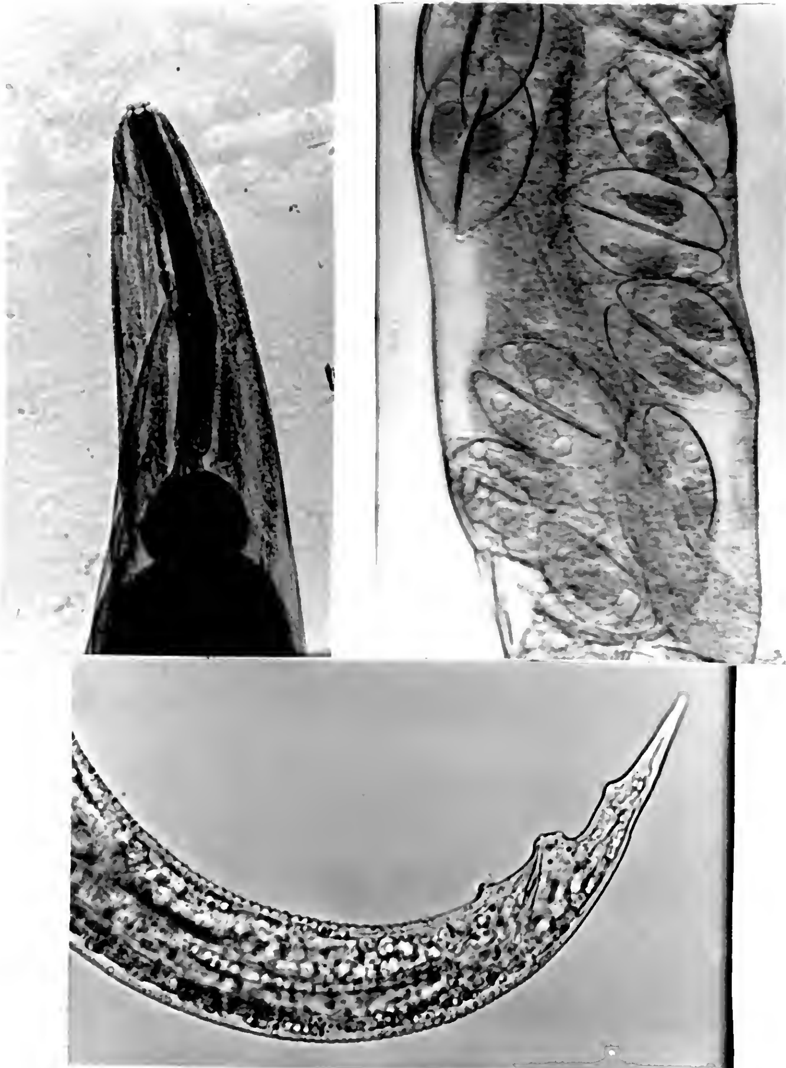


Fig. 23. The nematode Cameronia sp. found in the mole crickets Scapteriscus acletus, S. vicinus, Neocurtilla hexadactylla. Left: anterior part. Right: egg in the body. Bottom: male tail.

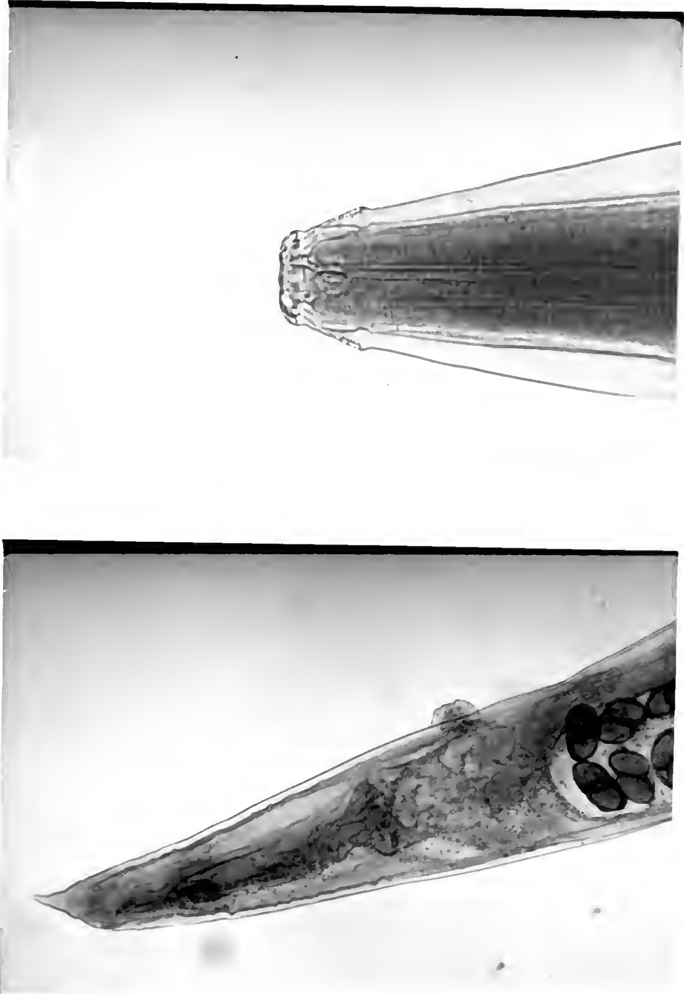


Fig. 24. The nematode <u>Talpicola</u> sp. found in the mole cricket <u>Scapteriscus</u> <u>acletus</u>. Top: anterior part. Bottom: Posterior part showing eggs in the body.

Scapteriscus vicinus

Thirty seven adults and 10 nymphs were examined.

Cameronia sp. A range of 1-9 specimens were found in each of 16 of the 37 adults, and in all 10 of the nymphal mole crickets examined.

Neocurtilla hexadactylla

Only 10 adults and 2 nymphs of this mole cricket were examined because population densities are low.

Binema sp. A range of 5-11 specimens were found in each of 6 of the 10 adult mole crickets examined.

Cameronia sp. A range of 4-8 specimens were found in each of the 2 nymphal mole crickets examined.

Pulchrocephala sp. (Fig. 25). A range of 5-11 specimens were found in each of the 10 adult mole crickets examined.

Bacteriophagus nematodes

One or more of the bacteriophagus nematodes in the genera Diplogaster, Pelodera, Mesorhabditis and Rhabditis was found on all dead mole crickets. Diplogaster was present on almost every cadaver, and Pelodera was present least often.

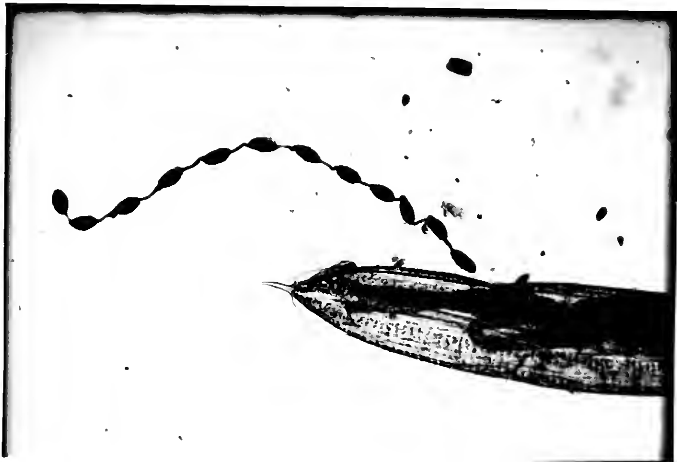


Fig. 25. The nematode Pulchrocephala sp. found in the mole cricket Neocurtilla hexadactylla. Top: Anterior part. Bottom: Posterior part of a female, showing eggs in a chain.

Conclusions

Parasitic nematodes in the genus Steinernema, occur very infrequently in mole crickets in the genus Scapteriscus. Since just 3 specimens of S. vicinus and no specimens of S. acletus were found to be infected with Steinernema sp. in the field during this survey, it seems likely that Steinernema spp. are not important natural enemies of those two mole crickets accidentally introduced into Florida. However, another Steinernema sp. was found with a rather high frequency of occurrence in Neocurtilla hexadactylla, a mole cricket native to Florida. This mole cricket is not very prevalent and it may be that the nematode is an important factor in keeping the populations below economically damaging levels.

Since one or more of the nematode genera Cameronia, Cruznema, Binema, Pulchrocephala and Talpicola were found in the rectum and hind gut of several mole crickets, and since populations of the mole crickets remain high, it is likely that the nematodes are at best weak parasites.

CHAPTER 12
SUMMARY AND CONCLUSIONS

A search for natural enemies of mole crickets in South America revealed a steinernematid nematode parasite which may reduce population density of the mole crickets. The nematode was brought to the University of Florida to determine its potential as a biological control agent of mole crickets imported accidentally from South America about the turn of the century. Studies have shown that the nematode does not fit nominal species, and it is described herein as *Steinernema scapterisci* n. sp. This nematode can be distinguished from other species of the genus as follows: female with thick cheilorhabdions, an excretory duct that forms a large doughnut-shaped structure near the base of the esophagus, and an epiptygma. The spicules of the male taper gradually to a narrow point, the shaft of the spicules bears a sheath, and the gubernaculum has a long neck, which is bent upward anteriorly. The body length of the juvenile is in the range of that of *S. carpocapsae*, but it differs by having a ratio of the measurement from the head to the excretory pore divided by the tail length, which is 0.73 for the new species and 0.60 for *S. carpocapsae*. It does not reproduce well, if at all, in *Galleria mellonella*, which is

a universal host for all other described species of the genus; and it does not cross hybridize with *S. carpocapsae* strain Breton.

Biology and Ecology

S. scapterisci enters the host through the mouth and spiracles, then either penetrates the digestive system or breaks through the tracheal system to enter the body cavity. The life cycle and sex ratio of the nematode is influenced by temperature. At 10 to 15 C the life cycle is not completed, at 20 C the cycle takes 12 days to complete, at 24 C 10 days and at 30 C 8 days. At 15-24 C the number of females in the population is greater than the number of males, but at 30 C the reverse occurs.

When released on the soil surface, the nematode moves down to seek out, enter and kill mole crickets at least 10 cm deep in the soil. When placed in the center of a column of soil, some of the nematodes move upward and some downward, with more moving downward.

S. scapterisci can survive in soil without a host for at least 10 weeks and retain its ability to kill mole crickets.

Some factors that may influence the effectiveness of the nematode to kill mole crickets are the encapsulation of the nematode by the blood cells of the mole cricket, and the presence of a nematode-eating mite, *Rhizoglyphus* sp., on the bodies of mole crickets. There are many other factors,

however, that increase the killing ability of the nematode. Infected mole crickets die in the soil, which increases the survival chances of the nematode. Since mole crickets in soil usually use tunnels made by other mole crickets chances are increased of a mole cricket being infected by nematodes that were produced in the cadaver of a mole cricket which died in its tunnel. Mole crickets can fly a long distance which increases the dissemination of the nematode.

The nematode can be produced *in vivo* in mole crickets and house crickets, and *in vitro* on liver medium.

Steinernema scapterisci as a Biological Control Agent of Mole Crickets

This new species appears to have a relatively narrow host range being quite specific to mole crickets and a few other Orthoptera. In a host range experiment with mole crickets, house crickets, field crickets, granulate cut worms, wax moth larvae, fall army worms, cockroaches, honey bee adults, velvet bean caterpillars, earth worms and two different beetles which prey on mole crickets, only mole crickets and house crickets were killed at a rate of 100%. Field crickets were killed at 22 %, and all others at 10% or less. None of the earth worms or the two predatory beetles were killed.

When released in the field 3 1/2 years ago in North Florida, the nematode became established, continues to kill mole crickets, and has become disseminated at least 24,000

meters from the original release sites.

Results of these investigations indicate that *S. scapterisci* is a good candidate for biological control of mole crickets.

Nematode Parasites and Associates of Mole Cricket

A survey of nematodes associated with mole crickets revealed the following nematodes:

Parasite

Steinernema spp.

Associates

Internal: *Cameronia* sp., *Cruznema* sp., *Binema* sp., *Pulchrocephala* sp., *Talpicola* sp. were found in the rectum and hind gut of one or more species of mole crickets.

External: *Diplogaster* sp., *Mesorhabditis* sp., *Pelodera* sp., and *Rhabditis* sp. were found on all species of mole crickets both living and dead.

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BIOGRAPHICAL SKETCH

Khuong B. Nguyen was born on July 07, 1941, in Ben Tre, Viet Nam. After graduating from high school in that country, he attended the Agricultural University in Saigon from 1963 to 1967 obtaining his engineering degree in agriculture. After his graduation, he worked for this university for 4 years as an instructor. In 1972, he came to the University of Florida and studied for his Master of Science degree in nematology, which he earned in 1974. He then returned to his country to work as an instructor and to conduct research in nematology. After more than six years in Viet Nam he returned to the University of Florida in 1981 where he was employed as a Biological Scientist under the direction of Dr. G. C. Smart, Jr. In 1985, he enrolled in the Graduate School, University of Florida, Entomology and Nematology Department to pursue the Doctor of Philosophy degree while retaining his position as a Biological Scientist.

He married Sam Ngoc Nguyen in 1971, and they have three children, Ngoc Bao (Marjorie), Anh Bao (Annie), and Minh Bao (Mindy).

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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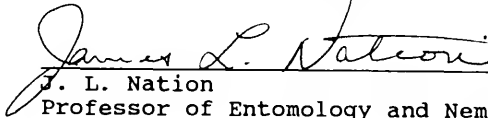

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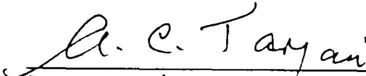

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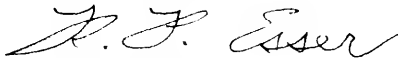

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