PLANT PARASITIC NEMATODES OF THE U.S. VIRGIN ISLANDS WITH THE DESCRIPTION, LIFE CYCLE AND MORPHOLOGY OF *Meloidogyne cruciani* n.sp. (NEMATODA: MELOIDOGYNIDAE) AND ITS INTERACTION WITH *Rotylenchulus reniformis*

BY

ROBERTO GARCIA MARTINEZ

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PARTIAL FULFILLMENT OF THE REQUIREMENTS
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1981

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

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By

Roberto Garcia Martinez

March, 1981

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

A nematode survey was conducted on St. Croix and St. Thomas, U.S. Virgin Islands. Soil samples were collected and processed from all agricultural and some non-agricultural lands. The nematodes recovered were preserved and brought to the University of Florida for identification. Eleven species in nine genera of plant parasitic nematodes were recovered: *Rotylenchulus reniformis*, *R. parvus*, *Helicotylenchus dihystera*, *H. multicinctus*, *Tylenchorhynchus mashhoodi*, *Xiphinema americanum*, *Pratylenchus pratensis*, *Criconemoides citri*, *Meloidogyne cruciani* n.sp. and *Hemicriconemoides cocophillus* on both islands, and *Hoplolaimus columbus* only on St. Croix.

Meloidogyne cruciani n.sp. differs from other species of the genus by having punctations around the anus of the female and by the larvae possessing extremely long tri-lobed esophageal glands. Females, males and larvae possess a uninucleate gland excretory system. Post-infection developmental stages of females of M. cruciani were dissected from tomato roots, killed and fixed in lactophenol-cotton blue and mounted in glycerin. Eleven days after inoculation, the procorpus, metacorpus and esophageal glands of the second stage larva were enlarged and prominent. Two small lobes were present just posterior to the metacorpus. The excretory duct of the second stage larva was directed anteriorly and seemingly connected to the cuticle of the third stage larva opposite the procorpus. The esophagus of the adult female appeared typical for the species, having a prominent procorpus, metacorpus and five nucleated lobes. The excretory pore was opposite the procorpus with the excretory duct directed posteriorly and terminating in a uninucleate gland.

Rotylenchulus reniformis was distributed throughout the two islands while M. cruciani was restricted in occurrence. The interaction between these two species was investigated in a clay vs. a sandy soil and at two temperatures. While R. reniformis populations developed better in clay soils, M. cruciani developed better in sandy soils. When M. cruciani was present, R. reniformis populations did not increase as much as when the latter

was alone. M. cruciani populations also were suppressed when R. reniformis was present. This indicates competition for available feeding sites. The soil texture of the U.S. Virgin Islands and the faster reproduction rate of R. reniformis may be reasons why R. reniformis was found throughout those islands, while M. cruciani was restricted in distribution.

CHAPTER I
SURVEY OF THE PLANT PARASITIC NEMATODES OF THE U.S. VIRGIN ISLANDS

Introduction

The U.S. Virgin Islands consists of three islands, St. Croix, St. Thomas and St. John. They are located between 17°40' and 18°24' latitude north, 64°30' and 65°04' longitude west and have an area of 207,199; 77,699; and 51,799 square kilometers, respectively. These islands are of volcanic origin with elevations up to 366 meters; they have a tropical trade wind climate, with an average temperature of 27 C and an average annual rainfall of 965 millimeters.

The tropical and subtropical regions of the world have climates that will permit year round production of a great variety of agricultural crops. These continuous crop productions favor nematode pests, since they are able to reproduce continuously, increasing their numbers and their damage. In 1978, I conducted a survey on St. Croix and St. Thomas Islands to determine the genera of plant parasitic nematodes present, their relative abundance and geographic distribution, and the crops with which they were associated. All cultivated areas and some non-cultivated areas were sampled on each island. St. John Island was not included

in the survey because most of the island is a national park with very little agriculture on the remainder.

Materials and Methods

Large maps of St. Croix and St. Thomas Islands were constructed with aerial photographs and the areas under cultivation and those areas that could be cultivated were marked on the maps for easy reference and location. The survey extended over a period of four months covering agricultural fields, home gardens, golf courses, nurseries, lawns and non-cultivated areas that had a potential for agricultural development. Soil subsamples were taken with a cone-type soil sampler (22) and combined in the field to form a composite sample that was used for the extraction of nematodes. The number of subsamples comprising a composite sample was determined by the size of the area and diversity of crops sampled, but ranged from three from about 3 m^2 garden plots to 10 from 2 hectare fields. The subsamples were mixed thoroughly and approximately 500 cm^3 of soil were placed in plastic bags, numbered, and the number recorded on the map of each island. The crop or plants from which a sample was taken also was recorded. A total of 80 composite soil samples were taken from the root zone of 30 different plants on St. Croix and 26 samples from the root zone of 16 different plants on St. Thomas. Samples were processed on St. Croix using a modification of the centrifugation-

flotation technique described by Caveness and Jensen (5). The nematodes recovered from 100 cm^3 of soil were killed in hot water, fixed and preserved in 4% formalin-2% glycerin, placed in vials and brought back to the Nematology Laboratory, University of Florida, Gainesville, Florida, where the plant parasitic nematodes were identified to genus and the number per sample determined.

Twenty adult nematodes of each genus were mounted in 2% formalin on glass slides and using an Olympus Vanox compound microscope with a Nomarski reflected light differential interference contrast attachment, measurements and other morphological characters of the nematodes were recorded and the species determined.

Results

There were nine genera and 11 species of plant parasitic nematodes recovered from soil associated with 30 different host plants (Table 1). Rotylenchulus reniformis Linford and Oliveira, 1940 (34), R. parvus (Williams, 1960) Sher, 1961 (48), Helicotylenchus dihystera (Cobb, 1893) Sher, 1961 (48), H. multicinctus (Cobb, 1893) Golden, 1956 (27), Tylenchorhynchus mashhoodi Siddiqui and Basir, 1959 (51), Xiphinema americanum Cobb, 1913 (14), Pratylenchus pratensis (de Man, 1880) Filipjev, 1936 (25), Criconemoides citri Steiner, 1949 (58) = Macroposthonia sphaerocephala (Taylor, 1936) De Grisse and Loof, 1965 (18), Meloidogyne

Table 1

Plants from which soil samples were taken through the root zones and nematodes recovered on St. Croix and St. Thomas, U.S. Virgin Islands

Host plants	Nematodes										
	Rotylenchulus reniformis	*Rotylenchulus parvus*	*Helicotylenchus dihystera*	*Helicotylenchus multicinctus*	*Tylenchorhynchus mashhoodi*	*Xiphinema americanum*	*Pratylenchus pratensis*	*Criconemoides citri*	*Meloidogyne arenaria* n.sp.	*Hemicriconemoides cocophilus*	*Hoplolaimus columbus*[1]
Bahia grass (*Paspalum notatum* Flügge)	+		+	+	+	+	+	+	+	+	
Banana (*Musa acuminata* Colla)	+	+	+	+	+	+	+	+	+	+	
Bean (*Phaseolus vulgaris* L.)	+		+		+	+			+	+	
Bermuda grass [*Cynodon dactylon* (L.) Pers.]	+		+		+	+	+	+	+	+	+
Citrus [*Citrus aurantiifolia* (Christm.) Swingle]	+		+		+	+	+	+	+	+	
Corn (*Zea mays* L.)	+	+	+		+	+		+	+	+	
Grapes (*Vitis rotundigolia* Michx.)	+					+	+		+		
Guayaba (*Psidium guajaba* L.)	+		+		+	+		+		+	
Guinea grass (*Panicum maximum* Jacq.)	+		+		+	+			+	+	
Hairy crabgrass [*Digitaria sanguinalis* (L.) Scop.]	+		+			+	+	+		+	
Hibiscus (*Hibiscus rosa-sinensis* L.)	+		+		+	+					
Hurricane grass [*Sporobolus cryptandrus* (Torr.) Gray]	+				+	+	+				
Mango (*Mangifera indica* L.)	+		+		+	+			+		

Table 1--continued

Host plants	Nematodes									
	Rotylenchulus reniformis	*Rotylenchulus parvus*	*Helicotylenchus dihystera*	*Helicotylenchus multicinctus*	*Tylenchorhynchus mashhoodi*	*Xiphinema americanum*	*Pratylenchus pratensis*	*Criconemoides citri*	*Meloidogyne cruciani* n.sp.	*Hemicriconemoides cocophillus*	*Hoplolaimus columbus*[1]
Okra [*Abelmoschus esculentus* (L.) Moench]	+	+	+	+	+	+	+	+	+	+	
Onion (*Allium cepa* L.)	+		+		+	+		+			
Pangola grass (*Digitaria decumbens* Stent)	+	+	+		+		+	+			
Papaya (*Carica papaya* L.)	+	+	+	+	+	+	+	+	+		
Pepper (*Capsicum annuum* L.)	+		+		+			+			
Pineapple [*Ananas comosus* (L.) Merrill]	+	+	+	+	+	+		+			
Spanish bayonet (*Yucca aloifolia* L.)	+		+		+	+	+	+			
Sorghum [*Sorghum bicolor* (L.) Moench]	+	+	+		+	+	+	+	+	+	
Squash (*Cucurbita pepo* L.)	+		+		+	+		+		+	
Sugar apple (*Annona squamosa* L.)	+		+		+	+		+		+	
Sugar cane (*Saccharum officinarum* L.)	+	+	+			+	+	+	+		
Sour sop (*Annona muricata* L.)	+		+		+	+		+		+	
Sweet potato [*Ipomoea batatas* (L.) Lam.]	+		+			+	+	+			
Tomato (*Lycopersicon esculentum* Mill.)	+		+		+	+	+	+	+	+	
Watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai]	+	+	+	+		+					
Yam (*Dioscorea alata* L.)	+		+	+	+	+	+	+		+	
Yuca (*Manihot esculenta* Crantz)	+		+		+	+		+		+	

[1] Hoplolaimus columbus recovered from St. Croix only.

cruciani n.sp. and Hemicriconemoides cocophillus (Loos, 1949) Chitwood and Birchfield, 1957 (8) were found on both St. Croix and St. Thomas (Tables 2 and 3). Hoplolaimus columbus Sher, 1963 (49) were recovered only on St. Croix from the three golf courses on the island (Table 2).

Rotylenchulus reniformis were present in every sample taken from both islands while R. parvus was present in 8 and 5% respectively, of the samples taken from St. Croix and St. Thomas (Tables 2 and 3, Figs. 1 and 10). Helicotylenchus dihystera, H. multicinctus, Tylenchorhynchus mashhoodi, Xiphinema americanum and Pratylenchus pratensis were recovered from 70, 4, 65, 58 and 58%, respectively, of the samples taken from St. Croix (Table 2, Figs. 2-5), and from 74, 3, 45, 16 and 13%, respectively, of the samples taken from St. Thomas (Table 3, Figs. 11-14).

Meloidogyne, an economically important nematode genus which is widespread in most tropical regions (9), was found in only 11 samples from St. Croix (Table 2, Fig. 7) and five samples from St. Thomas (Table 3, Fig. 15). It was found only in home gardens, but not in all home gardens sampled.

Criconemoides citri and Hemicriconemoides cocophillus were recovered in relatively low numbers from both St. Croix and St. Thomas. Criconemoides was found in 15% of the samples from St. Croix (Table 2, Fig. 6) and only in 8% of the samples from St. Thomas (Table 3, Fig. 17). Hemicriconemoides was recovered from 9% of the samples from St. Croix (Table 2, Fig. 8) and in 11% of the samples from St. Thomas (Table 3, Fig. 16).

Table 2

Incidence and percentage frequency of occurrence of plant parasitic nematodes in St. Croix

Nematode genera	Incidence	% Frequency of occurrence
Rotylenchulus reniformis	80	100
Rotylenchulus parvus	6	8
Helicotylenchus dihystera	56	70
Helicotylenchus multicinctus	3	4
Tylenchorhynchus mashhoodi	52	65
Xiphinema americanum	46	58
Pratylenchus pratensis	46	58
Criconemoides citri	12	15
Meloidogyne cruciani n.sp.	11	14
Hemicriconemoides cocophillus	7	9
Hoplolaimus columbus	4	5

Table 3

Incidence and percentage frequency of occurrence of plant parasitic nematodes in St. Thomas

Nematode genera	Incidence	% Frequency of occurrence
Rotylenchulus reniformis	38	100
Rotylenchulus parvus	2	5
Helicotylenchus dihystera	28	74
Helicotylenchus multicinctus	1	3
Tylenchorhynchus mashhoodi	17	45
Xiphinema americanum	6	16
Pratylenchus pratensis	5	13
Meloidogyne cruciani n.sp.	5	13
Hemicriconemoides cocophillus	4	11
Criconemoides citri	3	8

Discussion

Rotylenchulus reniformis was found to be more widely distributed than R. parvus. Dasgupta et al. (17) gave an extensive list of plants with which R. reniformis has been found associated and the localities from which they were reported. They found R. reniformis associated with banana, citrus, corn, papaya, sugarcane, sweet potato and tomato. These crops also were found to be common hosts of R. reniformis in St. Croix and St. Thomas (Table 1).

Helicotylenchus dihystera was found to be the most widely distributed species of the genus. Sher (50) gave an extensive list of plants with which H. dihystera has been found associated and the localities from which they were reported. He found H. dihystera associated with banana, citrus, corn, mango, onion, papaya, pineapple and sugarcane. These crops also were found to be common hosts of H. dihystera in St. Croix and St. Thomas (Table 1).

The occurrence of Hoplolaimus columbus on golf courses associated with bermuda grass indicates that these genera of nematodes might have been introduced to St. Croix with the turfgrass.

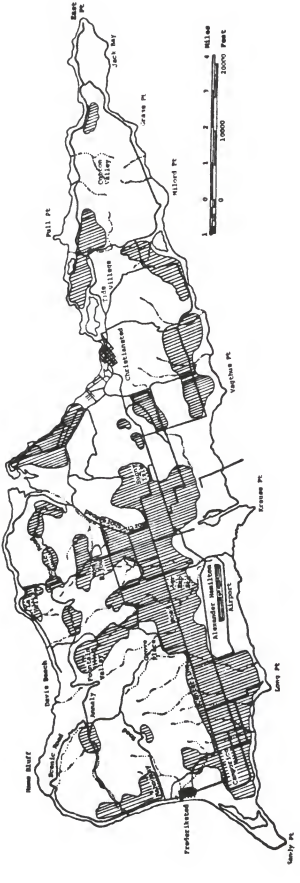
During this survey, a new species of the genus Meloidogyne was discovered. The complete description and a host differential test of the new species, named Meloidogyne cruciani, are reported in Chapter II.

The extensive distribution of R. reniformis throughout these islands indicates favorable environmental conditions for the growth, development and reproduction of these nematodes. The widespread distribution of this nematode may be due to the extensive and intensive production of sugarcane on both islands in the past (63). Contrary to this, the low recovery rate of M. cruciani indicates the presence of biotic or abiotic factors that are restricting further establishment of these nematodes. With this in mind, experiments were initiated to determine whether certain factors favored Rotylenchulus reniformis and were detrimental to Meloidogyne cruciani. These experiments are reported in Chapter V.

Figure 1

St. Croix, U.S. Virgin Islands.

Shading indicates total area sampled.
Rotylenchulus spp. were found in all of the above areas.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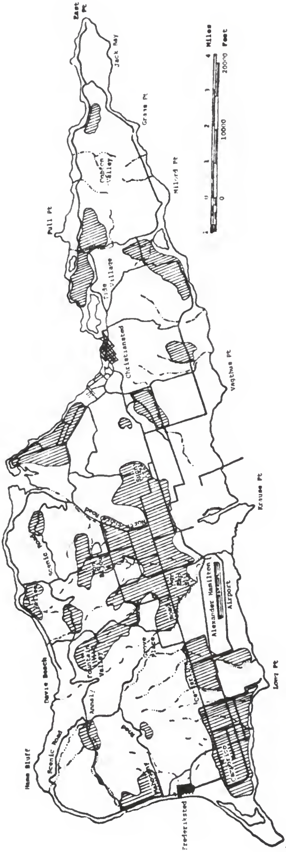
Grass Pt
Milord Pt
Cotton Valley
Tide Village
Christiansted
Vagthus Pt
Krause Pt
Alexander Hamilton Airport
Long Pt
Davis Beach
Ham Bluff
Scenic Road
Annaly
Frederiksted
Sandy Pt
1 0 1 2 3 4 Miles
0 10000 20000 Feet

Figure 2

St. Croix, U.S. Virgin Islands.

Shaded areas were found infested
by Helicotylenchus spp.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Jack Bay
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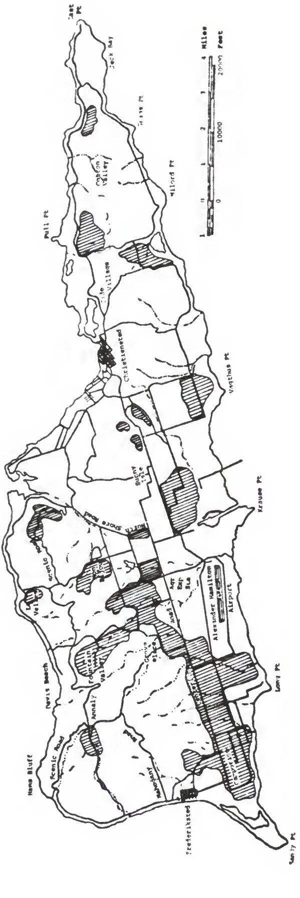
Pull Pt
Christiansted
Vagthus Pt
Krause Pt
Alexander Hamilton
Airport
Long Pt
Davis Beach
Ham Bluff
Scenic Road
Frederiksted
Sandy Pt
Miles
Feet
0
10000
20000

Figure 3

St. Croix, U.S. Virgin Islands.

Shaded areas were found infested
by *Tylenchorhynchus mashhoodi*.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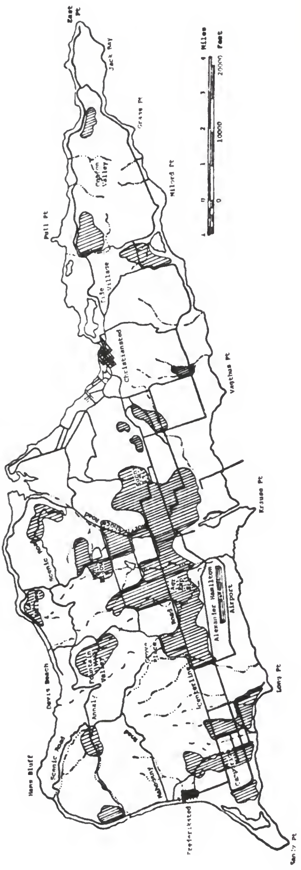
Milord Pt
Village
Vagthus Pt
Krause Pt
North Shore Road
Scenic Road
Alexander Hamilton
Airport
Fountain Valley
Davis Beach
Annaly
Long Pt
Miles
Feet
10000
0

Figure 4

St. Croix, U.S. Virgin Islands.

Shaded areas were found infested by *Xiphinema americanum*.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Milord Pt
Christiansted
Vagthus Pt
Krause Pt
Alexander Hamilton
Airport
Long Pt
Sandy Pt
Frederiksted
Hams Bluff
Scenic Road
Davis Beach
Annaly
Mahogany
Centerline
4 Miles
20000 Feet
10000
0

Figure 5

St. Croix, U.S. Virgin Islands.

Shaded areas were found infested by *Pratylenchus pratensis*.

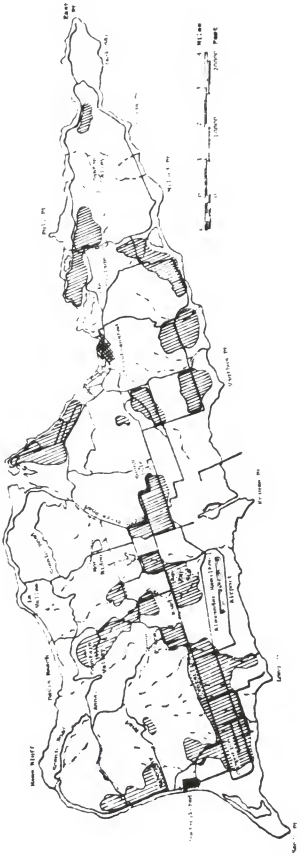
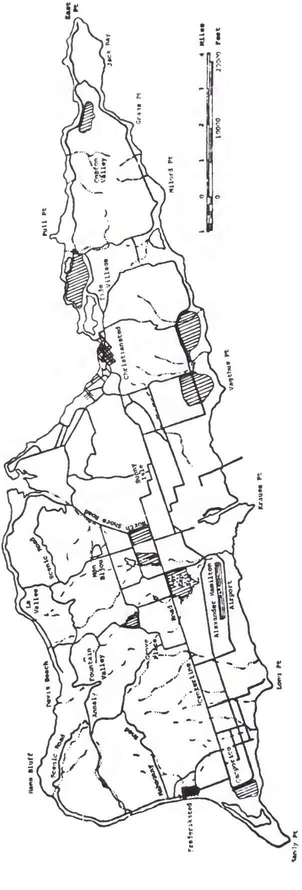


Figure 6

St. Croix, U.S. Virgin Islands.

Shaded areas were found infested by *Criconemoides citri*.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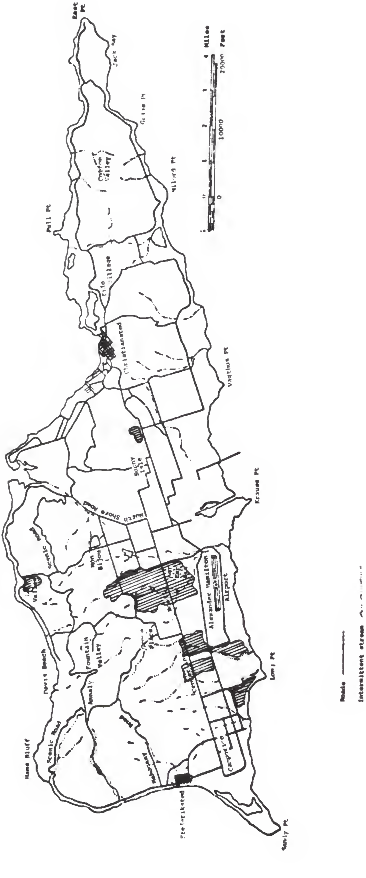
Sunny Isle
North Shore Road
Krause Pt
Scenic Road
Mon Bijou
La Vallee
Alexander Hamilton Airport
Centerline Road
Grove Place
Fountain Valley
Davis Beach
Annaly
Long Pt
Ham Bluff
Scenic Road
Mahogany Road
Frederiksted
Sandy Pt
4 Miles
Feet
10000
20000

Figure 7

St. Croix, U.S. Virgin Islands.

Shaded areas were found infested
by *Meloidogyne cruciani* n.sp.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Milord Pt
Pull Pt
Christiansted
Vagthus Pt
North Shore Road
Sunny Isle
Krause Pt
Scenic Road
Mon Bijou
Alexander Hamilton Airport
Fountain Valley
Davis Beach
Annaly
Hams Bluff
Scenic Road
Mahogany Road
Frederiksted
Sandy Pt
0 1 2 3 4 Miles
0 10000 20000 Feet
Roads
Intermittent stream

Figure 8

St. Croix, U.S. Virgin Islands.

Shaded areas were found infested
by Hemicriconemoides cocophillus.

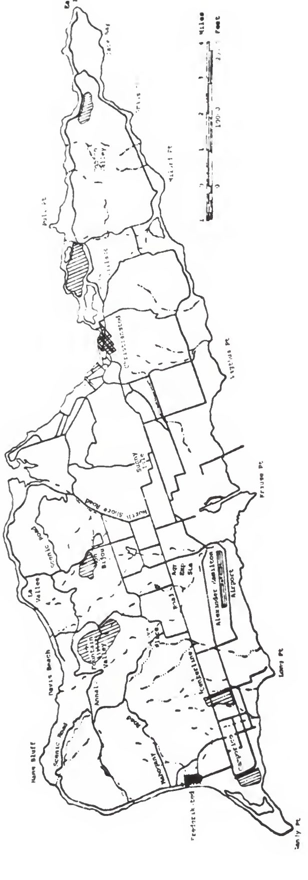
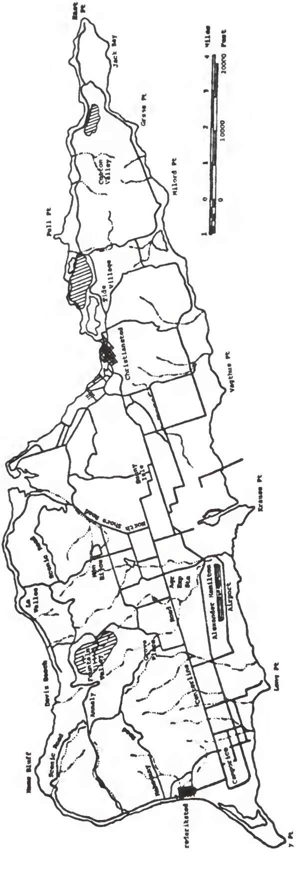

East Pt
Scenic Road
La Vallee
Davis Beach
North Shore Road
Sunny Isle
Agr Exp Sta
Alexander Hamilton Airport
Centerline
Mahogany
Krause Pt
Long Pt
Feet

Figure 9

St. Croix, U.S. Virgin Islands.

Shaded areas were found infested by *Hoplolaimus columbus*.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Pull Pt
Tide Village
Christiansted
Vagthus Pt
Sunny Isle
Krause Pt
North Shore Road
Scenic Road
Mon Bijou
La Vallee
Alexander Hamilton
Airport
Davis Beach
Annaly
Centerline
Long Pt
Ham Bluff
Scenic Road
Frederiksted
1 0 1 2 3 4 Miles
0 10000 20000 Feet

Figure 10

St. Thomas, U.S. Virgin Islands.

Shading indicates total area sampled.
Rotylenchulus spp. were found in all of the above areas.

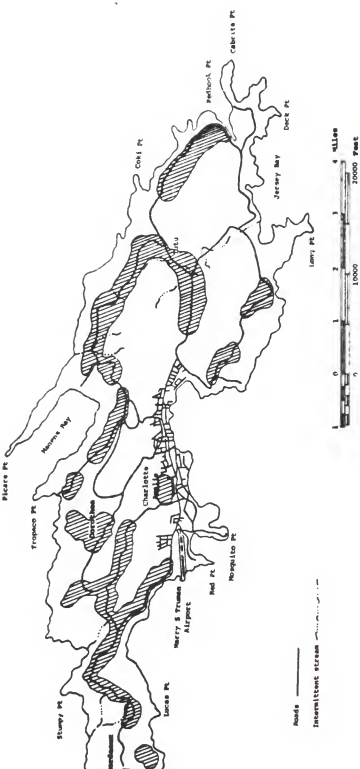

Point Pleasure
Tropaco Pt
Nazareth Bay
Charlotte
Red Pt
Mongoose Pt
Harry S Truman Airport
Lucas Pt
Stumpy Pt
Rotary Pt
Little St Thomas
Coki Pt
Pettlock Pt
Cabrita Pt
Jersey Bay
Duck Pt
Long Pt
Roads
Intermittent stream
0
1
2
3
4 Miles
10000
20000 Feet

Figure 11

St. Thomas, U.S. Virgin Islands.

Shaded areas were found infested by Helicotylenchus spp.

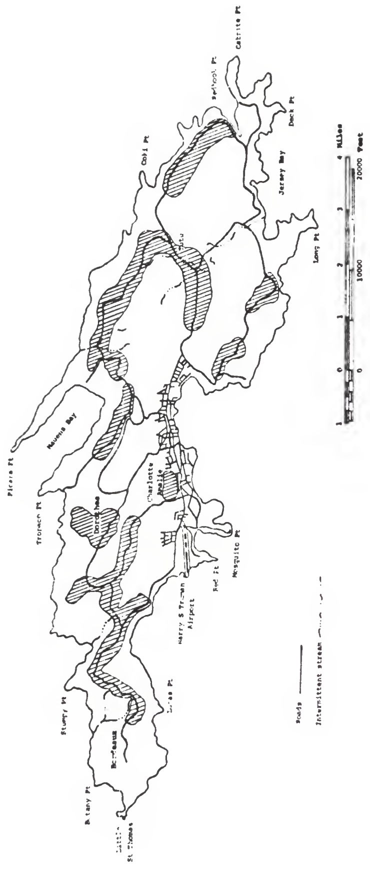
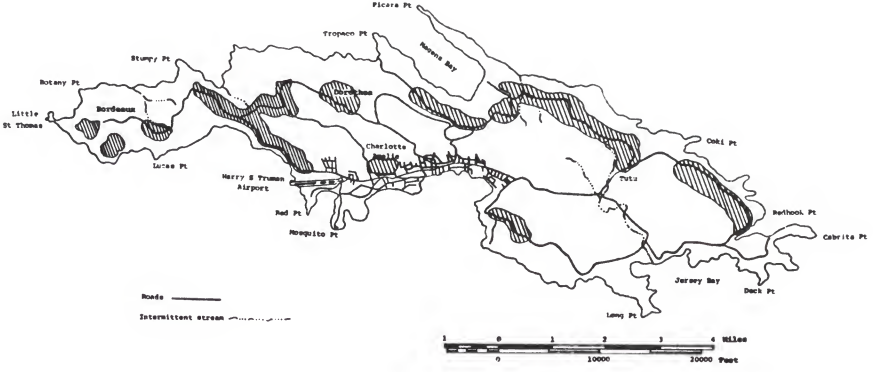

Cabrita Pt
Dock Pt
Jersey Bay
Coki Pt
Tutu
Long Pt
Magens Bay
Picara Pt
Dorothea
Charlotte Amalie
Mosquito Pt
Red Pt
Harry S Truman Airport
Stumpy Pt
Bordeaux
Botany Pt
St Thomas
Scale
Intermittent stream
1 0 1 2 3 4 Miles
0 10000 20000 Feet

Figure 12

St. Thomas, U.S. Virgin Islands.

Shaded areas were found infested by *Tylenchorhynchus mashhoodi*.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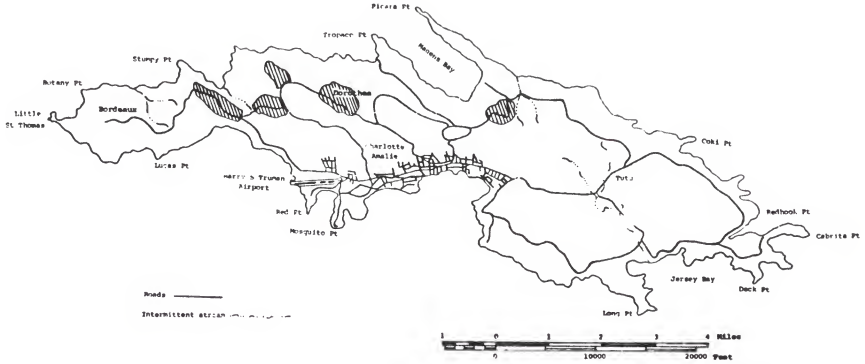
Stumpy Pt
Botany Pt
Little
St Thomas
Bordeaux
Lucas Pt
Harry S Truman
Airport
Red Pt
Mosquito Pt
Charlotte
Amalie
Magens Bay
Coki Pt
Tutu
Redhook Pt
Cabrita Pt
Jersey Bay
Buck Pt
Long Pt
Roads
Intermittent stream
1 0 1 2 3 4 Miles
0 10000 20000 Feet

Figure 13

St. Thomas, U.S. Virgin Islands.

Shaded areas were found infested
by Xiphinema americanum.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Tropaco Pt
Stumpy Pt
Mahoma Bay
Botany Pt
Bordeaux
Little
St Thomas
Coki Pt
Charlotte
Amalie
Lucas Pt
Tutu
Redhook Pt
Red Pt
Mosquito Pt
Cabrite Pt
Jersey Bay
Dock Pt
Long Pt
Roads
1 0 1 2 3 4 Miles
0 10000 20000 Feet

Figure 14

St. Thomas, U.S. Virgin Islands.

Shaded areas were found infested
by Pratylenchus pratensis

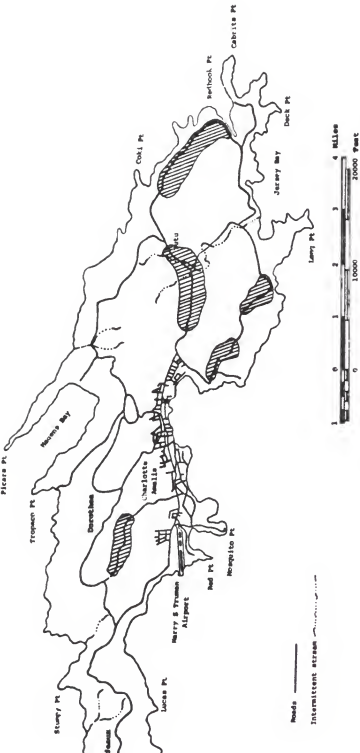

Cabrite Pt
Redhook Pt
Coal Pt
Duck Pt
Jersey Bay
Larry Pt
Picara Pt
Magens Bay
Tropaco Pt
Charlotte Amalie
Mangolia Pt
Red Pt
Harry S Truman Airport
Stumpy Pt
Lucas Pt
Northside
Botany Pt
Little St. Thomas
Roads
Intermittent stream
Miles
Feet
10000
20000

Figure 15

St. Thomas, U.S. Virgin Islands.

Shaded areas were found infested by *Meloidogyne cruciani* n.sp.

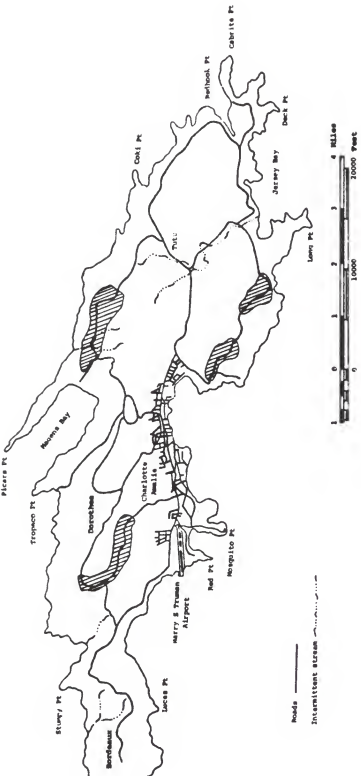

Picara Pt
Magens Bay
Coki Pt
Pothook Pt
Cabrita Pt
Deck Pt
Jersey Bay
Long Pt
Tropaco Pt
Donkeybay
Charlotte Amalie
Harry S Truman Airport
Red Pt
Mosquito Pk
Stumpy Pt
Bordeaux
Lovos Pt
Rotary Pt
Little St Thomas
Roads
Intermittent stream
1 0 1 2 3 4 Miles
0 10000 20000 Feet

Figure 16

St. Thomas, U.S. Virgin Islands.

Shaded areas were found infested
by Hemicriconemoides cocophillus.

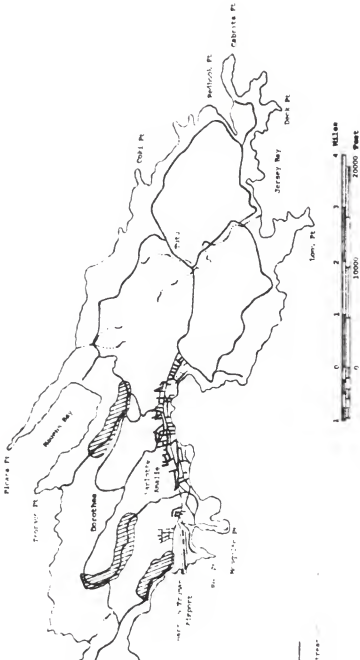
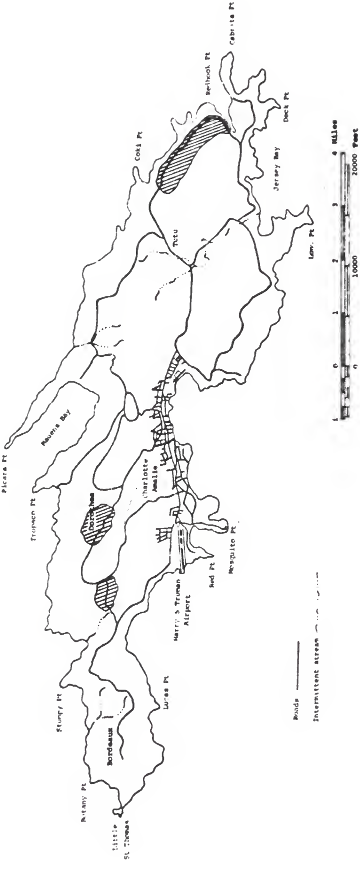

Point
Transmission grease
0
1
2
3
4 Miles
0
5000
10000
20000 Feet

Figure 17

St. Thomas, U.S. Virgin Islands.

Shaded areas were found infested by *Criconemoides citri*.


Cabrita Pt
Redhook Pt
Coki Pt
Dock Pt
Jersey Bay
Tutu
Long Pt
Magens Bay
Picara Pt
Charlotte
Amalie
Mosquito Pt
Red Pt
Harry S Truman
Airport
Stumpy Pt
Bordeaux
Botany Pt
Little
St Thomas
Roads
Intermittent stream
0 1 2 3 4 Miles
0 10000 20000 Feet

CHAPTER II
Meloidogyne cruciani n.sp., A ROOT-KNOT NEMATODE FROM ST. CROIX, U.S. VIRGIN ISLANDS

Introduction

During a survey to determine the plant-parasitic nematode complement in the U.S. Virgin Islands, an undescribed species of *Meloidogyne* was recovered from tomato roots. Specimens of the nematode were brought to the University of Florida and populations established on 'Rutgers' tomato plants. Single egg mass isolations were made to establish a population originating from a single female. One isolate was selected and used for the taxonomic and morphologic studies reported herein.

Materials and Methods

Egg masses dislodged from 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) roots were teased apart to expose the eggs. These eggs were placed in water and left overnight at 28-29 C. Newly-hatched second stage larvae were mounted in 2% formalin on slides. The specimens were measured and drawn immediately using a camera lucida (23). Males were dissected from large root galls and from egg masses and prepared as above. Females were dissected from roots, placed in 2% formalin and their posterior ends were excised. These sections

were transferred to 45% lactic acid, cleaned of debris, trimmed to the perineal region and mounted in glycerin for observation. Anterior ends of females were prepared by fixing and staining whole females in lactophenol-cotton blue (21), after which the anterior ends were excised and mounted in glycerin. All type material was prepared using the same technique as for the female anterior ends.

Photomicrographs of perineal patterns were made with an automatic 35-mm camera using an interference contrast system (Nomarski) attached to a compound microscope. In making the scanning electron microscope (SEM) micrographs, females were killed and fixed in 2.5% glutaraldehyde solution with phosphate buffer for 12 hours, transferred to 2% osmium tetroxide for 24 hours at 8 C, dehydrated in an ethanol series (10-100%) for 15 minutes in each concentration, dried to critical point, coated with gold, and examined with a SEM.

A differential host test (59) was conducted by transplanting seedlings of the following plants into steam-sterilized, sandy soil contained in 10-cm clay pots and inoculating the plants with 5000 eggs per pot: sweet corn (Zea mays L. var. rugosa Bonaf. cv 'Silver Queen'), cotton Gossypium hirsutum L. cv 'Delta pine'), peanut (Arachis hypogaea L. cv 'Florunner'), pepper (Capsicum annuum L. cv 'California Wonder'), strawberry (Fragaria ananassa Duch. cv 'Albritton' and 'Florida 90'), sweet potato [Ipomoea batatas (L.) Poir. cv 'Allgold' and 'Porto Rico'], tobacco

(Nicotiana tabacum L. cv 'NC 95'), watermelon [Citrullus lanatus (Thunb.) Matsum and Nakai cv 'Charleston Gray'], and tomato (Lycopersicon esculentum Mill. cv 'Rutgers'). Each treatment was replicated five times. Inoculum was prepared by shaking egg masses for two minutes in a 1% NaOCl solution to obtain a suspension of eggs and larvae (31). Sixty days after inoculation at a greenhouse temperature of 22-26 C, roots were removed from soil, washed and examined for galls and egg masses.

Results

Meloidogyne cruciani n.sp.

Females. (21): Length: 426.0-1121.8 µm (mean 787.5 µm, 95% confidence interval ± 86.2); body width: 315.7-770.0 µm (505.1 µm ± 61.5); a: 1.2-2.1 (1.5 ± 0.1); stylet length: 11.4-16.2 µm (14.2 µm ± 0.6); stylet knob height: 2.1-2.9 µm (2.4 m ± 0.1); stylet knob width: 3.8-5.1 µm (4.5 µm ± 0.2); dorsal gland orifice to base of stylet knobs: 3.2-5.1 µm (3.9 µm ± 0.2); excretory pore to anterior end: 25.7-45.1 µm (32.2 µm ± 2.2); center of median bulb to anterior end: 65.4-93.3 µm (78.3 µm ± 3.4); vulva slit length: 20.0-25.7 µm (23.2 µm ± 0.7); vulva slit to anus: 15.9-20.3 µm (18.1 µm ± 0.6); interphasmidial distance: 25.7-36.8 µm (30.3 µm ± 1.2).

Holotype. (female): Length: 949.9 µm; body width 536.4 µm; a: 1.8; stylet length: 12.7 µm; stylet knob

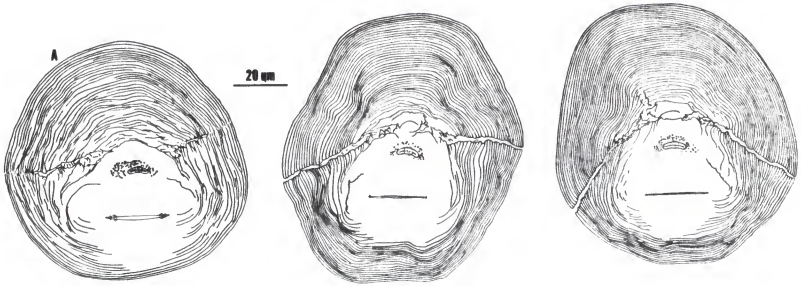
height: 2.7 µm; stylet knob width: 4.4 µm; dorsal gland orifice to base of stylet knobs: 4.1 µm; excretory pore to anterial end: 31.7 µm; center of median bulb to anterior end: 93.3 µm; vulva slit length: 23.8 µm; vulva slit to anus: 20.0 µm; interphasmidial distance: 31.7 µm.

Description. Females white, pear-shaped to globular, without prominent posterior protuberance (Fig. 18B). Neck tapers, curving gently (Figs. 18B, 19A). Head offset slightly with labial cap and one or two cephalic annules. Labial or cephalic sensillae not observed. Amphidial openings oval, inconspicuous, obscured by labial cap. Cephalic framework with lateral sectors larger than ventral or dorsal sectors. Stylet robust, with rounded knobs. Excretory pore about one stylet length from base of stylet knobs, variable in exact position; excretory duct seen easily throughout anterior region terminating in a uninucleate gland (Fig. 28C). Esophageal lumen between base of stylet knobs and valve of median bulb well sclerotized with an average width of 2.4 µm. Prominent metacorpus with strongly sclerotized valve. Esophageal glands consisting of five distinct nucleated lobes (Fig. 19A, C). One lobe always larger than the other four. Perineal pattern (Figs. 18A, 20-23) with subcuticular punctations (stippling) almost surrounding the anus on lateral and posterior sides (Figs. 18A, 20-23). Striae deep, wavy, sometimes broken. Lateral field fairly deep with distinct phasmids. Phasmidial ducts often visible. Vulva lips faintly serrated, margins with very fine striae.

Figure 18

A) Drawings of perineal patterns of *Meloidogyne cruciani* n.sp.

B) Outlines of females in varying sizes and shapes.


A
20 µm

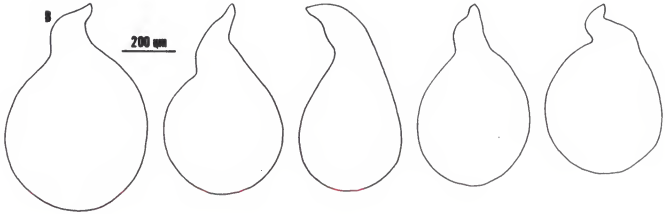
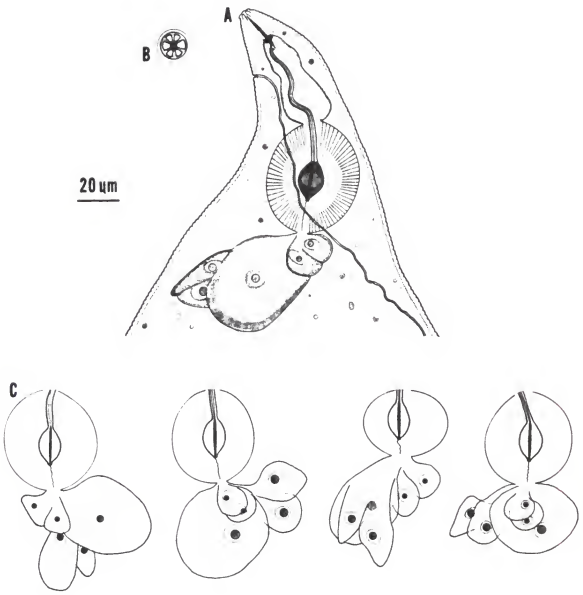

B
200 µm

Figure 19

A) Anterior region of female.

B) Face view showing cephalic framework.

C) Variations in size and shape of esophageal glands.

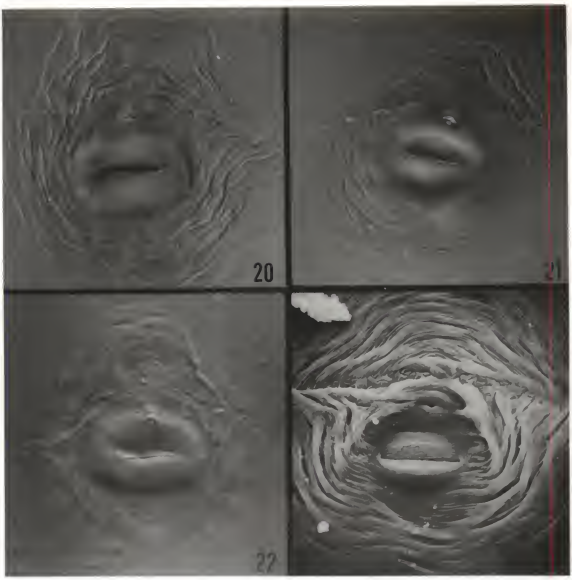

A
B
20 µm
C

Figures 20-23

Perineal patterns of *Meloidogyne cruciana* n.sp.

20-22) Photomicrographs showing subcuticular punctations.

23) Scanning electron micrograph.


20
21
22

Males. (25): Length: 1160-1620 µm (mean 1378.8 µm, 95% confidence interval ± 52.3); body width: 23.2-46.3 µm (33.8 µm ± 2.4); stylet length: 19.4-24.1 µm (22.0 µm ± 0.5); stylet base to anterior end: 21.2-26.3 µm (24.4 µm ± 0.5); stylet knob height: 2.7-3.8 µm (3.3 µm ± 0.1); stylet knob width: 4.1-6.0 µm (5.2 µm ± 0.2); dorsal esophageal gland orifice to base of stylet knobs: 3.2-7.9 µm (4.9 µm ± 0.4); center of metacorpus valve to anterior end: 66.7-118.1 µm (88.9 µm ± 4.1); excretory pore to head end: 127.3-189.5 µm (149.2 µm ± 5.8); anterior end of testis to posterior end: 489.4-1127.0 µm (823.4 µm ± 60.1); spicule length: 28.7-38.0 µm (31.3 µm ± 0.9); gubernaculum: 6.7-11.1 µm (8.8 µm ± 0.5); phasmid to posterior end: 8.3-22.9 µm (16.7 µm ± 1.4); a: 31.9-71.2 (43.2 ± 3.7); c: 89.2-238.2 (132.6 ± 13.1); c': 0.3-0.7 (0.5 ± 0.1); 0 (distance from dorsal esophageal gland orifice to base of stylet knobs, expressed as % of stylet length): 14.3-36.8 (22.6 ± 1.9); T% (distance from anterior end of testis to posterior end, expressed as % of body length): 39.8-79.7 (60.1 ± 4.7).

Allotype. (male): Length: 1440 µm; body width: 29.9 µm; stylet length: 22.8 µm; stylet base to anterior end: 24.8 µm; stylet knob height: 3.5 µm; stylet knob width: 4.8 µm; dorsal esophageal gland orifice to base of stylet knobs: 4.9 µm; center of metacorpus valve to anterior end: 97.4 µm; excretory pore to anterior end: 139.2 µm; anterior end of testis to posterior end: 933.8 µm; spicule

length: 28.7 µm; gubernaculum: 8.1 µm; phasmid to posterior end: 18.4 µm; a: 48.0; c: 151.3; c': 0.4; 0: 21.5; T% 64.9%.

Description. Body long, vermiform, tapering at both ends (Fig. 24A). Head offset with two annules and distinct head cap. Labial or cephalic sensillae not observed. Cephalic framework with lateral sectors larger than ventral or dorsal sectors; ends of framework slightly forked when viewed laterally. Stylet robust, with rounded knobs. Amphidial glands prominent posterior to stylet knobs. Cephalids not observed. Metacorpus poorly developed, slightly larger than procorpus with well sclerotized valve. Esophageal glands consisting of three distinct nucleated lobes. Excretory pore prominent (139.1 µm from anterior end). Hemizonid 3.5 annules anterior to excretory pore. Excretory duct long, terminating in a sac-like gland. Lateral fields begin anteriorly as two lateral lines opposite stylet knobs and become four near metacorpus. There is anastamosis of the lateral lines in the posterior end of the body. Testis predominantly one, two occasionally. Spicules slightly arcuate, their tips rounded (Fig. 24D). Gubernaculum with fine serrations on the cuneus. Phasmids 5.9 µm anterior to cloaca.

Second stage larvae. (20): Length: 418.6-479.8 µm (mean 435.3 µm, 95% confidence interval $\pm$ 8.7 µm); width: 14.6-18.7 µm (17.2 µm $\pm$ 0.5); stylet length: 9.8-12.1 µm

Figure 24

Male of Meloidogyne cruciani n.sp.

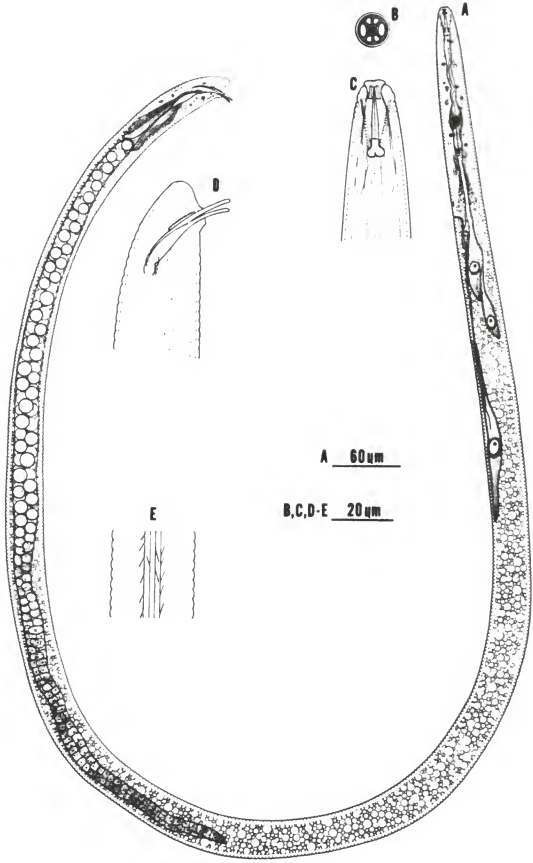
A) Entire specimen (curvature of specimen for convenience in illustrating).

B) Face view showing cephalic framework.

C) Anterior portion.

D) Tail (lateral view).

E) Lateral field.


B
A
C
D
A 60µm
B,C,D-E 20µm
E

(10.6 μm ± 0.2); stylet base to anterior end: 14.3-17.6 μm (15.2 μm ± 0.4); stylet knob height: 1.1-1.6 μm (1.4 μm ± 0.1); stylet knob width: 2.1-2.7 μm (2.3 μm ± 0.1); dorsal esophageal gland orifice to base of stylet knobs: 3.2-3.9 μm (3.5 μm ± 0.1); center of metacorpus valve to anterior end: 51.7-61.9 μm (57.8 μm ± 1.4); distance from cardia to anterior end: 69.5-86.7 μm (76.3 μm ± 2.1); distance from posterior end of glands to anterior end: 190.4-250.4 μm (202.0 μm ± 6.4); excretory pore to anterior end: 74.6-103.2 μm (88.1 μm ± 3.4); genital primordium to posterior end: 148.2-175.5 μm (163.5 μm ± 4.3); phasmid to posterior end: 34.9-43.8 μm (39.2 μm ± 1.2); tail length (anus to posterior end): 41.3-51.7 μm (46.6 μm ± 1.3); tail width (at anus): 9.8-13.0 μm (11.2 μm ± 0.4); a: 22.9-29.8 (25.4 ± 0.8); b: 5.0-7.1 (5.8 ± 0.2); b': 1.8-2.4 (2.2 ± 0.1); c: 8.6-10.5 (9.4 ± 0.3); c': 3.7-4.6 (4.2 ± 0.1); 0 (distance from dorsal esophageal gland orifice to base of stylet knobs, expressed as % of stylet length): 28.9-37.9 (33.1 ± 0.9).

Description. Body vermiform, tapering slightly anteriorly and much more posteriorly (Fig. 25A). Head offset slightly with one annule; head cap with weakly visible cephalic framework with lateral sectors larger than ventral or dorsal sectors. Labial or cephalic sensillae not observed. Stylet robust, rounded knobs slanting posteriorly. Cephalids not observed. Amphidial glands prominent, posterior to stylet knobs. Esophagus extremely long, from

Figure 25

Larvae of Meloidogyne cruciani n.sp.

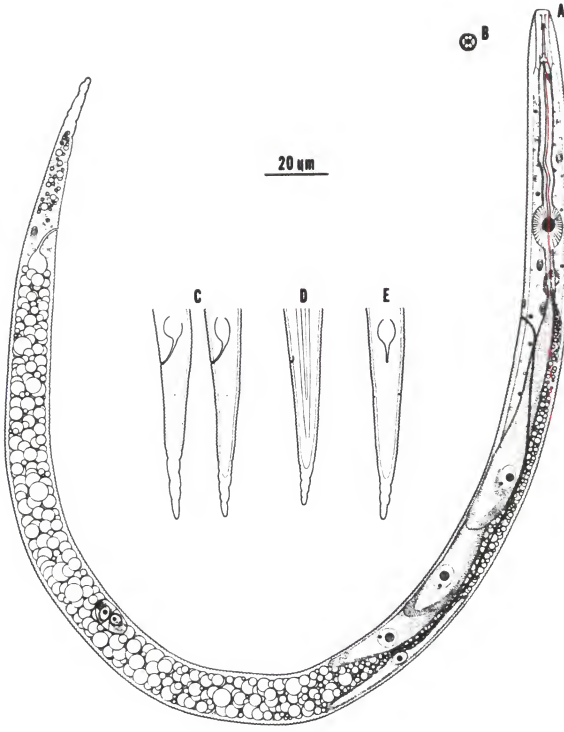
A) Entire specimen (curvature of specimen for convenience in illustrating).

B) Face view showing cephalic framework.

C) Tails (lateral view).

D) Lateral field at tail region.

E) Tail (ventral view).


A
B
20 µm
C
D
E

tip of head to posterior extremity of glands averaging 46.4% of the total body length. Metacorpus well developed with well sclerotized valve. Esophageal glands contained in three distinct nucleated lobes, each with a smaller satellite nuclear body. Excretory pore position variable; always posterior to esophago-intestinal valve. Hemizonid 2-4 annules anterior to excretory pore. Excretory duct long, terminating in a sac-like gland. Lateral fields originate as two lines one stylet length posterior to base of knobs, becoming four near metacorpus. Two inner lateral lines terminate at phasmids and outer two terminate posteriorly. Genital primordium in the two-cell stage, seen easily. Rectum dilated. Phasmids small and difficult to see; one anal body width posterior to level of anus. Tail gradually tapering, with annules disappearing near hyaline area. Tail terminus notched, with smooth, bluntly conoid tip.

Holotype. (whole female): Originally recovered in tomato roots from the Agricultural Community Gardens, St. Croix, U.S. Virgin Islands in September 1977. It was grown subsequently on 'Rutgers' tomato in an isolated greenhouse. [Slide T-333t, USDA Nematode Collection, (USDANC)], Beltsville, Maryland, USA.

Allotype. (male): Isolated from 'Rutgers' tomato roots cultured in a greenhouse and established from type locality. Slide T-334t, USDANC, Beltsville, Maryland, USA.

Paratypes. Females (whole mounts, perineal patterns), males and larvae. Same data as allotype. USDANC, Beltsville, Maryland; Laboratorie voor Nematologie, Binnehaven, Wageningen, The Netherlands; Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; Canadian National Collection of Nematodes, Ottawa, Canada; Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida; and Entomology and Nematology Department, University of Florida, Gainesville, Florida.

Diagnosis. Meloidogyne cruciani differs from other published descriptions of species of the genus by its perineal pattern with punctations around the anus. The only other species with punctations in the perineal area is M. hapla Chitwood, 1949 (8) but the punctations of M. hapla are around the tail terminus. The larvae of M. cruciani differ from most other species of the genus in possessing extremely long, distinctly tri-lobed esophageal glands.

Other morphological characters found in this species and not reported for other members of the genus are: 1) The presence of a guiding ring around the stylet shaft of males and larvae (Figs. 24C, 25A). (These rings are often associated with the order Dorylaimida, class Adenophorea (1), but have not been reported in the class Secernentea.) 2) Three distinct lobes of the esophagi of males and larvae of this species (Figs. 24A, 25A). Original descriptions and illustrations of 29 other species of this genus examined

report one single esophageal lobe with three nuclei (23), but post-infection studies of Meloidogyne naasi (52) and M. incognita (61) illustrate the parasitic second stage larvae as having 3-lobed esophageal glands. 3) Inside each nucleus, a small chromocenter is present beside the nucleolus in each lobe of the esophagi of the second stage larvae (Figs. 25A). This has not been reported for other species of this genus. 4) The esophageal glands of the females (Fig. 19A, C) consist of five separate and distinct lobes. 6) A uninucleate gland (renette-type) excretory system. 7) The gubernaculum of the males (Fig. 24D) has fine serrations on the cuneus; this condition is also present in males of Verutus volvingentis (23).

In the host-differential test, peanut, strawberry, and cotton were not hosts. Tomato, watermelon, sweet potato, tobacco, corn and pepper were hosts. Based on these results, Meloidogyne cruciani seems to have a similar host range as that of M. incognita Race 2. One other plant, cabbage (Brassica oleracea L. cv 'Greenback') also was found to be a suitable host.

Type host and type habitat. tomato, Lycopersicon esculentum Mill., roots

Type locality. Agricultural Community Gardens, College of the U.S. Virgin Islands, St. Croix, U.S. Virgin Islands

Discussion

Perineal patterns of Meloidogyne cruciani differ from perineal patterns of other species of the genus in having punctations around the anus. Some variations of the perineal patterns (Figs. 18A, 20-23) resemble perineal patterns of Meloidogyne javanica Chitwood, 1949 (7) in their pronounced lateral fields; however, length of larvae easily separate the two species.

Andrássy (1) stated that as far as the triradial symmetry of the esophagus is concerned, the 5-gland condition in nematodes represents a more advanced evolutionary stage. This condition would certainly aid the feeding process of highly advanced sedentary parasites such as members of the genus Meloidogyne. I have found that females of M. incognita and M. arenaria also have 5-lobed esophageal glands.

Andrássy (1) further stated that the uninucleate gland (renette-type) excretory system is considered typical of Torquentia and Penetrantia and does not occur in the Secernentea (1), but this excretory system which was first called a renette cell-type by Cobb (13), has been shown in other genera of the Secernentea (11, 12, 14-16, 41).

The development of five glands in the esophagi of females of Meloidogyne cruciani and the type of excretory system are reported in Chapter III.

CHAPTER III
POST-INFECTION DEVELOPMENT OF FEMALES OF *Meloidogyne cruciani* n.sp.

Introduction

In most genera of nematodes, the adult females resemble the larval stages in many of their morphological characters. In the genus *Meloidogyne*, the developing larvae undergo a series of morphological changes and the mature females appear very different than the second stage larvae. The former have a saccate pear-shaped body, a very large robust stylet, very prominent esophageal glands and an enlarged metacorpus. The larvae are filiform with a small stylet and small esophageal glands.

Before Chitwood (7) placed what was known as *Heterodera marioni* into the genus *Meloidogyne* and created four species and one subspecies, the life cycle and morphological studies of unknown species of *Meloidogyne* (*Heterodera marioni*) were carried out by Nagakura (37) and Christie and Cobb (10). Nagakura described three molts taking place within the plant roots and the existence of third and fourth larval stages. Christie and Cobb disagreed with Nagakura, stating there is no third larval stage and that the fourth is just theoretical.

More recently, Bird (3), Triantaphyllou and Hirschmann (61) and Siddiqui and Taylor (52) studied the morphology and developmental stages of females of M. javanica, M. incognita and M. nassi, respectively. They all agree with the early studies of Nagakura and report the presence of third and fourth larval stages.

The work reported herein is an attempt to determine the initiation, development and formation of morphological structures in the developmental stages of Meloidogyne cruciani n.sp. with emphasis on the esophageal region, excretory system and genital region.

Materials and Methods

Seeds of 'Rutgers' tomato (Lycopersicon esculentum Mill.) which is susceptible to M. cruciani, were germinated in sterile vermiculite at 30 C in a water bath. When seedlings were two weeks old, they were removed from the vermiculite, their roots washed and trimmed to 1 cm in length and the seedlings placed in sterile water for two days. These seedlings were exposed to freshly hatched second stage larvae for 24 hours. (Larvae were obtained by placing egg masses in distilled water at 30 C overnight.) After the 24 hour exposure period, roots of the seedlings were washed to remove any larvae that had not penetrated and the seedlings were transplanted into sterile white sand in 33 cm^3 plastic cups with a small hole punched in the bottom for drainage.

The experiment was carried out at a constant temperature of 28 ± 1 C in a temperature controlled growth chamber. The seedlings were fertilized twice a week with 2 ml of a 390 ppm Nutrisol^{R} (12-10-20) nutrient solution. Every 24 hours, five seedlings were removed and the roots washed and fixed using De Guiran's (29) method.

After fixation, the developmental stages were recovered by dissecting them from roots. The nematodes then were mounted in glycerin on glass slides, a cover glass applied and sealed with Zut.

Morphological observations and drawings were made with the aid of a camera lucida attached to an Olympus Vanox compound microscope equipped with a Nomarski reflected light differential interference contrast attachment.

Results

For the first 7-8 days, the post-infective second stage larva underwent very few changes but generally decreased in length when compared with the pre-infective stage and increased in diameter (Fig. 26A, B). After 7-8 days, the genital primordium in the four-cell stage began to migrate posteriorly, the esophageal glands became shorter in length but larger in diameter and volume, and there was a slight increase in the width of the metacorpus and an increase in the body width around the esophageal region (Fig. 26B). Inside the nuclear envelopes of the

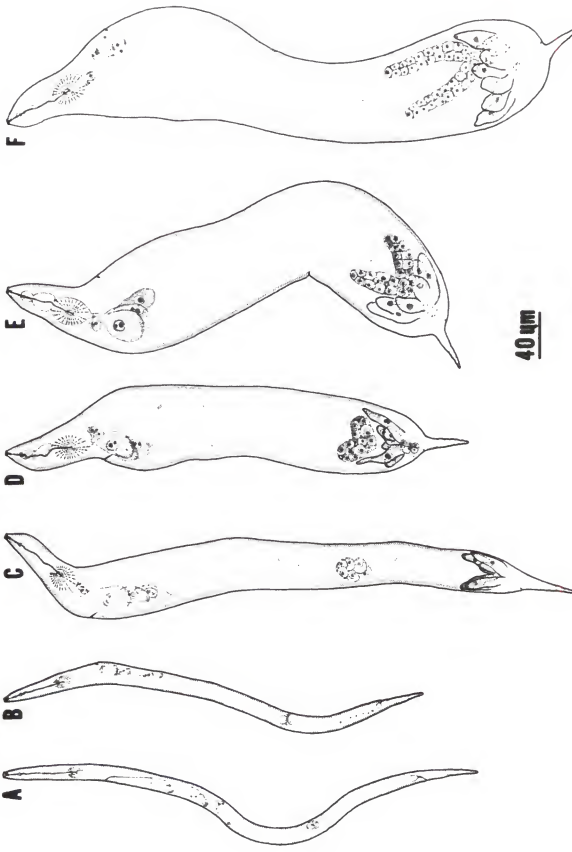
Figure 26

Second stage larvae of *Meloidogyne cruciani* n.sp.

A) Infective second stage larva.

B) Post-infective sexually undifferentiated second stage larva.

C-F) Sexually differentiated, female second stage larvae.


A
B
C
D
E
F
40 µm

esophageal glands, the nucleoli and the chromocenters enlarged. At this point, the rectal glands were not seen, but six irregularly arranged nuclei were present near the anal region (Fig. 26B).

Eleven days after inoculation the second stage larva had enlarged considerably in size (Fig. 26C). At this time, the esophagus had decreased in length, but increased in width and volume; the procorpus and metacorpus were enlarged and more prominent. For the first time, two small lobes were seen connected to the esophagus just posterior to the metacorpus; chromocenters of the esophageal glands were very prominent. The dorsal gland had enlarged more than the subventral esophageal glands. The genital primordium was in the six-cell stage and had enlarged and migrated further posteriorly. The rectal glands were visible in this stage. Triantaphyllou and Hirschmann (61) and Siddiqui and Taylor (52) refer to this stage as the "developed but sexually undifferentiated" second stage larva based on the genital primordium. The genital primordium had not reached the V-shape characteristic of the developing female gonad, but the presence of rectal glands indicated the sex as a developing female.

The "developed and sexually differentiated" second stage larva continued to enlarge. By fourteen days after inoculation (Fig. 26D) the procorpus, metacorpus and esophageal glands decreased still further in length, but

became wider and more voluminous. The two esophageal lobes seen first at day 11 were larger and nuclei could be seen for the first time. The nucleoli and chromocenters inside the nuclei of the other three esophageal glands were prominent. The genital primordium in this stage had assumed the V-shape characteristic of the developing female gonad. The genital primordium was associated closely with the now visible and prominent rectal glands but had not attached to the body wall.

Sixteen days after inoculation the early second stage female larva (Figs. 26E, F) showed further enlargement of the esophagus. The genital primordium now assumed a definite V-shape with two branches directed anteriad. The branches grew in length as the gonad moved towards the anal region and attached to the body wall (Fig. 26F); the female second stage larva possessed six well-developed rectal glands. At this point, a new cuticle was evident posteriorly as the second stage cuticle began to separate from it.

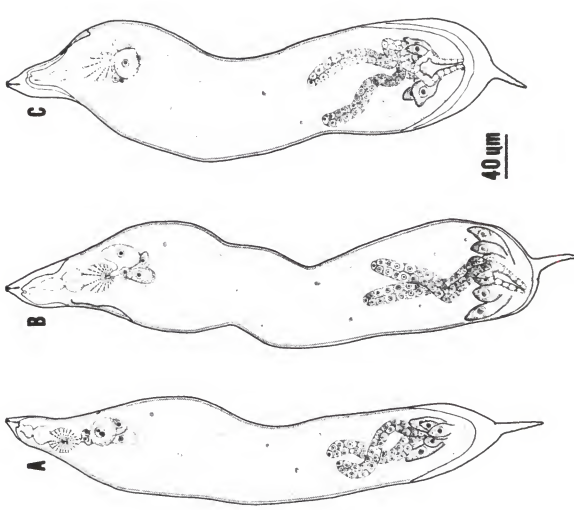
The third stage larva (Figs. 27A, B) could be recognized enclosed in the second stage larval cuticle; it did not possess a stylet and the posterior end was round. The second stage larval stylet remained attached to the old cuticle. During the molt the cone and shaft of the stylet and the lumen of the stylet knobs were shed with the second stage cuticle, the stylet knobs disappeared and there was a void at the anterior part of the body where the stylet and stylet knobs normally would be.

Figure 27

Third and fourth stage larvae of *Meloidogyne cruciani* n.sp.

A-B) Third stage larvae encased in the second stage cuticle.

C) Fourth stage larva encased in the second and third stage cuticles.


A
B
C
40 µm

The esophagus compressed, with the procorpus and metacorpus enlarging. The esophageal lumen and metacorpus valve were visible but very faint. The esophageal glands lost their chromocenters but the nucleoli remained very prominent. In the early third stage (Fig. 27A) the excretory pore was located opposite the esophageal glands with the duct pointing anteriorly. In the late third stage larva (Fig. 27B) the excretory pore was located opposite the esophageal glands with the anteriorly directed duct penetrating the body opposite the procorpus. The ovaries of the third stage larva continued to elongate and the uterus and vagina began to develop.

With the onset of the third molt, the body of the fourth stage larva formed and separated from the third stage cuticle but was still enveloped in the third and second stage cuticles. The early fourth stage larva (Fig. 27C) differed from the late third stage larva (Fig. 27B) in being enclosed in the old second and third larval cuticles. There was no stylet visible, and a void was seen at the anterior end where the stylet would have been. The esophageal lumen and metacorpus valve were very faint; the valve was now located in the posterior portion of the metacorpus. The excretory duct was similar to that of the third stage larva; it ran anteriorly and penetrated the body opposite the procorpus. At the posterior end, the gonads continued to elongate, the uterus and vagina formed completely.

In the early stages of the adult female, shortly after the fourth molt (Fig. 28A) and while still enclosed in the second, third and fourth larval cuticles, the stylet could be seen. The esophagus appeared typical of adult females with the procorpus and metacorpus enlarged and prominent. The lumen of the esophagus and valve of the metacorpus were reformed and appeared faintly at first. Inside the nuclear envelope, the chromocenters had disappeared but the nucleoli remained prominent. On the old cuticle of the second stage larva the excretory pore could be seen below the metacorpus with its duct leading anteriorly where it appeared to penetrate the female body opposite the procorpus. From here the excretory duct was seen leading posteriorly, as normally found in adult females. At the posterior end, the gonads continued to elongate. The uterus, vagina and vulva were prominent, and the perineal pattern could be detected. Nineteen days after inoculation all organs of the adult female were developed, and molting of the second, third and fourth cuticles occurred simultaneously (Fig. 28B).

Immediately after molting, feeding was resumed and the female enlarged from a sausage-shape (Fig. 28C) to the pear-shape typical of the genus (Fig. 28D). The stylet was robust and well-developed. The enlarged procorpus had a prominent lumen. The massive metacorpus had a strong well-sclerotized valve. The esophageal glands consisted of five distinct lobes with prominent nuclei and nucleoli. The

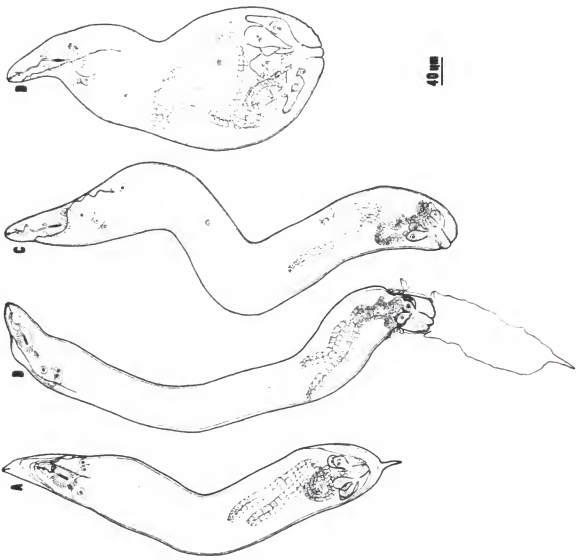
Figure 28

Adult females of *Meloidogyne cruciani* n.sp.

A) Early adult female still enclosed in old cuticles of the second, third and fourth stages.

B) Early adult female in the process of shedding the second stage cuticle.

C-D) Fully developed females.


B
40 µm
C
D
A

excretory pore was located opposite the procorpus with the duct leading posteriorly to a unicellular gland. The gonads elongated with one branch extending anteriorly close to the esophageal region. The rectal glands were large, with prominent nuclei and nucleoli.

Discussion

The post-infection development of Meloidogyne cruciani agrees in general with the studies done by Bird (3), Triantaphyllou and Hirschmann (61) and Siddiqui and Taylor (52).

The first noticeable changes that the post-infection larvae underwent were in body length and in the esophageal region (Fig. 26B). The esophagus increased in volume and the body around the esophageal region had a noticeable increase in width. These changes in the esophageal region may have been due to the intense feeding activity of the second stage larvae. The post-infection stage had a slight decrease in body length when compared to the pre-infective stage. Bird (3) also found a decrease in size of the infective second stage larvae after root penetration; he attributed the decrease in size to the depletion of food reserves used during penetration and migration into the roots.

Triantaphyllou and Hirschmann (61) reported the shape of the genital primordium (V-shape for females; straight cylindrical shape for males) could be used to differentiate sex in the early second stage larva. I found that sex could

be determined in the second stage larva of M. cruciani as early as 11 days after inoculation and before the genital primordium had assumed the V-shape typical of developing females (Fig. 26C). This determination was based on the presence of rectal glands in second stage immature females and the absence of rectal glands in males (61).

I found that the stylet cone, shaft and lining of the lumen through the stylet knobs are molted. This differs from previous reports on other species of Meloidogyne. According to Christie and Cobb (10), Bird (3) and Siddiqui and Taylor (52) only the anterior conical portion of the stylet is shed, with the basal portion of the stylet and stylet knobs disappearing. However, my findings agree with the illustrations of M. incognita presented by Triantaphyllou and Hirschmann (61).

The formation of two extra esophageal lobes in females but not in males indicates that females have different digestive requirements than do males or larvae. (There is no evidence that adult males feed.) Original descriptions of other Meloidogyne females illustrate the esophagi as tri-lobed glands or as an amorphous mass below the metacorpus. Bird (3) made careful examination of the esophageal region of M. javanica throughout larval development, and reported only one lobe in the esophagus. Chitwood's (7) illustrations showed three lobes.

Andrássy (1) stated that the five-gland condition represents a more advanced evolutionary state. The

sedentary parasite feeding habit of Meloidogyne sp. can be interpreted as one of the most advanced evolutionary states of parasitism. This high degree of specialization in feeding habit of these nematodes would undoubtedly require also a highly specialized digestive system. A five-gland esophageal condition should aid in the digestive process by increasing the quantity of digestive enzymes secreted by those glands. The two extra glands described in this study have always been found in close association with the dorsal esophageal gland. Baldwin and Sasser (2) and Eisenback et al.(19) found that the dorsal gland orifice of various species of Meloidogyne branched into three channels. That indicates that species other than M. cruciani may have five glands and that the two extra esophageal glands supplement secretions by the dorsal esophageal gland and thus aid in preoral digestion or may serve to stimulate the "nurse" cells of the plant.

As stated earlier, there are striking differences between the second stage larvae and the adult females of Meloidogyne sp. One of these differences is the position of the excretory pore. In the second stage larvae, it is usually found posterior to the metacorpus. In the adult females it is usually found anterior to the metacorpus adjacent to the stylet knobs. Christie and Cobb (10) illustrated the excretory pore opposite the metacorpus. In M. cruciani the excretory pore is opposite the procorpus, which agrees with the illustrations of Chitwood (7), Bird

(3) and Triantaphyllou and Hirschmann (61). Siddiqui and Taylor (52) did not illustrate an excretory system in M. naasi, and did not mention the location of the excretory pore. Bird (3) made careful examinations of the excretory pore and duct of M. javanica throughout the larval development, but he made no mention of the changes in position of the excretory pore opening between the second stage larva and the adult female.

Daily examinations of the developmental stages reveal the transformation that takes place in the position of the excretory pore (Figs. 27, 28). In the late second stage larva (Fig. 26F), the excretory pore is posterior to the metacorpus with the duct directed posteriorly for some distance. Immediately after the second molt (Fig. 27B) the duct is directed anteriorly. Subsequently in the late third state larva (Fig. 27B) and early fourth stage larva (Fig. 27C) the duct was observed penetrating the body opposite the procorpus. In the young adult female (Fig. 28A), the excretory pore on the second stage cuticle was posterior to the metacorpus with its duct directed anteriorly, attaching to the adult female body opposite the procorpus where the new excretory pore formed with the duct directed posteriorly. After the final molt (Fig. 28B), the excretory pore and duct became sclerotized and very prominent, ending in a renette-type cell (Fig. 28C). Observation of this excretory gland was possible in the adult female immediately after the last molt and before

enlargement (Figs. 28C, D). In older females the body is full of fat globules and ovaries making it impossible to observe.

It is probable that during the molting stages the excretory system is non-functional, forming a new duct directed anteriorly, a new excretory pore and a new and larger excretory duct in the final stage.

The post-infection development of the gonads of Meloidogyne cruciani agrees in general with the descriptions and illustrations presented by Triantaphyllou and Hirschmann (61) and Siddiqui and Taylor (52). The genital primordium enlarged by cell division and migrated posteriorly. As it approached the posterior end, the genital primordium assumed a V-shape, attached itself to the body wall, and formed two branches which grew anteriorly.

Maggenti and Allen (35) gave a complete account of the formation of the rectal glands and the origin of the gelatinous matrix in Meloidogyne sp. They found six rectal glands present in the early post-infective second stage larvae before enlargement. My study agrees with Maggenti and Allen's findings. Since males do not have rectal glands, it is possible, therefore, to determine the sex of the developing second stage larvae based on the presence of the rectal glands. Thus, sex can be determined in the very early stages of development, when the genital primordium is still in the six-cell stage (Fig. 26C) and has not migrated to the posterior end of the body or assumed a V-shape (Fig. 26D).

Previous studies of the life cycle and development of the genus Meloidogyne reveal few details on the molting of the three cuticles by the females. Siddiqui and Taylor (52) working with M. naasi were the first to mention shedding of the cuticles. They observed the old cuticles lying in the cortex in close proximity to the adult female bodies. Observations during my study indicate that the second stage larval cuticle is shed (Fig. 28B) while the third and fourth stage larval cuticles are either absorbed by the developing adult female or are lysed away. No feeding takes place during the third and fourth stages while the female is still enclosed within the second, and second and third stage larval cuticles, respectively. Before the female resumes feeding it has to get rid of the barrier that the second, third, and fourth stage larval cuticles present. By absorption or lysing action, the third and fourth stage cuticles are eliminated, while by force (mechanical action from body movements) the second stage cuticle is broken in half and molted (Fig. 28B).

CHAPTER IV
ESOPHAGEAL GLANDS OF ADULT FEMALES
OF Meloidogyne cruciani n.sp.

Introduction

Under the light microscope, the esophageal glands of Meloidogyne cruciani appear composed of five individual lobes. This is a deviation from the typical tylenchoid esophagus which is considered to be composed of only three esophageal glands. Previous original descriptions of Meloidogyne spp. illustrate the esophageal region as one, two or three lobes with three nuclei. Bird (3) reported that the esophageal region in the developmental stages of M. javanica was composed of only one gland. This differs from Chitwood's (7) original description of M. javanica in which he reported three glands.

The purpose of this study was to develop a fixation technique that could be used to study internal structures of nematodes with the scanning electron microscope (SEM) and to corroborate the existence of five esophageal lobes that can be seen with the light microscope.

Materials and Methods

Egg laying females were dissected from galled 'Rutgers' tomato roots (Lycopersicon esculentum Mill.) in 2% formalin

and divided into two groups. One group of females was placed in a glass cylinder (10 mm long x 6 mm inside diameter) and both ends covered with a fine mesh nylon screen (6 µm openings). The cylinder containing the females was placed in 5 ml of 2.5% glutaraldehyde solution in pH 7.2 phosphate buffer and fixed for 24 hours. Subsequently they were transferred to 2% osmium tetroxide for 24 hours at 8 C. The specimens, still inside the glass cylinder, were dehydrated for 15 minutes in a series of 10, 20, 30, 40, 50, 75, 95 and 100% ethyl alcohol. They were dehydrated in two changes of 100% ethyl alcohol for 15 minutes each. Then the specimens were placed in a Pelco Critical Point Dryer with an ethanol-liquid CO_2 system and dried. The dried specimens were removed from the glass cylinder, transferred by means of a dental root canal file onto a stub covered with double-sided adhesive tape. Under a dissecting microscope the anterior end of the specimens were cut with an eye knife to expose the internal organs. They were coated with gold with an Iako Sputter Coater, viewed and photographed with a Hitachi S-450 scanning electron microscope operated at 20 KV.

The other group of females was placed in 2% formalin in a stendor dish, heated to 68-70 C and fixed and stained in lactophenol-cotton blue (21). The females were transferred to glycerin and the cuticle was cut and removed (using an eye knife under a dissecting microscope) exposing the esophageal region. These sections were placed in a

glass cylinder and fixed, dehydrated, critical point dried, coated, mounted and examined as for the first group.

Results

The first group of females was not satisfactory for studying the esophageal region. All the internal structures had been fixed properly but the osmium tetroxide made the internal contents of the females very brittle and, when cut with the eye knife, the internal structures crumbled (Fig. 29).

The second method was successful. By first staining the females, the esophageal glands could be seen thus facilitating cutting and removing the cuticle. Once the cuticle was punctured, the pseudocoelomic fluids flowed out, the cuticle was removed, exposing the esophageal glands. The SEM corroborated the presence of two additional lobes in the esophageal region of M. cruciani as seen with the light microscope (Figs. 30-33).

Discussion

The use of the SEM to observe internal organs of nematodes requires further study. Most of the SEM studies of Meloidogyne females have been limited to external features such as perineal patterns (20, 28, 32, 36, 57, 68) and to the anterior ends (20, 28). Hogger and Estey (30) used cryofracturing techniques to observe internal structures

Figure 29

Scanning electron micrograph of *Meloidogyne cruciani* female internal structures crumbled.

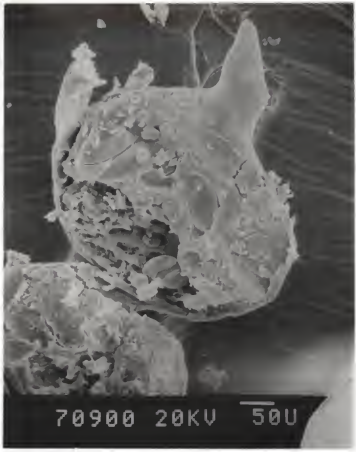

70900 20KV 50U

Figure 30

Scanning electron micrograph of *Meloidogyne cruciani* female dissected anterior region.


08729 20KV 50U

Figure 31

Enlarged portion of Fig. 30
showing esophageal glands.

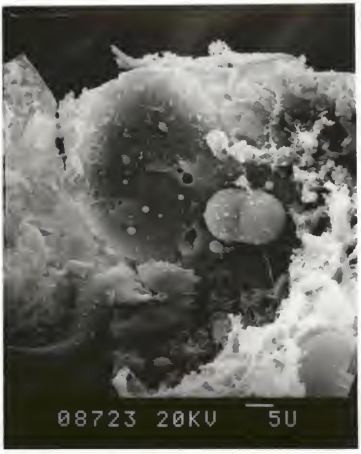

08723 20KV 5U

Figure 32

Scanning electron micrograph of *Meloidogyne cruciani* female dissected anterior region.

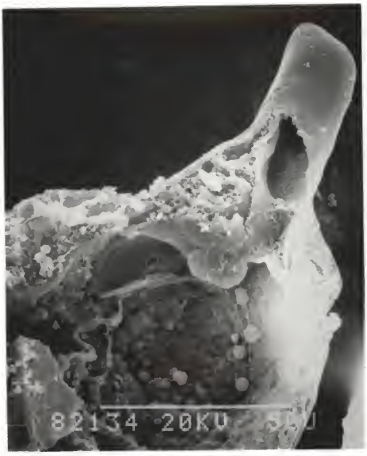
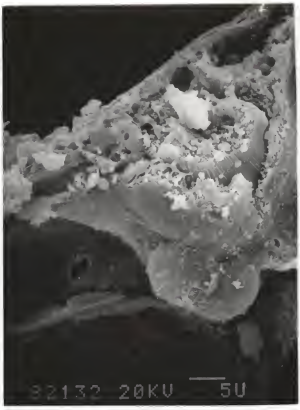

82134 20KV

Figure 33

Enlarged portion of Fig. 32
showing esophogeal glands and metacorpus.


32132 20KV 5U

of Xiphinema americanum and Caenorhabditis briggsae. The method they used presents the problem of not being specific as to where the cuts are going to occur. Furthermore, the observation plane is a cross section of the nematode, not a tridimensional view of the organs exposed during fracturing. More recently, Eisenback et al. (20) used the SEM and light microscopes to study the excised stylet, lumen and metacorpus valve of Meloidogyne hapla, M. arenaria, M. incognita, and M. javanica females. These are the first accounts of using the SEM to study excised internal organs of nematodes. Their results show that it is possible to excise sclerotized internal structures of nematodes and view them with the SEM. My results show that with adequate techniques, non-sclerotized internal structures also can be studied (Figs. 30-33).

Light microscope studies of Meloidogyne cruciani revealed five-lobed esophageal glands; one large dorsal gland, two small dorsal glands and two subventral glands. SEM micrographs confirm the presence of the two small dorsal glands (Figs. 30-33). The external surface of the metacorpus appears ridged (Figs. 32, 33).

CHAPTER V
INTERACTION OF Rotylenchulus reniformis AND
Meloidogyne cruciani ON TOMATO

Introduction

During a survey of two of the U.S. Virgin Islands, as described in Chapter I, Rotylenchulus reniformis Linford and Oliveira, 1940 (34) was recovered from all samples while Meloidogyne cruciani n.sp. was recovered from only 14% of the samples. Since both islands surveyed, St. Croix and St. Thomas, are tropical and would appear to have conditions, including ample host plants, suitable for both nematode species, the question arises as to why the low incidence of Meloidogyne.

Experiments were initiated to determine the influence of soil type and temperature on each nematode. Since the islands have predominently clay-type soils, a clay soil and a sandy soil were compared as to their influence on population development at two different temperatures.

Materials and Methods

Seeds of tomato, Lycopersicon esculentum Mill. cv 'Rutgers', which is susceptible to both R. reniformis and M. cruciani, were germinated in moist sterile vermiculite in a temperature controlled water bath set at 29 C. When

seedlings were one month old, they were transplanted to 15-cm clay pots containing either sterile clay-loam (Esto series) soil or sandy (Arrendondo fine sand) soil. Each soil type was inoculated as follows: 200 R. reniformis/0 M. cruciani, 0 R. reniformis/200 M. cruciani, and 200 R. reniformis/200 M. cruciani. The larval stages were introduced at random in holes about 5 cm from the base of each seedling. The inoculated seedlings were placed at two different temperature conditions: at 30 ± 1 C in a constant temperature chamber or at 22 ± 2 C in a growth room. The plants were watered daily and fertilized once a week with 100 ml of a solution containing 390 ppm of Nutrisol^{R} (12-10-20), a commercially obtained fertilizer solution. Nine replicates of each treatment were sampled at one and three months after inoculation.

Inoculum was obtained as follows: Roots of tomato plants heavily infested with M. cruciani were washed free of soil under a gentle stream of water. The roots were blotted dry, placed in a petri dish in water, set on the stage of a dissecting microscope and egg masses dislodged from the roots with a dissecting needle. Individual egg masses were transferred to a BPI dish containing distilled water, teased apart with a dissecting needle to expose the eggs and left overnight at 30 C. The next morning freshly hatched second stage larvae were transferred, using a capillary pipette apparatus, in lots of 100 to vials containing distilled water. Immature females of R.

reniformis were obtained by processing infested soil using a sieving and sugar flotation-centrifugation technique (5). As for M. cruciani, these nematodes were transferred in lots of 100 to vials containing distilled water.

One month and three months after inoculation, nine pots of each treatment were sampled as follows: the soil in each pot was placed in a container and water added to bring the total volume to 10 liters. The soil in the container was mixed thoroughly with the water and an aliquot of 500 ml was poured through a 500 mesh sieve (pore size 30 μm). The residue on the sieve was washed into 50 ml centrifuge tubes and the nematodes extracted using a modification of the sugar flotation-centrifugation technique described by Caveness and Jensen (5). The extracted nematodes were placed in Syracuse dishes and counted.

A computerized statistical analysis of variance procedure (ANOVA) was run on all the different treatments and two way tables used to compare means followed by a Tukey's HSD (honestly significant difference) test of those comparison of means that were found significant with the tables (6).

Results

Statistical analyses were used to interpret the following variables:

1) two types of soil (sand and clay);
2) two sampling times (one and three months after inoculation);
3) two different temperature conditions (22 and 30 C); and
4) three different inoculum levels (0/200, 200/0, and 200/200--Rotylenchulus reniformis/Meloidogyne cruciani).

Rotylenchulus reniformis

The results of the analysis of variance of the two treatments containing R. reniformis (200/0 and 200/200) showed the following interactions statistically significant:

Interaction:

Soil by time by treatment; and

Soil by treatment by temperature.

At one month sampling time, there was no significant effect of the treatments in either sand or clay, while at three months sampling time, the treatment effect was significant in both sand and clay (Table 4). The treatments were significant in time in both sand and clay. At one month sampling time, soil type had no significant effect on the population development of R. reniformis alone or with concomitant populations of M. cruciani. There was a significant increase in population growth at three months sampling time in both soil types with both treatments.

While R. reniformis alone had a significant increase within soil types at three months sampling time, in the presence of M. cruciani, it was not significant.

Table 4

Effects of soil type and time of sampling on populations of Rotylenchulus reniformis when inoculated alone and simultaneously with an equal number of Meloidogyne cruciani.

	Soil type			
	Sand		Clay	
	Sampling time		Sampling time	
Inoculum[1] density	1 mo.	3 mo.	1 mo.	3 mo.
(No./pot)	(No./pot)			
200/0	4021_{d}[2]	94297_{b}	4845_{d}	226000_{a}
200/200	4677_{d}	51201_{c}	4608_{d}	40483_{c}

[1]Inoculum density of Rotylenchulus reniformis/Meloiodgyne cruciani, respectively.

[2]Means followed by the same letter are not significantly different at 5% level according to Tukey's HSD comparison of means. Significance applies to vertical and horizontal columns.

Treatment effect was significant in both soil types regardless of temperature conditions (Table 5). Temperature conditions had no significant effect on the treatments in

either soil type. Soil type was significant with R. reniformis alone but not significant when both R. reniformis and M. cruciani were together.

Table 5

Effects of soil type and temperature on populations of Rotylenchulus reniformis when inoculated alone and simultaneously with an equal number of Meloidogyne cruciani.

	Soil type			
	Sand		Clay	
	Temperature		Temperature	
Inoculum[1] density	22 C	30 C	22 C	30 C
(No./pot)	(No./pot)			
200/0	54413_{b}[2]	43906_{b}	120364_{a}	110481_{a}
200/200	30065_{c}	25812_{c}	20870_{c}	24221_{c}

[1]Inoculum density of Rotylenchulus reniformis/Meloidogyne cruciani, respectively.

[2]Means followed by the same letter are not significantly different at 5% level according to Tukey's HSD comparison of means. Significance applies to vertical and horizontal columns.

Meloidogyne cruciani

The results of the analysis of variance of the two treatments containing M. cruciani (0/200 and 200/200--Rotylenchulus reniformis/Meloidogyne cruciani) showed the

following interactions statistically significant:

Soil by time by treatment; and

Soil by treatment by temperature.

Sampling time had a significant effect on both treatments, with both soil types (Table 6). Treatment effect was not significant one month after inoculation regardless of soil type, but it was significant at three months in both soil types. Soil type had a significant effect three months after inoculation with M. cruciani alone, but its effect was not significant for any of the other treatments or sampling times.

Temperature effect was significant with M. cruciani alone in the clay soil; it was not significant with the other treatments (Table 7). Treatment effect was not significant in the clay soils at the variable temperature conditions, but was significant in the other interactions. Regardless of temperature conditions, soil type was significant with M. cruciani alone but not significant when both genera of nematodes were together. Soil type has a significant effect on M. cruciani reproduction, while temperature does not.

Table 6

Effects of soil type and sampling time on populations of *Meloidogyne cruciani* when inoculated alone and simultaneously with an equal number of *Rotylenchulus reniformis*.

	Soil type			
	Sand		Clay	
	Sampling time		Sampling time	
Inoculum[1] density	1 mo.	3 mo.	1 mo.	3 mo.
(No./pot)	(No./pot)			
0/200	261_{d}[2]	24467_{a}	369_{d}	7505_{b}
200/200	282_{d}	3607_{c}	562_{d}	3431_{c}

[1]Inoculum density of *Rotylenchulus reniformis*/*Meloidogyne cruciani*, respectively.

[2]Means followed by the same letter are not significantly different at 5% level according to Tukey's HSD comparison of means. Significance applies to vertical and horizontal columns.

Table 7

Effects of soil type and temperature on populations of *Meloidogyne cruciani* when inoculated alone and simultaneously with an equal number of *Rotylenchulus reniformis*.

	Soil type			
	Sand		Clay	
	Temperature		Temperature	
Inoculum[1] density	22 C	30 C	22 C	30 C
(No./pot)	(No./pot)			
0/200	12332_a[2]	12396_a	2455_c	5418_b
200/200	1316_c	2573_c	1283_c	2710_c

[1]Inoculum density of *Rotylenchulus reniformis*/*Meloidogyne cruciani*, respectively.

[2]Means followed by the same letter are not significantly different at 5% level according to Tukey's HSD comparison of means. Significance applies to vertical and horizontal columns.

Discussion

Rotylenchulus reniformis populations increased over two-fold more in the clay soils than in sandy soils while Meloidogyne cruciani increased over three-fold more in sand than in clay. Other researchers (38, 39, 42, 44, 46, 66) have found that Meloidogyne spp. develop better in sandy soils than in fine textured soils. This study corroborated their findings. The lack of population increase of M. cruciani in the clay soil indicates the presence of an abiotic factor that restricts their reproductive capacity under these conditions. Their inability to reproduce and increase in numbers in clay soils as compared to sandy soils will restrict their distribution. R. reniformis, on the other hand, had a large increase in numbers in the clay soils. This indicates that R. reniformis is more adapted to and develops better in fine texture soils than does M. cruciani. This advantage that R. reniformis has over M. cruciani answers the question of why R. reniformis was distributed throughout the U.S. Virgin Islands, while M. cruciani was found only in isolated areas. Since the U.S. Virgin Islands are composed mainly of clay-type soils, R. reniformis will be able to reproduce and increase their numbers at a faster rate than M. cruciani. While the former has the ability to spread throughout, the latter is restricted to areas where the soil conditions are more suitable to their development.

When M. cruciani was present, R. reniformis did not increase as much as when the latter was alone. This influence that M. cruciani had on R. reniformis was detected in both sand and clay soils. Singh (55) and Thomas and Clark (60) found that R. reniformis populations are suppressed in the presence of M. incognita.

M. cruciani numbers were lower under both soil types when R. reniformis was present than when M. cruciani was alone. The lack of reproduction of M. cruciani in sand was greater proportionally than for R. reniformis. Other studies with R. reniformis and Meloidogyne spp. have shown that in the presence of R. reniformis, populations of Meloidogyne spp. are inhibited, their growth retarded, or penetration and developmental rates are slower (33, 40, 43, 67).

The mutual antagonistic effect found in this study between R. reniformis and M. cruciani indicates a competition between both nematodes for available feeding sites. This agrees with the work reported by Estores and Chen (24). They found that when Pratylenchus penetrans and M. incognita were together on tomato, populations of both species were lower than when alone. Turner and Chapman (62) also found that when P. penetrans and M. incognita were together on alfalfa or red clover, root invasion by M. incognita was reduced because of fewer suitable feeding sites. Other researchers also have reported that M. incognita (26, 47, 56, 64), M. hapla (45), M. javanica (65), and M. naasi

(53, 54) had lower reproduction, less galling, and lower root penetration when in the presence of other genera of plant parasitic nematodes.

When comparing the population densities of R. reniformis and M. cruciani together in sand and clay, there was no significant difference in their numbers between either soil type. Soil type had no effect on either nematode when they were together.

There was a significant increase in population development with both nematodes alone and combined in both soil types from one month to three months. Sampling at one month after inoculation is too short a period of time to detect any significant effect soil type might have on the nematodes. After three months, R. reniformis increased greater in clay than in sand while M. cruciani showed the opposite effect. Regardless of soil type, the increases shown by R. reniformis were much greater than the increases shown by M. cruciani. This could be due to R. reniformis having a shorter life cycle than M. cruciani. Rao and Prasad (43) found that R. reniformis increased in numbers faster than did M. javanica; and they attributed this to R. reniformis having a shorter life cycle.

Populations of R. reniformis alone or with concomitant populations of M. cruciani increased with no significant difference between 22 or 30 C. The experiments showed that the temperature conditions used had no significant effect on R. reniformis growth.

Temperature had no significant effect on M. cruciani in the sandy soil when alone or with R. reniformis, but in clay, populations increased more at 30 C than at 22 C. The temperature ranges for M. cruciani have not been determined; therefore, it is possible that the lower temperatures in the growth room (20-24 C) might have slowed the development of these nematodes.

The soil texture conditions of the U.S. Virgin Islands plus the faster reproduction rates of R. reniformis may be the reasons why reniform nematodes have adapted themselves throughout these islands. Those same conditions may have kept M. cruciani from further spreading. The former were found in every sample examined, while the latter were recovered only in specific sites--intensively worked vegetable gardens.

Experiments of longer duration (more than three months) are needed to determine whether R. reniformis is capable of displacing Meloidogyne spp. under field conditions in sand or clay soils. Bird et al. (4) reported that when field populations of Hoplolaimus columbus and M. incognita were together on cotton, H. columbus populations increased in number, suppressing populations of M. incognita and eventually replacing them.

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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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