Pathogenicity of the Nematodes *Rotylenchulus reniformis*,
Helicotylenchus dihystra, and *Tylenchorhynchus claytoni*
to Shade Tobacco

By

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

UNIVERSITY OF FLORIDA

1969

ACKNOWLEDGEMENTS

The author expresses his sincere appreciation to members of his supervisory committee: Dr. Grover C. Smart, Jr., Chairman; Dr. V. G. Perry; and Dr. Howard N. Miller. A special debt of gratitude is due Dr. Smart for his guidance, consultation, and criticism of manuscript.

He is indebted to Dr. William G. Eden, Chairman of the Department of Entomology and Nematology, and Dr. Perry, Professor of Nematology, who made a teaching assistantship available making it possible for the author to continue his education.

Appreciation is extended to Mr. William B. Tappan, Associate Entomologist, North Florida Experiment Station, for providing soil, original nematode populations, tobacco seeds and valuable information regarding shade-grown tobacco.

He expresses his gratitude to Mr. R. W. Patrick for assistance on several occasions during the investigation.

A recommendation of Mr. A. L. Taylor, Nematologist, formerly with FAO mission in Bangkok, to AID officials in Bangkok granting the extension of training to the author in order to complete this investigation is greatly appreciated.

Finally, he thanks his wife for her understanding, encouragement, and assistance.

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

PATHOGENICITY OF THE NEMATODES ROTYLENCHULUS RENIFORMIS,
HELICOTYLENCHUS DIHYSTERA, AND TYLENCHORHYNCHUS CLAYTONI
TO SHADE TOBACCO

By

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March, 1969

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Helicotylenchus dihyстера, Rotylenchulus reniformis, and
Tylenchorhynchus claytoni are plant parasitic nematodes frequently
encountered in shade-grown tobacco fields in North Florida. Pathogeni-
city experiments were conducted in the greenhouse using each species
alone and in all combinations with the other two species at different
inoculum levels. T. claytoni significantly retarded the root system
of shade-grown tobacco variety 'Dixie Shade' with an exposure period of
128 days. However, height and yield of inoculated plants were not
affected by root retardation. Smaller root systems of plants were
always accompanied by some discoloration and shriveled appearance of
roots. At exposure periods of 80, 70, or 42 days at inoculum levels
comparable to those used for 128 days' exposure, root retardation by T.
claytoni did not occur. H. dihyстера and R. reniformis inoculated
alone or in all combinations at any inoculum level did not affect plant

growth. At any given time of exposure T. claytoni inoculated to tobacco alone or in combination with the other species reproduced at a greater rate than did the other nematodes. Terminal populations increased several times over the inoculum. Terminal populations of H. dihystrera and R. reniformis inoculated alone or in combination were in most cases greater than the inoculum, but numbers were not as great as were terminal populations of T. claytoni. Inoculation with 1,500 specimens each of T. claytoni, H. dihystrera, and R. reniformis resulted in significantly fewer numbers of R. reniformis 42 days after inoculation than when 1,500 specimens of R. reniformis were inoculated alone. Similarly, inoculation with 1,500 specimens each of H. dihystrera, R. reniformis, and T. claytoni resulted in fewer numbers of T. claytoni 42 days after inoculation than when 1,500 specimens of T. claytoni were inoculated alone.

Histopathological studies of tobacco roots infected by R. reniformis revealed that eelworm-shaped females penetrated tobacco roots near the root tips to initiate infection. The parasites penetrated the epidermis, cortical parenchyma, and endodermis and fed in the pericyclic region. Only the stylet penetrated into pericycle cells. Hypertrophy and enlargement of nuclei in the pericyclic region extended from the cell where a nematode stylet was inserted to adjacent pericyclic cells. Cytoplasmic content of hypertrophied cells stained darker than uninfected surrounding cells. The above-ground portions of parasitized plants exhibited no symptoms.

INTRODUCTION

Records maintained at the Department of Entomology and Nematology, University of Florida, Gainesville and the North Florida Experiment Station, Quincy, show that plant parasitic nematodes of the genera Helicotylenchus, Rotylenchulus and Tylenchorhynchus are frequently encountered in shade-grown tobacco areas in North Florida. Experiments at Quincy indicated that yields were increased and a better quality of tobacco was produced when nematicides were used (Tappan and Kincaid, 1962 and 1964). Records also revealed that Helicotylenchus dihystrera (Cobb) Sher (syn. H. nannus Steiner), definitely affected crop growth. This parasite damaged shade-grown tobacco roots reducing plant height, yield, and quality of the tobacco (Tappan and Kincaid, 1963). The reniform nematode, Rotylenchulus reniformis Linford and Oliveira, has been suspected of reducing yield and quality of shade-grown tobacco. Tylenchorhynchus claytoni Steiner, though not reported in the Gainesville and Quincy records as a pathogen of tobacco, has been reported as a pest of tobacco in other areas.

The purposes of these investigations were to determine the effects of Helicotylenchus dihystrera, Rotylenchulus reniformis and Tylenchorhynchus claytoni on shade-grown tobacco variety 'Dixie Shade' by using each species alone and in all combinations with the other species, and to investigate the histopathology of R. reniformis on tobacco.

LITERATURE REVIEW

Members of the nematode genus Helicotylenchus are encountered frequently in the roots and surrounding soil of many host plants. The parasites have been found repeatedly in soil samples from turf grasses, ornamental plants and field crops by various workers (Perry et al., 1959; Taylor, 1960; McGlohon et al., 1961; Springer, 1964; Roman, 1965; Sher, 1966). H. dihystrera is the predominant species found in shade-grown tobacco areas of North Florida (Tappan and Kincaid, 1963), yet little is known about its pathogenicity to tobacco.

Sledge (1956) studied the pathogenicity of H. dihystrera to certain varieties of corn and found that the nematode multiplied readily but caused little damage. The symptoms included those of stunting and some root necrosis at high inoculum levels. Minton and Cairns (1957) showed that H. dihystrera decreased root production by 'Ogden' soybean. However, seed yields of the infected and control plants were not significantly different. H. dihystrera was reported to be associated with gardenia decline by Konicek and Jensen (1961). The decline plants showed stunting, yellowing, premature dropping of leaves, failure to form flower buds, premature dropping of buds, and eventual death of plants. Ruehle (1966) reported that H. dihystrera parasitized and reproduced on six species of pine seedlings native to the southeastern United States. However, effects of this parasite on the pine seedlings were not determined.

Pathogenicity of a few other species of the genus have been studied by Perry et al. (1959). They demonstrated that H. digonicus Perry and, to a

lesser extent, H. microlobus Perry [= H. pseudorobustus (Steiner) Golden] and H. pumilus Perry [= Rotylenchus pumilus (Perry) Sher] parasitized roots of common Kentucky blue grass, Poa pratensis L., and produced a condition called "summer dormancy".

The reniform nematode, Rotylenchulus reniformis was described from roots of cowpea, Vigna sinensis Endl., in Hawaii (Linford and Oliveira, 1940). Since then the species has been reported to occur in various tropical and semitropical areas of the world and in the southern United States (Smith, 1940; Smith and Taylor, 1941; Steiner, 1949; Martin, 1955; Peacock, 1956; Timm, 1956; Minton and Hopper, 1959; Thorne, 1961; Sasser et al., 1962; Lambe and Horne, 1963; Swarup et al., 1967; Fassuliotis and Rau, 1967; and Dasgupta et al., 1968). This species attacks various host plants (Linford and Yap, 1940; Peacock, 1956; Luc and de Guiran, 1960; Birchfield and Brister, 1962; Ayala and Ramirez, 1964). Among the economic crops are cotton, sweet potato, pineapple, tomato and tobacco.

The importance of R. reniformis was recognized when Neal (1954) reported that the presence of the reniform nematode increased the incidence of Fusarium wilt of cotton in the wilt susceptible varieties. Birchfield and Jones (1961) stated that R. reniformis was found associated with cotton failures distributed over 2,000 to 2,500 acres in Louisiana. This parasite was associated with severe disease symptoms of dwarfing, premature decay with loss of secondary roots, and death of young cotton plants. These conditions resulted in poor stands, "grassy" areas in the fields, and ultimate yield reductions of 40 to 60 per cent. Jones et al. (1959) reported that the infestation of R. reniformis at the rate of 320 specimens per 473 ml (one pint) of soil caused a reduction in the green weights of cotton plants by 12.6 per cent, a 21.3 per cent reduction in total boll weights and 20.7 per cent reduction in number of bolls. Cotton seedling growth was reduced when

roots were parasitized by R. reniformis according to Brodie and Cooper (1964). Minton et al. (1964) reported that R. reniformis reduced emergence, plant height, and yield of cotton, and hastened crop maturity. They also noted that the incidence of Fusarium wilt of cotton was significantly increased by the presence of R. reniformis. Host-parasite relationships of R. reniformis on cotton were studied by Birchfield (1962). He found that young females initiated infection by extending the anterior portion of their bodies through the epidermis and cortical parenchyma to feed in phloem tissues of young roots. Phloem cells near the feeding site of the nematode stained darker than normal tissues; damage to the phloem extended several cells along the axis of the root. Birchfield concluded that necrosis in the phloem and parenchyma apparently resulted in severe root "pruning" of seedlings and subsequent dwarfing of cotton.

In Florida, R. reniformis was first reported by Steiner (1949) on tomato and coffee weed (Cassia tora L.) from Quincy. Later, the parasite was found parasitizing roots of mango tree (Mangifera indica L.) on the grounds of the University of Florida Subtropical Experiment Station at Homestead according to Van Weerdt et al. (1959). However, the damage by R. reniformis to mango in Florida has not been investigated.

Tylenchorhynchus claytoni, the tobacco stunt nematode, was described by Steiner (1937) from specimens collected around and in the roots of stunted tobacco plants growing near Florence, South Carolina. Graham (1954) found that species in 67 per cent of a series of soil samples taken from tobacco fields in Eastern South Carolina. He also stated that the species was encountered frequently in soil samples from cotton and corn fields. As an ectoparasite of tobacco, Graham reported that T. claytoni caused stunting of the tobacco plant and a much retarded root system as compared with normal

growth in nematode-free soil. Graham further stated that the roots did not show definite symptoms but became shriveled and sparsely developed. Drolsom and Moore (1955) reported that T. claytoni was one of the principal plant parasitic nematodes found in tobacco fields in North Carolina where stunting of plants occurred. Holdeman (1956) stated that the presence of T. claytoni attacking the roots of wilt susceptible tobacco variety 'Oxford 1-181,' greatly increased the incidence of wilt caused by Fusarium oxysporum. Krusberg (1959) reported that T. claytoni completed its life cycle in about one month on alfalfa roots growing on nutrient agar. He also found that males are necessary for reproduction. Root systems of tobacco growing in pots were considerably retarded by large populations of T. claytoni. No nematode injury to the roots of tobacco variety 'Dixie Bright 101' was observed. Host range studies, including 41 plant varieties, indicated that the tobacco variety 'Bottom Special' is the most suitable host. Reproduction of the nematode was best at 29.4° - 35.0° C on tobacco and at 21.1° - 26.6° C on wheat.

Nelson (1956) tested several corn hybrid lines in the greenhouse against T. claytoni and found that at high population levels root and shoot weights of plants were reduced.

Brodie and Cooper (1964) reported that T. claytoni reproduced in the presence of cotton seedling roots but did not cause excessive root injury or measurable retardation in growth of seedlings.

Pathogenicity of T. claytoni on turf grasses was investigated by Troll and Rhode (1966). They observed the parasite feeding on the roots of annual rye grass, creeping red fescue, and Kentucky blue grass but root injury was not discernible. Shoots of the three grasses inoculated with T. claytoni were not affected by the nematode. The lower dry weights of roots and shoots of inoculated plants compared with non-inoculated controls indicated that

T. claytoni was pathogenic to creeping red fescue (Festuca rubra L.)

Sumner (1967) reported that T. claytoni was one of the predominant stylet-bearing nematodes found in Kentucky blue grass lawns in Lincoln, Nebraska. However, attempts to demonstrate its pathogenicity on blue grass in greenhouse and laboratory experiments were unsuccessful.

T. claytoni is also found frequently in forest tree nurseries. Hopper (1958) reported that population levels of 1,500 to 2,000 specimens per 473 ml of soil caused severe injury to seedlings of slash pine, Pinus elliotii Engelm. He described the symptoms as ranging from green-, yellow-, and brown-colored seedlings to dead seedlings. However, low infestation of T. claytoni did not appear to affect pine seedlings. Sutherland and Adams (1964) reported that T. claytoni was parasitic on but not pathogenic to red pine seedlings (Pinus resinosa Ait.). Ruehle (1966) established that T. claytoni is parasitic on loblolly, longleaf, pond, sand slash, shortleaf, and Virginia pines.

Sher (1958), and Barker and Worf (1966) reported that parasitism by T. claytoni resulted in retarded growth of azalea plants. Barker and Worf also found that T. claytoni accentuates the appearance of foliage chlorosis of azalea plants.

MATERIALS AND METHODS

Pathogenicity

General Methods

In December, 1965, soil infested with moderate numbers of Rotylenchulus reniformis, Helicotylenchus dihystrera, and low numbers of Tylenchorhynchus claytoni was collected from the North Florida Experiment Station at Quincy, Florida. The soil was processed using the elutriation technique (Seinhorst, 1956) and a modified Baermann funnel technique (Christie and Perry, 1951). Larvae, eelworm-shaped females and males of R. reniformis were isolated individually and randomly and each of several pots were inoculated with a total of 100 specimens. The pots were divided into five groups and each group was planted to bean (Phaseolus vulgaris Savi.), cotton (Gossypium hirsutum L.), sweet potato (Impomoea batatus Lam.), tobacco (Nicotiana tabacum L.) or tomato (Lycopersicon esculentum Mill.). Large numbers were recovered from tomato and thus this host was selected for the increase and maintenance of populations of R. reniformis.

One hundred gravid females (males are not known to occur) of H. dihystrera were placed in 15-cm plastic pots and either tobacco or tomato seedlings were planted. Reproduction was greater on tomato and thus this host was used to maintain populations of H. dihystrera also. This soil did not contain sufficient numbers of T. claytoni to establish colonies.

In June, 1966, soil containing large numbers of T. claytoni was collected near Quincy, Florida. The samples were processed by the elutriation technique

(Seinhorst, 1956) and large numbers were recovered. Males and females were individually isolated and each of several pots was inoculated with a total of 200 specimens. A tobacco seedling then was planted in each pot and after three months populations of the parasites increased several times the original inoculum.

All nematode colonies were maintained in an air-conditioned greenhouse at $26 \pm 4^{\circ}\text{C}$. About 28-42 days prior to initiating the experiments, tobacco seeds were sown in a flat containing autoclaved soil. The flats of seedlings were maintained in the greenhouse and received fertilizer and water as needed.

The soil used to colonize the nematodes and used in Experiment I was collected from the North Florida Experiment Station, autoclaved at 18 psi for 30 minutes, and allowed to aerate a few days before use. Then 2,000 ml was placed in each of several 15-cm plastic pots.

Experiment I.

This experiment was initiated on October 14, 1966, and terminated on February 20, 1967. Tobacco seedlings were 36 days old at the time of inoculation. The following nine treatments for this experiment were replicated four times:

1.	Inoculated with	500H ^a
2.	" "	500T
3.	" "	1,000H
4.	" "	1,000T
5.	" "	500H + 500T
6.	" "	2,000H
7.	" "	2,000T

8. Inoculated 1,000H +
 1,000T

9. Uninoculated

^aH = Helicotyle hus dihystra, T = Tylenchorhynchus claytoni

Pot arrangement was a completely randomized block design. Specimens for inoculations were obtained from the greenhouse colonies described above. Nematodes were extracted from the soil by using an elutriation technique (Seinhorst, 1956). The specimens were used within 48 hours after isolation from the soil. Nematodes in all treatments were hand-picked with the aid of a dissecting microscope.

Inoculation was performed by adding the requisite numbers of nematodes as a water suspension into a hole made in the soil in a pot, then a tobacco seedling was planted in the same hole and water was added. Pots then were randomly arranged on a greenhouse bench. A liquid fertilizer solution (Ortho[®] 12-6-6) was applied every two weeks, and plants were watered as required. Plants heights were recorded at monthly intervals, and "ripe" leaves were primed on three different dates and the green weights recorded. Upon termination of the experiment the remaining leaves were primed and weighed. Weights of leaves at the three different primings and at the termination of the experiment were combined. The stalks then were cut off at the soil line and plant heights were measured. After the stalks were cut the roots were removed from the soil, washed gently in running water, blotted with paper towels, and weighed. The roots then were examined for nematode injury with the aid of a dissecting microscope. The soil was removed from the pots, thoroughly mixed, and two 100 ml samples were processed by using a rapid centrifugal-flotation technique (Jenkins, 1964). The nematodes recovered were rinsed into a graduate cylinder and enough water was added to

make 250 ml. Specimens were counted in five aliquots of 10 ml each and populations for each sample determined. An average was taken of the two samples and the numbers of nematodes per pot calculated.

Experiments II, III, and IV

Soil used in these experiments was collected from a field near the North Florida Experiment Station. This soil was fumigated with methyl bromide at the rate of two pounds per cubic yard for 48 hours and allowed to aerate in an open shade area for two weeks. Then 2,000 ml were placed in each of several 15-cm plastic pots. At this time some of the soil was processed for nematodes and none was found.

Experiment II, a repetition of Experiment I, was initiated on June 16, 1967, and terminated on September 4, 1967. The tobacco seedlings were 41 days old when transplanted and inoculated.

Experiment III was initiated on June 3, 1967, and terminated on August 12, 1967. The seedlings used were 28 days old. The 15 treatments each replicated four times were as follow:

1.	Inoculated with	500H ^a
2.	" "	500R
3.	" "	500T
4.	" "	1,000H
5.	" "	1,000R
6.	" "	1,000T
7.	" "	500H +
500R
8.	" "	500R +
500T
9.	" "	500H +
500T

10.	Inoculated with	500H +
500R +
500T
11.	" "	1,500H
12.	" "	1,500R
13.	" "	1,500T
14.	" "	1,500H +
1,500R +
1,500T
15.	Uninoculated	

²H = Helicotylenchus dihystra, R = Rotylenchulus reniformis,

T = Tylenchorhynchus claytoni

Experiment IV, a repetition of Experiment III, was initiated on July 28, 1968, and terminated on September 8, 1968. Tobacco seedlings were 42 days old at the time of inoculation.

The pot arrangements in these three experiments were completely randomized block designs. Nematode specimens for inoculations were obtained from the greenhouse colonies described above. Specimens were isolated from the soil by using either an elutriation technique (Seinhorst, 1956) or a modified Baermann funnel technique (Christie and Perry, 1951) or both. The specimens were used within 36 hours after isolated from the soil. To obtain the numbers of specimens needed for inoculation, the number of specimens in one ml of water was determined and the correct number of milliliters of water was used for each treatment. Inoculations and maintenances were performed as previously described for Experiment I. On termination of the experiments leaves were primed and weighed. The stalks were cut off at the soil line and plant heights were measured. The soil and roots were removed from the individual pots and placed in a 10-liter plastic pail to which 5,000 ml of water were

added. The roots were moved about in the water to separate them from the soil after which they were removed from the pail, washed in running water, blotted with paper towels, weighed and examined. The water and soil in each pail was roiled, an aliquot of 1,000 ml taken immediately and then processed using a modified Baermann funnel technique (Christie and Perry, 1951) for Experiments II and III, and a rapid centrifugal-flotation technique (Jenkins, 1964) for Experiment IV. The specimens recovered were counted by the aid of a dissecting microscope and the numbers of specimens in each pot were computed. Females of R. reniformis which were embedded in the roots were not included in the computations.

Histopathology of Rotylenchulus reniformis on Tobacco Roots

On July 4, 1968, thirty day-old seedlings of the shade-grown tobacco variety 'Dixie Shade' were placed in 14-cm plastic petri dishes painted black. The plants were placed through slits made in the edge of the petri dishes with stems and leaves outside. At the point of exit, the stems were wrapped in cotton. Nematode inoculum composed of a mixture of larvae, males and eelworm-shaped females of R. reniformis was pipetted onto the roots which then were covered with autoclaved builder's white sand and watered with rain water which had been collected for this purpose. Uninoculated plants served as controls. Plants were watered alternately with rain water and Hoagland solution as required by using polyethylene squeeze bottles. The experimental plants were kept in a growth room at approximately 27°C. A 16-hour photo period was used.

One month after inoculation the plants were removed from the petri dishes and the roots were examined for nematode infection and injury. Some of the

inoculated roots were stained in acid fuchsin-lactophenol and cleared in lactophenol (McBeth et al., 1941). Sections of roots 2-3 cm in length in which saccate females were embedded and comparable sections of uninfected roots were placed for at least 24 hours in Craf III fixative (Sass, 1958) containing chromic acid (30 ml of one per cent), acetic acid (20 ml of 10 per cent), formaldehyde (10 ml of 37.5 per cent) and water (40 ml). They were dehydrated by a normal butyl alcohol series, embedded in 'Tissuemat' (60°C. m.p.) and sliced in cross- and longitudinal-sections, 10 micron thick, with a rotary microtome. Sections were affixed to glass slides with Haupts adhesive and stained using Johansen's quadruple stain method (Johansen, 1940). The stained sections were mounted in 'Permunt' and examined with the aid of a compound microscope.

RESULTS

Pathogenicity

Experiment I

Plant growth was almost uniform throughout the experiment and no symptoms on the above-ground plant portions were observed at any time. Data on plant heights taken at 30, 73, 90 and 128 (terminal) days after inoculation showed no significant differences between inoculated and uninoculated plants (Table 1). Green weights of leaves taken at three different primings and at the termination of the experiment were combined. An analysis of variance of the data on leaf weights disclosed no significant differences between the inoculated and uninoculated plants (Table 2). An analysis of variance of the data on root weights disclosed highly significant differences between treatments (Table 2). Duncan's multiple range test revealed that the root weights of the uninoculated plants were significantly higher at the one per cent level than those of plants inoculated with each of the three population levels of T. claytoni. Root weights of the uninoculated plants were also significantly higher at the five per cent level than those of plants inoculated with 500 specimens of H. dihystrera + 500 specimens of T. claytoni. Differences were not significant between the roots of uninoculated plants and those of plants inoculated with 1,000 and 2,000 specimens of H. dihystrera or 1,000 specimens of H. dihystrera + 1,000 specimens of T. claytoni.

Table 1.--Means of heights (cm) of tobacco plants measured on four different dates after inoculation on October 14, 1966.

Treatments	Days after inoculation			
	30*	73*	90*	128*
1. 500H ^a	26.0	56.0	77.2	113.5
2. 500T	27.5	57.2	77.5	113.7
3. 1,000H	25.2	55.2	76.2	112.7
4. 1,000T	29.2	53.7	71.7	103.7
5. 500H + 500T	27.0	58.7	80.7	118.5
6. 2,000H	31.2	60.2	81.0	120.2
7. 2,000T	26.7	52.7	72.5	101.2
8. 1,000H + 1,000T	29.2	55.7	73.2	102.2
9. Uninoculated	29.5	61.0	84.0	122.2

* = Differences not significant at the 5 per cent level of probability.

^aH = Helicotylenchus dihystrera, T = Tylenchorhynchus claytoni

Table 2.--Means of tobacco leaf and root weights (g) and the populations of nematodes 128 days after inoculation on October 14, 1966.

Treatments	Leaves ^a	Roots ^b	Nematodes	
			Populations	Increase
1. 500H ^c	80.2	28.9 ^a	3,345	6.5X
2. 500T	87.2	28.1 ^a	58,000	116X
3. 1,000H	85.3	41.4 ^{de}	4,250	4X
4. 1,000T	83.6	29.9 ^{abc}	117,875	118X
5. 500H + 500T	86.9	32.9 ^{ab}	1,995	4X
			93,625	187X
6. 2,000H	83.6	36.8 ^{cde}	10,830	5.5X
7. 2,000T	87.9	29.7 ^{ab}	211,500	105X
8. 1,000H + 1,000T	86.2	36.0 ^{bcde}	4,337	4X
			115,000	115X
9. Uninoculated	84.4	42.7 ^e	0	0X

^a Differences not significant at the 5 per cent level of probability

^b Differences isgnificant at the 1 per cent level of probability.
Treatment means having the same letter are not significantly different.

^c H = Helicotylenchus dihystrera, T = Tylenchorhynchus claytoni

Root systems of plants inoculated with T. claytoni exhibited some discoloration and shriveled appearances. Well defined lesions or extensive root necrosis were not observed.

In every case the number of nematodes increased over the initial inoculum. The numbers of H. dihystrera increased as follows: on plants inoculated with 500 specimens about six and one-half times, with 1,000 specimens about four times, and with 2,000 specimens about five and one-half times. The numbers of T. claytoni increased as follows: on those plants inoculated with 500 specimens about 116 times, with 1,000 specimens about 118 times and with 2,000 specimens about 105 times. Nematodes on those plants inoculated with H. dihystrera + T. claytoni increased as follows: with 500 specimens of each, H. dihystrera increased about four times whereas T. claytoni increased about 187 times; and with 1,000 specimens of each, H. dihystrera increased about four times and T. claytoni about 115 times (Table 2).

Experiment II

Symptoms of injury were not detected during the experiment. Inoculated and uninoculated plants were uniform in general appearances in all treatments and plant heights were not significantly different (Table 3). Green weights of the leaves and weights of the roots at termination of the experiment were not significantly greater for uninoculated plants than for inoculated plants (Table 3). Examination of the root systems of inoculated plants revealed no discoloration or other symptoms of root injury.

Populations of T. claytoni increased during the course of the experiment at all three levels of inoculum. The nematodes increased about 30 times when 500 specimens were used as inoculum, about 17 times when 1,000 specimens were used and about 10 times when 2,000 specimens were used. Numbers of

Table 3.--Means of plant heights (cm), and green leaf and root weights (g) of tobacco, and the terminal populations of nematodes, 80 days after inoculation on June 16, 1967.

Treatments	Heights ^a	Weights ^a		Nematode Populations
		Leaves	Roots	
1. 500H ^b	160.0	197.6	75.7	257
2. 500T	162.0	200.2	78.8	15,063
3. 1,000H	158.5	190.4	75.4	725
4. 1,000T	171.2	188.1	86.9	17,648
5. 500H + 500T	163.7	197.9	72.5	182
6. 2,000H	161.7	188.9	81.8	9,572
7. 2,000T	157.7	192.8	69.6	1,617
8. 1,000H + 1,000T	169.5	196.8	81.4	20,552
9. Uninoculated	164.7	190.0	89.4	393
				17,741
				0

^a Differences not significant at the 5 per cent level of probability.

^b H = Helicotylenchus dihystrera, T = Tylenchorhynchus claytoni

Each treatment replicated four times.

H. dihystrera at termination of the experiment averaged fewer than the initial numbers in all cases. In combination with H. dihystrera, T. claytoni increased about 19 times when 500 specimens of each species were used as inoculum, and about 18 times when 1,000 specimens of each species were used (Table 3).

Experiment III

Differences in growth between the inoculated and uninoculated plants could not be detected during the course of the experiment. Symptoms of injury to above-ground portions of the inoculated plants were not observed. The heights, green weights of leaves and fresh weights of roots of inoculated and uninoculated plants were not significantly different (Table 4). No discoloration or other symptoms of root injury were evident.

Saccate females, some with egg masses, of R. reniformis were found embedded in roots of plants inoculated with specimens of this parasite. Malformation or swelling of the infected root, at or near the feeding sites were not observed. Neither necrosis nor lesions were evident.

Final populations of T. claytoni in every case increased over the inoculum but the numbers of H. dihystrera and R. reniformis decreased whether inoculated alone or in combination (Table 4).

Experiment IV

The results of this experiment were very similar to those of Experiment III, differing only in the final populations of the parasites. Symptoms of damage to the above-ground plant parts due to nematodes were not observed. Data on plant heights, green weights of leaves and fresh weights of roots revealed no significant differences between inoculated and uninoculated plants (Table 5).

Table 4.--Means of plant heights (cm) green leaf and root weights (g) of tobacco and terminal populations of nematodes, 70 days after inoculation on June 3, 1967.

Treatments	Heights ^a	Weights ^a		Nematode Populations
		Leaves	Roots	
1. 500H ^b	128.2	148.2	48.9	175
2. 500R	123.7	158.5	43.2	86
3. 500T	122.2	147.5	53.9	6,328
4. 1,000H	134.7	154.7	45.8	261
5. 1,000R	131.2	159.7	52.9	108
6. 1,000T	126.5	154.0	45.8	8,025
7. 500H + 500R	122.7	152.7	45.2	130
8. 500R + 500T	123.5	154.7	45.6	125
9. 500H + 500T	130.0	154.2	52.1	70
10. 500H + 500R + 500T	130.7	147.7	49.5	2,475
11. 1,500H	122.5	142.5	46.8	120
12. 1,500R	126.5	145.5	45.1	1,971
13. 1,500T	131.5	161.7	52.3	160
14. 1,500H + 1,500R + 1,500T	133.2	151.7	50.7	96
15. Uninoculated	123.5	153.0	38.1	2,752
				388
				130
				13,137
				560
				247
				12,271
				0

^a Differences not significant at the 5 per cent level of probability.

^b H = Helicotylenchus dihystrera, R = Rotylenchulus reniformis,
T = Tylenchorhynchus claytoni

Each treatment replicated four times.

In each case the average number of nematodes on termination of the experiment was greater than the number used as inoculum (Table 5).

Histopathology

Adult females of Rotylenchulus reniformis ranging from eelworm to kidney-shaped (Fig. 1 A-E) were found in the stained tobacco roots. No life cycle stage was found with the body totally within a root. The majority of specimens were found in the secondary roots with developing females near the root tips and more mature kidney-shaped females (Fig. 1 D, E) some distance away from the root tips. Some of these females had deposited eggs into the gelatinous matrices, others had secreted the matrices but had not deposited eggs, and still others had not yet secreted the matrix. In all cases the posterior part of the female and the matrix and eggs were outside the roots (Fig. 3). On several occasions two or more specimens were found feeding in the same area (Fig. 4), but this was not the usual case.

This parasite fed in the stele of the roots, apparently on pericyclic cells (Fig. 5, 6, 7 A). The head was never found in the pericycle but appeared to remain in the endodermal region with the stylet penetrating the pericyclic cells. Cells fed upon enlarged in size to crush cells of the stele and endodermis. The hypertrophied pericycle cells in many cases encircled the vascular cylinder (Fig. 3) and extended longitudinally 15-19 cells along the root (Fig. 8). This condition was most often associated with females which had produced eggs. A lesser number of cells was affected where the females were immature (Fig. 9 A-E).

The enlarged cells stained darker than other root cells and contained enlarged nuclei (Fig. 7 A, 8, A, B). Affected cells near the head of a

Table 5.--Means of plant heights (cm) green leaf and fresh root weights (g) of tobacco, and terminal populations of nematodes, 42 days after inoculation on July 28, 1968.

Treatments	Heights ^a	Weights ^a		Nematode Populations
		Leaves	Roots	
1. 500H ^b	70.7	71.6	27.5	1,400
2. 500R	66.7	72.1	31.0	1,356
3. 500T	70.5	76.0	31.1	19,293
4. 1,000H	69.7	72.4	29.5	2,843
5. 1,000R	69.7	73.2	31.7	3,517
6. 1,000T	67.0	73.1	34.5	32,558
7. 500H + 500R	70.2	76.1	34.2	866 1,566
8. 500R + 500T	67.0	72.0	30.9	1,251 9,878
9. 500H + 500T	67.2	73.3	35.0	761 5,442
10. 500H + 500R + 500T	69.0	70.4	31.5	866 1,488 7,761
11. 1,500H	69.5	70.3	31.6	3,576
12. 1,500R	69.5	80.5	36.0	5,136
13. 1,500T	64.0	74.1	35.3	48,921
14. 1,500H + 1,500R + 1,500T	65.7	73.3	36.5	1,767 2,563 16,021
15. Uninoculated	67.7	74.1	35.7	0

^a Differences were not significant at the 5 per cent level of probability.

^b H = Helicotylenchus dihystris, R = Rotylenchulus reniformis,
T = Tylenchorhynchus claytoni

Figure 1.--Photomicrographs of adult females of Rotylenchulus
reniformis. A, Eelworm shape; B, C, D, developing stages; E, mature
kidney-shape. F, An enlargement of A, showing vulva (v). Didelphic
reflexed ovaries are not discernible in this photograph.

Figure 2.--A, Cross section view of a tobacco root from an uninoculated plant. B, An enlarged view of A.

Figure 3.--A-D, Photomicrographs of cross sections of tobacco roots infected with Botryotrichum paniformis showing various shapes of hypertrophied cells in the pericycle region surrounding vascular cylinders.

Figure 4.--Photomicrograph of two females of Rotylenchulus reniformis feeding in the same area of a tobacco root. Note the dissolution of cell walls in the hypertrophied cells.

Figure 5.--Cross section view of a tobacco root infected with Rotylenchulus reniformis showing the nematode head positioned near the hypertrophied pericycle. Note the dark stained structure (a) in the cell near the nematode head (b).

Figure 6.--An enlarged view of a portion of Fig. 5. Note the dark stained structure in Fig. 6 is a tubular strand (a) connecting from the point of stylet insertion (b) into the hypertrophied cell.

Figure 7.--A, Cross section view of a tobacco root infected with *Rotylenchulus reniformis*. Note that some hypertrophied cells are in double layers. B, A comparable uninfected root.

nematode were larger than those more distant, giving a spindle-shaped appearance in longitudinal section (Fig. 8 A). Occasionally cell walls near the feeding site were dissolved (Fig. 10), a condition which was more prevalent and usually more extensive where more than one specimen fed at the same site (Fig. 4).

The walls of two or more cells near the head of a parasite were thickened (Fig. 10). Pink-stained tubular strands were sometimes seen in a cell to which the lips of a nematode were appressed (Fig. 6). These strands were curved and appeared to be connected to or near the stylet of the nematode.

Figure 8.--A, View of a longitudinal section of tobacco root infected by *Rotylenchulus reniformis* showing hypertrophied cells and enlarged nuclei. Note the decreasing size of the hypertrophied cells away from the site of feeding (a). B, An enlarged view of a portion of A.

Figure 9.--A-E, Photomicrographs of serial sections of a tobacco root infected by Rotylenchulus reniformis showing the development of hypertrophied cells (arrows) which apparently originated in the pericycle. A is more distant and E is nearest the feeding site. Note the increasing numbers of hypertrophied cells from A to E. Anterior parts of the nematode are not seen but posterior portions can be seen at the top of D and E. F, A comparable cross section view of an uninfected root.

Figure 10.--Photomicrograph of a tobacco root showing two nuclei (a) of a hypertrophied cell which had been fed upon by Rotylenchulus reniformis. Note the thick cell walls near the stylet insertion (b). The nematode head (c) was moved away from the point of stylet insertion during the preparation process.

DISCUSSION

Pathogenicity Experiments

Experiment I

Specimens of T. claytoni fed as ectoparasites on tobacco and reproduced in large numbers under the conditions of the experiments. The spiral nematode, H. dihystrera, also parasitized tobacco roots but did not reproduce in numbers as large as did T. claytoni. Single and multiple inoculations of these two species caused no differences in top growth of plants when compared to the uninoculated plants. Single or multiple inoculations with different inoculum levels of T. claytoni caused no stunting of plants, in contrast to the results reported by Graham (1954). Quantitative yields of the plants as indicated by green weights of leaves were not affected by this parasite. It is possible that quality of the tobacco might have been affected due to parasitism by T. claytoni.

All three inoculum levels of T. claytoni with an exposure period of 128 days resulted in reduced root systems of parasitized plants when compared to those of the control plants. Growth of the above-ground portions of the plants was not affected by the root reduction.

No explanation can be offered for the root symptoms expressed in the experiment when the plants were each inoculated with 500 specimens of H. dihystrera. The fact that no symptoms were visible at the higher population levels indicates, however, that the root retardation was due to factors

other than the presence of this parasite. Root retardation in the T. claytoni treatments was always accompanied by some discoloration and shriveled appearance.

Inoculations with 500 specimens of T. claytoni + 500 specimens of H. dihystrera also resulted in retarded root systems of the plants. It is probable that T. claytoni was primarily responsible for the retardation. Where T. claytoni and H. dihystrera were inoculated in combinations (500 or 1,000 specimens each), terminal populations of each species were about the same as when each was inoculated alone at comparable levels. It was concluded that neither T. claytoni nor H. dihystrera suppressed or stimulated reproduction of the other species.

Experiment II

Treatments in this experiment were similar to those in Experiment I but differed in time of year, a shorter period of exposure and the use of fumigated soil for Experiment II. Nevertheless, plant growth was better than that in Experiment I. The better growth was probably due to a longer day length and the use of fumigated soil.

Terminal populations of T. claytoni in every case were several times greater than the inoculum level (Table 3). However, the terminal numbers were not so great as those of Experiment I, possibly due to the shorter duration of the experiment, the different method of extracting nematodes from soil and the use of fumigated soil.

The numbers of H. dihystrera recovered on termination of the experiment were less than the inoculum used, whether inoculated alone or in combination with T. claytoni (Table 3). Nonetheless, all the recovered specimens had

dark intestines indicating continuous feeding. It is probable that the small number recovered was due to inefficiency of the extraction technique. Weber and Williams (1968) reported recovery of only 53.3 per cent of H. erythrinae with the centrifugal-flotation technique. Reproductive potential of H. dihystrera might also be low. The life cycle of this species is not known, but Taylor (1961) reported that the life cycle of H. microlobus, a similar species, was completed on tomato in 35 days at 22.8°C and at least 30 days at 26.7°C. If the life cycle of H. dihystrera is of similar duration, the exposure period of 80 days would have been sufficient for two or at most three generations in this experiment. Furthermore, tobacco may not be a favorable host for H. dihystrera.

Experiment III

All three nematode species were used in this experiment in single and multiple inoculations. No significant differences occurred between inoculated and uninoculated plants (Table 4) but means of plant heights, leaf weights and root weights were smaller than those of plants in Experiment II, presumably due to the shorter period of the experiment. Less space was allowed between the pots and this may have hampered plant growth. Terminal populations of nematodes were determined using the modified Baermann funnel technique (Christie and Perry, 1951). Treatments that were inoculated with T. claytoni alone or in combination with one or both of the other species resulted in terminal populations of T. claytoni greater than the inoculum. However, terminal populations of H. dihystrera and R. reniformis recovered were less than the inoculum whether inoculated alone or in combination with one or both of the other species. The Baermann funnel as used was not an efficient method of extracting nematodes from soil and this was undoubtedly

the primary reason for the smaller numbers recovered. The relatively short period of exposure (70 days) might have been a contributing factor also. Other possible reasons were discussed under Experiment II.

Experiment IV

This experiment was a repetition of Experiment III except that the exposure period was shortened to 42 days. While plant heights, leaf weights and root weights were less than those of plants in previous experiments, no symptoms of injury were observed. In this experiment the rapid centrifugal-flotation technique (Jenkins, 1964) was used to isolate nematodes from the soil. In spite of the relatively short duration of the experiment all three nematode species were recovered in greater numbers than used as inoculum indicating that this method of removal is more efficient than the Baermann funnel. Also no determination was made of the number of females of R. reniformis embedded in the roots. The fact that T. claytoni was always recovered in larger numbers than were the other species demonstrates that the reproductive potential of T. claytoni obviously is greater than that of H. dihystra and R. reniformis under the conditions of these experiments.

When all three nematode species were inoculated alone at 500, 1,000 and 1,500 specimens terminal populations of each 42 days after inoculation increased in proportion to the inoculum level (Fig. 11). Helicotylenchus dihystra increased an average of about 2.6 times, Rotylenchulus reniformis about 3 times and Tylenchorhynchus claytoni about 34.5 times.

Populations of H. dihystra 42 days after inoculation were not significantly different when 500 specimens were inoculated alone than when 500 specimens were inoculated in combination with 500 T. claytoni or 500 R. reniformis or both (Table 6). The same relationship held when 1,500 specimens

Figure 11.--Comparison of terminal population levels of three nematode species at different levels of inoculum 42 days after inoculation.

Table 6.--Comparison of terminal populations of Helicotylenchus dihystera inoculated alone and in combinations as indicated.

Treatments	Replications				Means ^a
	I	II	III	IV	
500H ^b	945	1,295	1,260	2,100	1,400
500H +	525	665	1,435	840	866
500R					
500H +	805	560	1,155	525	761
500T					
500H +	595	735	945	1,190	866
500R +					
500T					

^a Differences were not significant at the 5 per cent level of probability.

^b H = Helicotylenchus dihystera, R = Rotylenchulus reniformis
T = Tylenchorhynchus claytoni

Table 7.--Comparison of terminal populations of Rotylenchulus reniformis^a inoculated alone and in combinations as indicated.

Treatments	Replications				Means ^b
	I	II	III	IV	
500R ^c	1,470	910	2,415	665	1,365
500R +	980	1,995	1,925	1,365	1,556
500H					
500R +	630	770	2,625	980	1,251
500T					
500R +	1,715	1,020	1,785	1,435	1,488
500H +					
500T					

^a Larvae, males and eelworm-shaped females were included.

^b Differences were not significant at the 5 per cent level of probability.

^c R = Rotylenchulus reniformis, H = Helicotylenchus dihystrera
T = Tylenchorhynchus claytoni.

of each species were used (Table 9). In both cases, however, the means were higher when H. dihystrera was used alone.

Populations of R. reniformis 42 days after inoculation were not significantly greater when 500 specimens were inoculated alone than when 500 specimens were inoculated in combination with 500 specimens of T. claytoni, 500 specimens of H. dihystrera or both (Table 7). However, when 1,500 specimens of R. reniformis were inoculated alone and when 1,500 were inoculated with 1,500 T. claytoni and 1,500 H. dihystrera differences were significant at the 5 per cent level (Table 10). While the presence of the other nematodes did not effect reproduction of R. reniformis at the lower inoculum levels, they did have an apparent effect at the higher level.

Populations of T. claytoni 42 days after inoculation were not significantly different when 500 specimens were inoculated alone than when 500 specimens were inoculated with 500 H. dihystrera, 500 R. reniformis or both. The means, however, were higher when inoculated alone (Table 8). When 1,500 T. claytoni were inoculated alone and when 1,500 were inoculated with 1,500 H. dihystrera and 1,500 R. reniformis differences were significant at the 1 per cent level (Table 11). The presence of the other two nematodes definitely influenced reproduction by T. claytoni. The influence probably was competition for feeding sites and/or food.

Histopathology

Penetration of roots by the parasite was not observed, but since eelworm-shaped females were observed near the root tip and more mature females farther away, it seems apparent that the eelworm-shaped females penetrated near the root tips. The anterior portion of their bodies

Table 8.--Comparison of the terminal populations of Tylenchorhynchus claytoni inoculated alone and in combinations as indicated.

Treatments	Replications				Means ^a
	I	II	III	IV	
500T ^b	5,635	18,340	10,850	42,350	19,293
500T +	4,410	4,690	8,365	4,305	5,442
500H					
500T +	7,105	11,970	10,780	9,660	9,878
500R					
500T +	7,945	8,925	8,015	6,160	7,761
500H +					
500R					

^a Difference were not significant at the 5 per cent level of probability.

^b T = Tylenchorhynchus claytoni, H = Helicotylenchus dihystrera,
R = Rotylenchulus reniformis.

Table 9.--Comparison of the terminal populations of Helicotylenchus dihystrera inoculated alone and in combination with Rotylenchulus reniformis and Tylenchorhynchus claytoni.

Treatments	Replications				Means ^a
	I	II	III	IV	
1,500H ^b	5,415	4,165	2,730	1,995	3,576
1,500H +	1,995	1,330	2,205	1,540	1,767
1,500R +					
1,500T					

^a Differences were not significant at the 5 per cent level of probability.

^b H = Helicotylenchus dihystrera, R = Rotylenchulus reniformis
T = Tylenchorhynchus claytoni.

Table 10.--Comparison of terminal populations of Rotylenchulus reniformis^a inoculated alone and in combination with Helicotylenchus dihystrera and Tylenchorhynchus claytoni.

Treatments	Replications				Means ^b
	I	II	III	IV	
1,500R ^c	3,745	3,955	6,405	6,440	5,136
1,500R +	2,555	1,715	3,500	2,485	2,563
1,500H. +					
1,500T					

^a Only larvae, males and eelworm-shaped females were included.

^b Differences significant at the 5 per cent level of probability.

^c R = Rotylenchulus reniformis, H = Helicotylenchus dihystrera
T = Tylenchorhynchus claytoni.

Table 11.--Comparison of terminal populations of Tylenchorhynchus claytoni inoculated alone and in combination with Helicotylenchus dihystrera and Rotylenchulus reniformis.

Treatments	Replications				Means ^a
	I	II	III	IV	
1,500T ^b	28,560	62,475	27,650	77,000	48,921
1,500T +	11,795	5,390	20,125	26,775	16,021
1,500H +					
1,500R					

^a Differences significant at the one per cent level of probability.

^b T = Tylenchorhynchus claytoni, H = Helicotylenchus dihystrera
R = Rotylenchulus reniformis.

penetrated the epidermis, cortical parenchyma and endodermis and only the stylet penetrated the stele. As the nematode began to feed on a pericyclic cell its esophageal gland secretions entered the cell to alter the protoplasmic contents. The cell began to enlarge and then stained darker than surrounding cells. As feeding continued other pericyclic cells were affected both in a circular and in a longitudinal direction. The cells near the head of the nematode were larger than those away from the head, due apparently to the fact that these cells received the stimulatory esophageal gland secretions in more concentrated form than did the more distant cells. In a few cases cells other than pericycle became involved, probably protophloem or protoxylem cells.

As the females matured the root continued to grow so that mature females were always found some distance from the root tip.

Males were never observed feeding on tobacco roots, which agrees with the statement of Linford and Oliveira (1940). Occasionally, males were found in the gelatinous matrix secreted by the female, but copulation was not observed.

Birchfield (1962) reported that R. reniformis fed on phloem in the cotton plant, and Linford and Oliveira (1940) reported that it fed on the cortex in cowpea. Apparently different tissue is used as the feeding site, depending on the host plant, the population of the nematode or both.

Cytoplasmic response of pericyclic cells to the feeding of R. reniformis on tobacco roots was similar to the response of cortical cells of susceptible citrus roots fed upon by Tylenchulus semipenetrans Cobb (Van Gundy and Kirkpatrick, 1964). However, no cell enlargement occurred in citrus nor was the area involved so large as in tobacco.

Mankau and Linford (1960) reported tubular strands in the syncytium of 'Ladino' clover fed on by Heterodera trifolii Goffart, but they reported

these strands as being hyaline. The strands in tobacco stained pink. The strands are apparently related in some way to feeding as suggested by Mankau and Linford (1960) but their exact nature remains unknown.

SUMMARY

Tylenchorhynchus claytoni, Helicotylenchus dihystrera, and Potytlenchulus reniformis are parasites of shade-grown tobacco, and are found frequently in shade-grown tobacco areas of North Florida.

Pathogenicity experiments using shade-grown tobacco variety 'Dixie Shade' indicated that single inoculation of T. claytoni at levels of 500, 1,000, or 2,000 specimens per 2,000 ml of soil significantly retarded root production of tobacco plants 128 days after inoculation. Some discoloration and shriveled appearance accompanied root retardation, but lesions or necrosis were not evident. Exposure periods of 42, 70 or 80 days did not result in root retardation.

Multiple inoculations of specimens of T. claytoni with either H. dihystrera or R. reniformis or both at any inoculum level usually did not affect root production of plants compared to the uninoculated plants.

Inoculations with H. dihystrera or R. reniformis alone or in combination did not affect top growth when compared to uninoculated plants.

Terminal populations of T. claytoni when inoculated alone or in combination with H. dihystrera, R. reniformis or both increased several times over the inoculum levels. Terminal populations of H. dihystrera and R. reniformis were in most cases greater than the inoculum levels but numbers were not as great as with T. claytoni. Populations of H. dihystrera 42 days after inoculation were not significantly different when 500 specimens were inoculated alone than when 500 specimens were inoculated in

combination with 500 T. claytoni, 500 R. reniformis or both. The same relationships held when 1,500 specimens of each species were used. Populations of R. reniformis 42 days after inoculation were significantly greater when 1,500 specimens were inoculated alone than when 1,500 specimens were inoculated in combination with the equal numbers of T. claytoni and H. dihystrera. The same relationships did not hold true when 500 specimens of each species were used. Populations of T. claytoni 42 days after inoculation were significantly greater when 1,500 specimens were inoculated alone than when 1,500 specimens were inoculated with 1,500 H. dihystrera and 1,500 R. reniformis. However, when 500 specimens of each species were used there were no significant differences in terminal populations of T. claytoni.

Histopathological studies of tobacco roots infected by Rotylenchulus reniformis disclosed:

1. Eelworm-shaped females penetrated tobacco roots near the root tips to initiate infection.
2. Parasites penetrated the epidermis, cortical parenchyma, and endodermis and fed in the pericyclic region. Only the stylet penetrated into pericyclic cells.
3. Hypertrophy and enlargement of nuclei occurred in the pericyclic region due to the feeding of the nematodes.
4. Hypertrophied cells extended from a cell in which the nematode stylet was inserted to cells in both a longitudinal and a circular direction.
5. The cytoplasm of hypertrophied cells stained darker than did uninfectd surrounding cells.

Considering the data on pathogenicity and the results of histological studies, H. dihystrera, T. claytoni, and R. reniformis all appear to be weak pathogens of tobacco but none a severe pathogen.

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BIOGRAPHICAL SKETCH

Sawart Ratanaworabhan was born on March 14, 1936 in Prae, northern Thailand. After attending public schools at Prae and Chiangmai, he enrolled at Kasetsart University, Bangkok, where he received the degree of Bachelor of Science in Agriculture in 1960. He worked as plant quarantine inspector until 1961, when he joined the staff of the Rice Protection Research and Training Center, Department of Rice, Bangkok, Bangkok. He came to the United States in March, 1963 and enrolled in the College of Agriculture, University of Florida in the summer trimester seeking a second bachelor's degree. He was awarded a B. S. A. degree in April, 1964. He entered the Graduate School in the spring of 1964 and was awarded the degree of Master of Science in December, 1965. He continued his graduate work toward the degree of Doctor of Philosophy while holding a teaching assistantship in the Department of Entomology and Nematology until he completed the requirements for the degree in March, 1969.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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