

Host-Parasite Relationships of *Hoplolaimus columbus* on Cotton and Soybean¹

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Abstract: *Hoplolaimus columbus* suppressed growth and pod yield of soybean in greenhouse tests. Although populations of *H. columbus* decreased in short-term experiments, increases occurred in long-term studies. The nematode caused extensive damage to the cortical parenchyma and occasionally to the endodermal-vascular region of both cotton and soybean roots. The nematode frequently entered secondary root primordia. Roots of soybean parasitized by *H. columbus* at high inoculum levels were severely damaged. The relationship of populations of *H. columbus* and stunting of soybean and cotton is discussed. *Key Words:* pathogenicity, histopathology, Columbia lance nematode.

The Columbia lance nematode, *Hoplolaimus columbus* Sher was found in Richland County, South Carolina, during the early 1950s by Q. L. Holdeman. Since then it has been detected in approximately

30,400 ha of some of the best agricultural land in the state and has been estimated to cause an annual loss in excess of \$4 million. Cotton, *Gossypium hirsutum* L., and soybean, *Glycine max* L., growing in fields heavily infested with the nematode, are often chlorotic and stunted (6). The nematode has a wide host range and can survive on a number of weeds, vegetables, and other field crops (5). Several aspects of the biology of the nematode have been described (4), but the pathogenicity and the pathological effects on cotton and soybean have not been studied under laboratory

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conditions. This paper deals with host-parasite relationships of *H. columbus* to soybean and cotton. Objectives included studies of pathogenicity on soybean and histopathological effects of *H. columbus* on soybean and cotton.

MATERIALS AND METHODS

Seeds of cotton, 'Deltapine-16', and soybean, 'Bragg', were surface sterilized in 95% ethanol for 5 min, and then immersed for 15-min in a 10% solution of commercial bleach (0.5% sodium hypochlorite). The seeds were then rinsed in four, 10-min washes of sterile distilled water; germinated; and grown, depending on the experiment, in plastic Petri dishes or in 250-ml bottles partially filled with sterilized quartz sand. Nematodes used in the study were extracted from Varina sandy loam by using centrifugal flotation (8) or Cobb's sieving and decanting method (3). They were then surface sterilized on a Baermann funnel assembly containing a solution of 86.6 µg/ml ethoxyethyl mercuric chloride (Aretan®, E. I. du Pont de Nemours & Co., Wilmington, Delaware) in distilled water, collected after 24 h, rinsed in sterile distilled water, and subsequently used as inoculum.

Pathogenicity: Two greenhouse experiments were conducted to determine the effects of *H. columbus* on soybean yields and to obtain information on its reproductive capacity on soybean. Fungicide-treated 'Hampton 266A' soybean seeds were planted singly in autoclaved Varina sandy loam in 20-cm diam clay pots containing 0, 500 or 4,000 nonsterile nematodes. Treatments were replicated six times and arranged in a random design. After 6 months, the plants were dried and weighed. Final numbers of nematodes/pot were determined by centrifugal-flotation for soil and by counting numbers in 0.5 g of roots stained with cotton blue lactophenol.

In the second experiment, cultures of *H. columbus* were increased on soybeans grown in autoclaved sandy loam soil in 5-liter glazed crocks in the greenhouse. Nematode counts were made after the soil was thoroughly mixed. The soil was then divided into three lots and, according to the number of nematodes from the laboratory assays, sterile soil was added to each in-

festated lot to provide initial population (Pi) levels of approximately 450, 900, and 2,000 nematodes/100 g soil. The soil was placed in 12.5-cm diam clay pots; soybean seeds were planted; and germinating seedlings were thinned to one/pot. Plants were maintained, harvested, and processed as described in the preceding experiment.

Histopathology: Fifty to five hundred specimens of all stages of *H. columbus* were added to single cotton or soybean plants 5 days after seeds were planted and grown as described earlier. Inoculated plants were harvested weekly, 7 to 28 days after inoculation. Root sections with nematodes actively feeding on soybean and cotton roots in plastic Petri dishes and glass bottles were processed, stained, and observed microscopically. A number of ca. 120-day-old soybeans also were dug from an infested field and processed for sectioning. Roots were fixed in FAA (formalin-acetic acid-alcohol) or CRAF III (chromic acid-alcohol-formalin), embedded in paraffin, and sectioned at 12 µm on a rotary microtome. Tissues were affixed to slides with celloidin and stained with safranin and fast-green. Picric acid-alcohol (0.5%) was used to help differentiate the stains (9).

RESULTS

Pathogenicity: The dry weights of tops, roots, and pods of soybean plants inoculated with *H. columbus* were lower than the controls containing no nematodes ($P = 0.05$) (Table 1). In the first experiment, which was concluded after 6 months, tops and roots of plants with Pi of 500 and 4,000 nematodes/pot weighed 21% and 23% less than plants without nematodes, respectively.

The nematode population increase was greater on plants receiving the lower inoculum level. Nematodes were found in stained roots of all nematode treatments. Roots of plants grown at high and medium inoculum levels were damaged severely, and the damage was reflected in top and root development. These roots were abbreviated, decayed, and had epidermal and cortical sloughing. Roots of plants grown at low inoculum levels were also damaged, but to a lesser extent. Plants growing in soil receiving the higher inoculum density were

TABLE 1. Growth of soybean as affected by inoculum levels of *Hoplolaimus columbus*.

Initial no. nematodes/pot (in 1000's)	Dry weight (gm)/plant [†]		Final no. nematodes/pot (in 1000's) [‡]
	Tops and roots	Pods	
<i>Experiment #1</i>			
4.0	51.8 a	9.0 a	5.2
0.5	53.3 a	11.8 b	2.2
0.0	67.2 b	16.4 c	0.0
<i>Experiment #2</i>			
32.6	0.3 a		12.7
14.5	0.6 a		6.7
7.0	1.8 b		4.9
0.0	5.9 c		0.0

[†]Means followed by the same letter are not significantly different ($P = 0.05$), according to Duncan's multiple range test. Experiments #1 and #2 harvested after 6 months and 2 months, respectively.
[‡]Represents estimates of nematodes in the soil only, not including those within roots.

discolored in comparison with other treatments.

In the second experiment, which was concluded after 60 days, dry weights of tops and roots of plants with the highest initial inoculum level were 95% less than those of the controls. Yields of plants at the two higher initial inoculum levels were lower than those at the low inoculum level and those at the control ($P = 0.05$). Nematode numbers declined in this and other tests in which naturally infested field soil was used. All stages of *H. columbus*, including eggs, were observed in roots of plants grown at the different inoculum levels.

Histopathology: Within 48 h after inoc-

ulation, *H. columbus* fed on roots of cotton and soybean seedlings growing in quartz sand. Nematodes were often present in the rhizoplane and within lateral roots that had emerged from the cortex of the primary root (Fig. 1-A). Two to eight nematodes were usually present at this region. The nematodes fed both ecto- and endoparasitically at one location for periods ranging from a few minutes to several hours. Surface depressions and epidermal-cortical browning were seen after 96 h. The lesions were several cell layers deep and were oriented parallel to the root axis. They enlarged in a longitudinal direction as the nematode advanced in the root cortex. In cotton, the nematode usually behaved as a semi-endoparasite and in soybean, an endoparasitic relationship was predominant. Differences were observed in the manner of penetration in two plant types. In cotton roots, nematodes most often were found situated in the cortex perpendicular to the stele (Fig. 1-B). The anterior end of the nematode was often reflexed to lie parallel and adjacent to the vascular region. In contrast, most individuals in the soybean cultivar were oriented parallel to the root axis but some nematodes were also seen partially encircling the vascular system. Eggs were found adjacent to the epidermis and in cortical areas vacated by the nematode 5-8 days after the plants were inoculated.

Examination of root-tissue sections showed that nematode migration through the cortex resulted in cavities; ruptured cells with associated debris; and red, abnormally dark, and enlarged nuclei. Cells with brown, granular cytoplasm were also

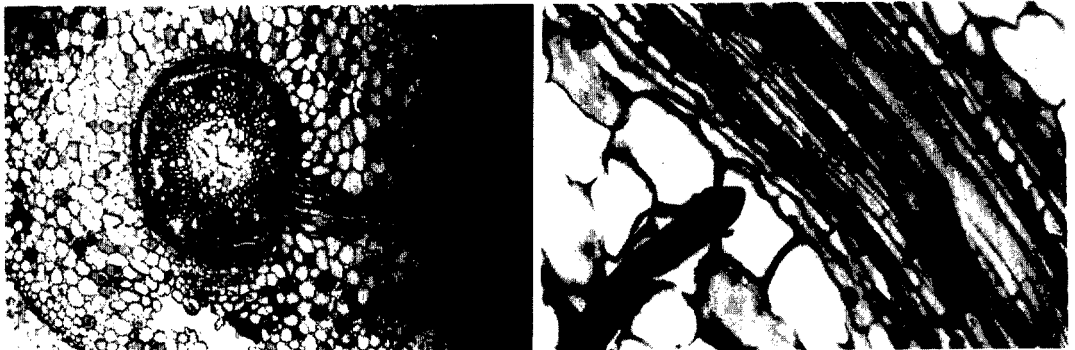


FIG. 1-(A-B). *Hoplolaimus columbus* in roots of cotton. A) Five *Hoplolaimus columbus* in region of lateral-root emergence from primary root. B) Nematode in inner cortex.

apparent in tissues where the nematodes had fed (Fig. 2 A-C). The nematodes were most often found in the cortex of both cotton and soybean roots. Often they penetrated as deep as the endodermis. The feeding sites stained darker red with the safranin, and vacuolated and ruptured cells were also present. None of these reactions were found in the stele.

Evidence of vascular feeding was inconclusive; however, there was some indication of phloem invasion in a tissue section of soybean root where one or two cells were ruptured and where the feeding cavity extended to the stele (Fig. 2-D). Rapid cell division or nuclear division was not observed.

DISCUSSION

Hoplolaimus columbus can cause extensive damage to soybean plants. We recovered as many as 1,800 individuals/100 cm³ of soil from about soybean roots. Soil fumigation can minimize the effects of the nematode, and plants growing in fumigated soil usually exhibit greater growth and

higher yields (2, 11). We observed that stunting and low yields have been especially marked during dry seasons. However, we were unable to demonstrate the same effects in the greenhouse when these plants were parasitized by field populations of the nematode. When higher population levels were added to plants, low yields of soybean were observed in the greenhouse where moisture stress was not a factor in plant growth.

Soybean plants growing in soil with a high nematode population were stunted and chlorotic under greenhouse conditions. These symptoms are similar to the severe stunting and discoloration reported by Fasuliotis et al. for soybeans grown in fields with high populations of this species (6). Low initial populations of *H. columbus* (500/12.5-cm pot) suppressed pod and plant growth under artificial conditions, but these differences were not easily seen. Soybean plants in the field may be damaged by low populations even when differences are not easily observed. These differences, however, are greater when the plants are grown with inadequate moisture or nutrients.

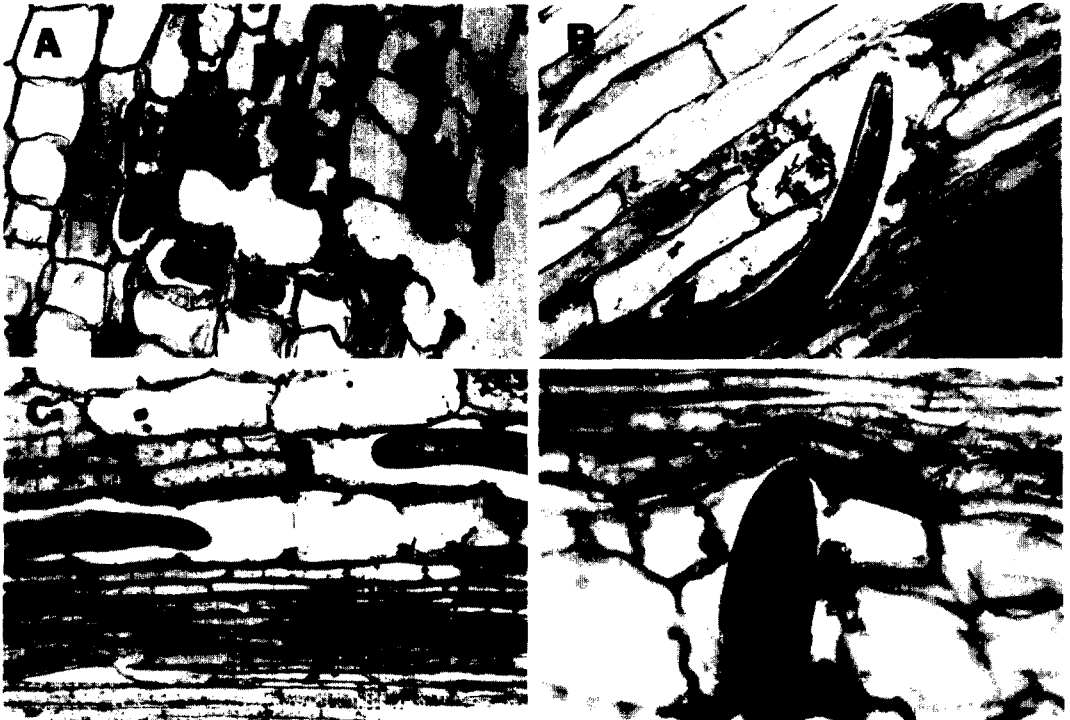


FIG. 2-(A-D). *Hoplolaimus columbus* in roots of soybean. A) Epidermal-cortical region infected and vacuolated by the nematode. B) Adult in cortex with broken cells and darkly stained areas. C) Nematode pathway through the cortex. D) Nematode penetrating endodermis.

The nematode population increased in the long-term test, but the final population was lower than the initial population in the short-term experiment. The lack of population increase may have resulted from a decrease in nematode reproductive rate due to nematode-damaged roots. A proportion of the high numbers of evenly distributed nematodes in the soil may not have had access to roots as a consequence of root damage. Egg production then would not have taken place since feeding is probably a prerequisite (4). Furthermore, there was only time for one generation (F. H. Smith, unpublished data).

Lance nematodes often congregated in the same locus. Groups were observed in stained primary and lateral roots and were often seen where lateral roots emerged from the cortex. This behavior has been noted for other nematodes (7, 12) and indicates that soybean roots may emit substances which are attractive and which may be more concentrated in primordial and wounded tissues.

Internal tissue damage was detected in differentially stained roots; cells ruptured by nematodes and nonpenetrated cells near the nematode's head were brown in live tissue and red in sections stained with safranin. These brown lesions were similar to those caused by *H. columbus* on alfalfa roots (4) and by *H. galeatus* on cotton (10). The vast amount of cortical damage observed in stained sections and sloughing of roots seen in fresh tissue account for the limited number of secondary roots found on heavily parasitized plants.

It is likely that *H. columbus* would increase the incidence of *Fusarium* wilt pathogens in soybean and cotton fields since endodermal disruption was observed. Several forms of this pathogen have been noted in South Carolina fields (1) and Mountain notes that, where there is synergism be-

tween nematodes and wilt, the endodermis is not formed, or disrupted, or killed (13).

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