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Pathogenicity of Five Isolates of Root-Knot Nematodes (*Meloidogyne* Spp.) to Sugarcane Roots[1]

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INTRODUCTION

The agricultural economy of the Island of Puerto Rico is mainly based on the production of sugarcane. A major part of the land available for cultivation has been devoted to this purpose for many years. Although a great deal of research has been conducted on the diseases and pests of this crop, the possible effects of nematodes have been considered very little.

During investigations carried out in Puerto Rico in 1957–58, to determine the types of parasitic nematodes associated with sugarcane roots, species within the following genera were found: *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Criconemoides*, *Xiphinema*, *Longidorus*, and *Ditylenchus*.

There are plans to conduct a series of investigations to determine whether any of these genera of plant-parasitic nematodes are detrimental in the cultivation of this crop. The present work is a part of this long-range project. The root-knot nematode genus, *Meloidogyne*, was selected for consideration first, because it is most frequently present in soil and root samples.

To date only three root-knot nematode species have been identified as occurring in the roots of sugarcane in the Island. These species are *Meloidogyne incognita incognita*, *M. incognita acrita*, and *M. arenaria*. Of these, *M. incognita acrita* seems to be the most widely distributed. It has been found that races or strains exist within this species which are morphologically indistinguishable, but which can be separated by host-range differences.

[1] This work was the basis for a thesis submitted to the Graduate Faculty of the Alabama Polytechnic Institute, Auburn, Ala., in partial fulfillment of the requirements for the degree of Master of Science.

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Although root-knot nematodes penetrate, form galls, and multiply in sugarcane roots, it has been reported (*1*, *5*)[3] that they do little or no damage to this crop. In contrast to this, other authors (*2*, *14*) have reported that these nematodes may be detrimental. This work was performed in an effort to determine objectively what damage, if any, this nematode does to sugarcane roots.

Two different but related lines of investigation were conducted, the first as to the nature of the pathogenic effects of the nematodes; and the second concerning the suitability of sugarcane as a host for root-knot nematodes. Special efforts were also made to determine whether *M. incognita incognita*, *M. incognita acrita*, and *M. arenaria* exhibit differences in pathogenicity to sugarcane roots, and to assess the possibility of the existence of strains within *M. incognita acrita*.

Several commercial varieties of sugarcane are grown in Puerto Rico, but, to limit the scope of this investigation, only one variety was used. A recently produced variety, Puerto Rico 980, was selected since it produces the highest yield of sugar per acre per month. It is also highly resistant to some of the major diseases, and it is expected that this variety will be widely used.

LITERATURE REVIEW

Treub (*21*) in 1885, reported that *Heterodera javanica* attacked sugarcane roots in Java. This is probably the earliest report of root-knot nematode infection in sugarcane. He said that the larvae entered through the root tip and also may have penetrated the root through breaks or openings in the root surface. He found that the larvae move intercellularly to the primordium of a lateral root to start their feeding. He also noticed that five or six giant cells formed around the region of the nematode's head. These cells have thick walls and 30 to 40 nuclei each. Treub reported that both the central cylinder and the cortex of the root were involved in the formation of a gall.

Matz (*14*) in 1925, on the other hand, reported that galls in sugarcane roots were enlargements of the outer fleshy layer of the root only, while the central part was not deformed. In the same paper, he reported results of field studies with sugarcane infected by root-knot nematodes on the south coast of Puerto Rico. He described the above-ground symptoms as intense, waxy, golden-yellow colored bands in the leaves starting from the tips and extending to the sheaths in older plants. In young plants, 3 to 4 months old, the leaves became shriveled and streaked longitudinally with red, copper-colored areas divided by long dry areas. He claimed that heavily infected plants were characterized by a marked stunting, and that galls,

[3] Italic numbers in parentheses refer to Literature Cited, pp. 83–4.

which occurred mostly at the root tips, were about twice the diameter of the unaffected root in size. Boyd (*2*), working on sugarcane in Georgia in 1925, reported that this marked stunting could be used to detect root-knot nematode infestations in sugarcane fields. He also observed that the size of the gall attained diameters up to one-quarter of an inch.

While these investigators emphasized the importance of root-knot in sugarcane, Cook (*5*) in 1925, stated that this disease was of little importance in Puerto Rico. In addition to this Muir (*16*) in 1926, Zwaluwenburg (*22*) in 1929, and Jensen, *et al.* (*9*) in 1959, reported that root-knot of sugarcane in Hawaii was not very important, because gall formation did not lead to a breakdown of the roots. Some investigators (*9*, *14*, *22*) also emphasized that excessive numbers of lateral roots were formed around the galled region.

Cavities or pits, about one-half millimeter in diameter, are frequently found on sugarcane roots. These pits are formed in the cortical tissue and, generally, the central cylinder is not affected. This condition has been noted by investigators for many years. Edgerton and Tims (*7*) in 1927, investigating the sugarcane-disease situation in Louisiana, considered pitting to be caused by soil animals such as snails, centipedes, springtails, and nematodes. However, Treub (*21*), as early as 1885, reported that the cavities he studied were associated with root-knot nematodes because there were giant cells in their vicinity. Evidently, he is the only worker to have published such an observation. Matz (*14*) also came to the conclusion that cavities were caused by nematodes, but did not mention the giant cells reported by Treub.

A few other investigators published interesting statements regarding sugarcane attacked by root-knot nematodes. Bessey (*1*) in 1911, found that nematodes were abundant in root-knot-infected sugarcane roots, but their injury to the roots apparently was not great. Spencer (*19*) in 1919, found that some of the most important sugarcane-growing sections of Florida were "seriously infected" with root-knot nematodes. Martin, *et al.* (*13*) in 1956, found *M. incognita acrita* in sugarcane in Louisiana. Details regarding symptoms and pathogenicity were not mentioned by any of these workers.

Other than the previously mentioned work by Treub, there apparently is no published information regarding the cytology of sugarcane roots attacked by root-knot nematodes. The literature review which follows, although based on other crops, is pertinent to the cytological studies performed by the author.

Kostoff and Kendal (*11*) in 1930, studying the cytology of root-knot nematode galls on tobacco roots, found that giant cells were formed by an abnormal type of mitotic division. In this division cell walls did not form between the separated chromosomes; this resulted in multinucleate cells

the nuclei of which appeared in multiples of two. These nuclei agglutinated, their nuclear membranes dissolved, and their contents coalesced.

Christie (*4*) in 1936, studying root-knot nematode galls on tomato roots, concluded that entering larvae migrated intercellularly and finally located with their heads in the plerome near the beginning of the region of cell elongation. In contrast to the reports of Kostoff and Kendal (*11*), Christie observed that cell walls of cells receiving stimulation by the nematode dissolved, and their cytoplasmic contents coalesced to begin the formation of a giant cell. He agreed with the results of Kostoff and Kendal that the nuclear membranes dissolved and nuclei coalesced. He further pointed out that the pericycle was stimulated by the nematode, and this resulted in lateral root formation.

Krusberg and Nielsen (*12*) in 1957, found the intercellular migration of larvae, as reported by Christie (*4*) to occur in tomato roots, and also in the roots of sweetpotato, but only through the cortical tissue. Migration through the vascular tissue was intracellular. The dispersed nuclei of an individual giant cell acquired a pyriform shape, aggregated together with their narrow ends towards a central point, and then coalesced. These authors (*12*) also reported what appeared to be an abnormal condition of the xylem. This abnormality apparently was formed directly from xylem parenchyma and was characterized by secondary wall thickenings of annular, reticulate, or pitted elements. Although commonly associated with giant cells, this abnormality was considered to be a response of the xylem to injury. Krusberg and Nielsen considered that this injury, perhaps, is not peculiar to root-knot nematodes since it was also noted in association with necrotic cells in traces of sloughed-off lateral roots.

MATERIALS AND METHODS

SOURCES OF NEMATODES AND PREPARATION OF CULTURES

Five isolates of root-knot nematodes were collected in 1958 from various host plants in Puerto Rico and identified as follows: *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949; *Meloidogyne incognita incognita* (Kofoid and White, 1919) Chitwood, 1949; and three selections of *Meloidogyne incognita acrita* Chitwood, 1949, from three different hosts.

M. arenaria was collected from roots of pepper (*Capsicum annuum* L.) from a field at Río Piedras used for growing vegetables the previous 5 years. *M. incognita incognita* was collected from tobacco (*Nicotiana tabacum* L.) roots from a field at Isabela which had been in tobacco the previous 3 years. One selection of *M. incognita acrita* came from the roots of pineapple (*Ananas comosus* Merrill) in a field at Manatí where pineapples had been grown for the previous 7 years following sugarcane. The second

selection of *M. incognita acrita* was taken from the roots of cucumber (*Cucumis sativus* L.) grown at Corozal where vegetables had been growing for 1 year. The field had been used for sugarcane for a number of years prior to this. The third selection of *M. incognita acrita* was obtained from the roots of "culantro del monte" (*Eryngium foetidum* L.) which, along with weeds, had been growing for 5 years in a field located at Río Piedras.

Cultures of the nematode isolates were initiated from single egg masses taken from a female of each selection possessing a typical perineal pattern for the nematode species desired. Each of the five selected egg masses was introduced into the root zone of a separate tomato seedling grown in steam-sterilized soil. After about 90 days, egg masses were removed from the 5 plants on which the nematodes had been cultured and were transferred to 20 additional tomato plants for the further increase of the nematode isolates. Each group of isolates was kept on an individual bench to avoid cross-contamination.

TREATMENTS

In preparation for the treatments, sugarcane cuttings of the variety Puerto Rico 980 (P.R. 980) were grown in 10-inch clay pots in steam-sterilized soil. These plants, when 8 to 10 inches high, were transferred to halved steel drums of 55-gallon capacity filled with steam-sterilized soil. The drums had holes drilled in the bottoms for drainage and were raised from the ground by cement blocks.

Inoculum for the treatments was then obtained from the tomato plants in which the nematodes were cultured. Egg masses were removed from the roots and placed in water, and, after most of the eggs had hatched, approximately 5,000 larvae were counted and placed in a beaker containing 75 ml. of water. After 4 hours, the top 50 ml. were decanted through a 325-mesh sieve that retained all nematodes but permitted passage of other micro-organisms which might be present. These nematode-free supernatant water collections were used as treatments to determine the effects, if any, of these micro-organisms. The remaining suspensions in the beakers containing the nematodes plus other micro-organisms were used as separate treatments. Inoculum was applied to the sugarcane plants in the steel drums by pouring the nematode suspension or supernatant water through a funnel inserted in the soil at the base of the plants.

A total of 44 sugarcane plants in individual drums was used. Four drums, each containing one sugarcane plant, were inoculated for each nematode and for each supernatant treatment from the five different nematode isolates. Plants in four drums were left uninoculated to serve as controls.

The treated plants were allowed to grow 5 months before being harvested for examination. No fertilization was used in this short growing period.

Routine leaf-insect control was accomplished by the use of Dieldrin. Cardboard shields placed over the drums prevented accumulation of insecticide in the soil.

PREPARATION OF ROOTS FOR EXAMINATION

The sugarcane plants were removed from the soil and their roots washed thoroughly. Individual quantities of 200 gm. of roots were obtained by taking small bunches of roots from different parts of each plant. These roots were fixed in formalin-aceto-alcohol following the method of Sass (*17*) before being transported from Puerto Rico to Auburn, Ala., where the laboratory phases of the work were done.

For histological studies, root-apex pieces about 1½ cm. in length were selected from roots of three categories. These were primary roots which developed directly from the shoot, secondary roots which developed from the primary roots, and tertiary roots which developed from the secondary roots. All root pieces were dehydrated, infiltrated, and embedded in paraffin according to the method of Johansen (*10*). Longitudinal and transverse serial sections were cut 12 μ in thickness with a rotary microtome and triple stained with safranin, fast green, and orange G combination. These sections were examined for details of larval penetration, migration, and other pathological effects on the tissues and cells. The nature of the cortical cavities or pits was also investigated.

Gross effects of the treatments on the sugarcane roots were studied by placing the FAA-fixed root samples in water trays. Observations on root distortions, coloration, cavities, lesions, and galling were made with the aid of a hand lens.

Quantitative studies were made of the roots as to the nematodes within, root galls, lateral roots, and cortical cavities. For this purpose samples of the FAA-fixed roots were taken for each treatment and divided into the primary-, secondary-, and tertiary-root categories. In all cases pieces of roots containing the root tips were used and stained with the simplified lacto phenol-acid fuchsin method of McBeth, Taylor, and Smith (*15*) for staining nematodes *in situ*.

For each treatment 10 primary roots, 33 secondary roots, and 48 tertiary roots were examined under a dissecting microscope. Counts were made of the numbers of the nematodes in various recognizable stages of their life history, root galls, lateral roots exposed at the surface, and the cortical cavities or pits. These data were recorded for the first centimeter measured from the root apex, the next 1 to 3 cm., 3 to 5 cm., and 5 to 7 cm. back from the apex. This was possible for the long primary roots, but secondary roots rarely exceeded 5 cm. in length and tertiary roots were only about 3 cm. long.

RESULTS

DETERMINATION OF THE GROSS AND QUANTITATIVE EFFECTS OF ROOT-KNOT NEMATODES ON SUGARCANE ROOTS

Tables 1–6 contain the detailed quantitative results. The most important of these data are summarized in table 7. In this table only data from the

TABLE 1.—*Results of the inoculation test with* **Meloidogyne arenaria** *from* **Capsicum annuum**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	70	63	62	23	0	7	62	2
	1–3	28	13	71	28	0	3	115	8
	3–5	35	0	38	18	0	2	106	5
	5–7	13	0	12	12	0	0	37	4
Secondary	0–1	21	19	22	2	1	3	74	2
	1–3	12	8	28	5	0	1	101	4
	3–5	3	1	4	0	0	0	38	3
Tertiary	0–1	1	24	7	1	0	3	—	1
	1–3	7	7	3	0	0	1	—	0

TABLE 2.—*Results of the inoculation test with* **Meloidogyne incognita incognita** *from* **Nicotiana tabacum**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	63	104	84	3	1	8	20	9
	1–3	27	31	216	25	9	12	94	13
	3–5	10	6	84	17	2	4	88	10
	5–7	25	2	41	11	4	2	50	7
Secondary	0–1	79	118	104	7	4	29	77	6
	1–3	5	5	26	0	0	5	161	10
	3–5	1	0	13	0	0	1	9	1
Tertiary	0–1	36	88	54	3	3	10	—	0
	1–3	18	20	43	6	4	3	—	0

first 3 cm. of the root tips are presented and these represent totals for all three root categories.

Roots from the plants inoculated with supernatant water were free from galls and nematodes (table 7), and, in appearance, were very similar to the

TABLE 3.—*Results of the inoculation test with* **Meloidogyne incognita acrita** *from* **Ananas comosus**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	27	3	1	0	0	1	25	6
	1–3	3	2	17	1	1	2	34	8
	3–5	2	1	9	2	0	1	37	7
	5–7	3	0	0	0	0	0	36	9
Secondary	0–1	8	11	5	0	0	2	52	2
	1–3	4	5	3	0	0	1	101	3
	3–5	1	7	0	0	0	1	38	1
Tertiary	0–1	4	5	2	0	0	2	—	0
	1–3	2	2	1	0	0	1	—	0

TABLE 4.—*Results of the inoculation test with* **Meloidogyne incognita acrita** *from* **Eryngium foetidum**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	19	89	71	0	0	8	34	7
	1–3	7	20	105	38	2	8	81	6
	3–5	4	5	114	82	2	6	92	7
	5–7	7	0	94	69	0	2	61	4
Secondary	0–1	17	76	48	0	0	8	35	3
	1–3	16	40	36	0	0	4	151	6
	3–5	0	3	0	0	0	0	24	1
Tertiary	0–1	18	59	43	0	3	16	—	2
	1–3	6	17	20	1	0	6	—	0

TABLE 5.—*Results of the inoculation test with* **Meloidogyne incognita acrita** *from* **Cucumis sativus**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	43	104	172	107	9	9	49	14
	1–3	49	46	109	125	5	5	150	9
	3–5	29	0	61	55	6	4	107	8
	5–7	37	0	26	34	0	3	78	11
Secondary	0–1	45	82	55	3	2	16	112	7
	1–3	8	16	24	0	1	8	130	6
	3–5	4	5	17	1	0	3	46	2
Tertiary	0–1	26	82	14	1	2	17	—	4
	1–3	2	18	3	0	0	0	—	0

TABLE 6.—*Results of the inoculation tests with supernatant water from the 5 root-knot nematode cultures and the noninoculated control*

Root category	Root zone (cm.)	Results when inoculated with supernatant water from root-knot nematodes										Noninoculated	
		M. arenaria from pepper		*M. incognita*, from tobacco		*M. incognita acrita*							
						From pineapple		From *E. foetidum*		From cucumber			
		Lateral roots	Root cavities	Lateral roots	Root cavities	Lateral roots	Root cavities	Lateral roots	Root cavities	Lateral roots	Root cavities	Lateral roots	Root cavities
Primary	0–1	19	4	45	4	28	1	30	3	35	1	31	1
	1–3	75	4	96	8	70	1	99	4	64	2	94	3
	3–5	131	7	108	5	101	2	89	2	73	3	120	4
	5–7	120	5	100	2	87	2	54	1	38	1	86	2
Secondary	0–1	126	0	144	2	40	3	104	0	75	2	57	1
	1–3	147	3	187	1	106	2	206	2	168	5	132	3
	3–5	51	0	94	1	161	1	46	2	72	0	126	2
Tertiary	0–1	—	0	—	0	—	0	—	0	—	0	—	0
	1–3	—	0	—	0	—	0	—	0	—	0	—	0

roots from the noninoculated control plants. Roots under both these treatments were straight and exhibited only the normal, slight, wavy appearance. Their tips were normally tapered. Root color varied from white in the young roots, to brown and black in the old ones. Brownish-black lesions and cavities similar to those noted in the roots infected with nematodes were also observed, but the frequency with which they occurred was slightly lower in the nonnematode-infected roots.

The roots of the plants that were inoculated with the five root-knot nematode isolates had distinct curvatures. In all these treatments, curva-

TABLE 7.—*Summary of the quantitative data obtained for the first 3 cm. of all the roots under different treatments*

Treatment	Nematodes	Galls	Cavities	Lateral roots
Meloidogyne arenaria	526	18	17	352
M. incognita incognita	1,186	67	38	352
M. incognita acrita from pineapple	106	9	19	212
M. incognita acrita from E. foetidum	751	50	24	301
M. incognita acrita from cucumber	1,153	55	40	441
Control	0	0	8	314
Supernatant water of *M. arenaria*	0	0	11	367
Supernatant water of *M. incognita incognita*	0	0	15	372
Supernatant water of *M. incognita acrita* from pineapple	0	0	7	244
Supernatant water of *M. incognita acrita* from *Eryngium foetidum*	0	0	9	439
Supernatant water of *M. incognita acrita* from cucumber	0	0	10	342

tures were most pronounced in the most highly galled roots. Root curvature occurred preceding a gall, after a gall, or in between galls (fig. 1).

The color of the roots under all nematode treatments ranged from white in the young roots, to brown and black in the old ones. No differences in root coloration were found between treatments.

Superficial, brownish-black lesions were observed scattered over the entire length of roots inoculated with each of the five nematode isolates. These lesions ranged from 1 to 3 mm. in length, and varied from elongated to irregular in shape (fig. 2). The older roots were so dark in color that the extent of their lesions could not be determined.

In contrast to these shallow lesions, cavities were found in roots of all ages extending through the cortex as far as the central stele. These cavities, as seen at the surface of the root cortex, appeared as tiny holes about ½ mm. in diameter (fig. 3). No external damage was seen in the tissue sur-

rounding the apertures of these cavities. No differences were observed regarding the superficial appearance of the cavities occurring in the roots

FIG. 1.—Contrast between infected curved and straight control roots of sugarcane.

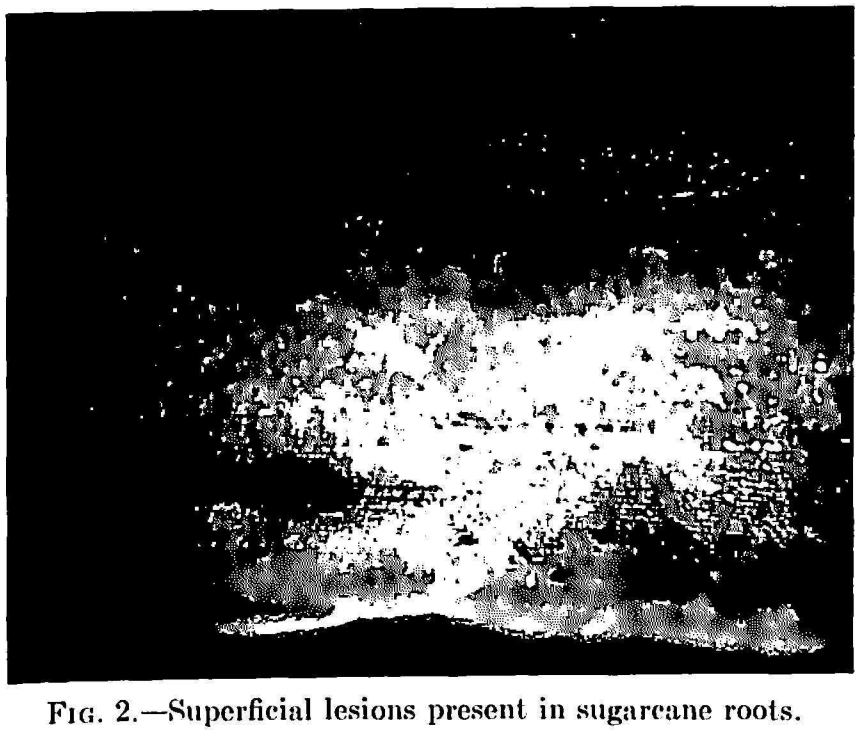


FIG. 2.—Superficial lesions present in sugarcane roots.

exposed to each of the root-knot nematodes and supernatant treatments. No differences in appearance of the cavities were found between the treated and uninoculated control roots. However, the numbers of cavities and galls

were found to be higher when the number of nematodes within the first 3 cm. of root tips was higher (table 7).

Galls in the primary roots were higher in number per centimeter of root within the first 3 cm. from the apexes than at locations farther back (tables 1–5). Gall shapes were similar in all the five sets of nematode-inoculated roots, being spheroidal or ellipsoidal. Both occurred at the proximal as well as at the distal parts of the roots (fig. 4).

The total number of lateral roots was higher in the nematode and supernatant-water treatments than for the uninoculated controls (table 7), with

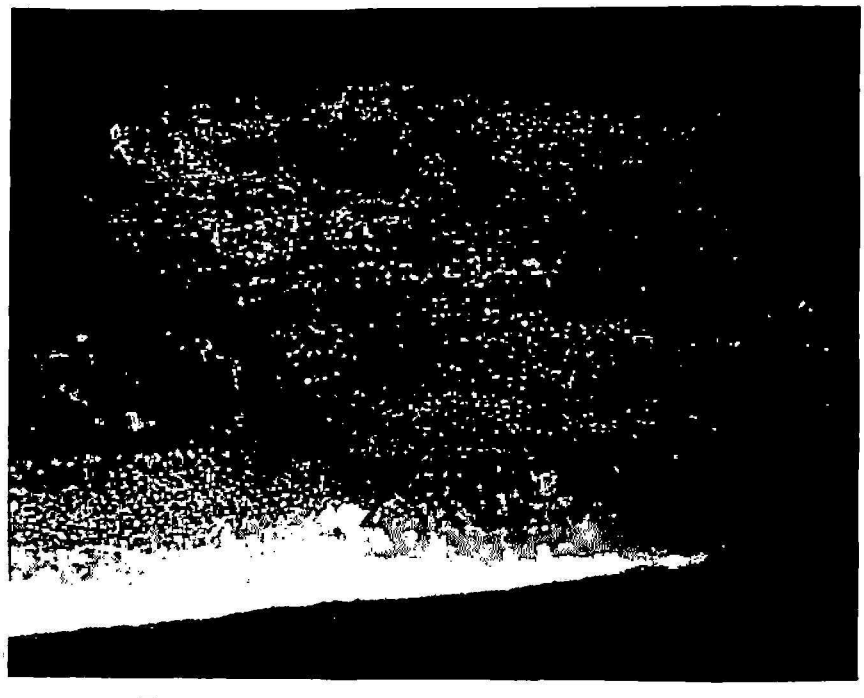


FIG. 3.—Surface view of cortical cavities.

the exception of those roots inoculated with *M. incognita acrita* from pineapple and its corresponding supernatant treatment.

In general, it was noted that, in each root category, the highest number of nematodes per centimeter of root usually occurred in the first centimeter from the root apex (tables 1–5). These nematodes represented all stages of development. However, the total number of males was found to be so low that no further study of them was conducted.

HISTOLOGICAL STUDIES

In the absence of root-knot nematodes, roots inoculated with the supernatant water and the noninoculated control roots gave no pathological responses. There were no differences observed in the internal responses of the roots infected with each of the five root-knot nematode isolates. Thus, the observations to be described in detail as to nematode penetration and

migration, sites for feeding and maturation, pathogenic effects, and root cavities, were found to apply equally to all five isolates.

Penetration and Migration

Penetration of root-knot nematode larvae into the roots of sugarcane appeared to occur only at the region of the root tip extending from root cap into the zone of differentiation. Both the infective larvae and the galleries or burrows made by them during penetration and migration were observed. The numbers of the infective larval stage ranged as high as 15 in the meristem of a single root. Infective larvae and galleries were less numerous in

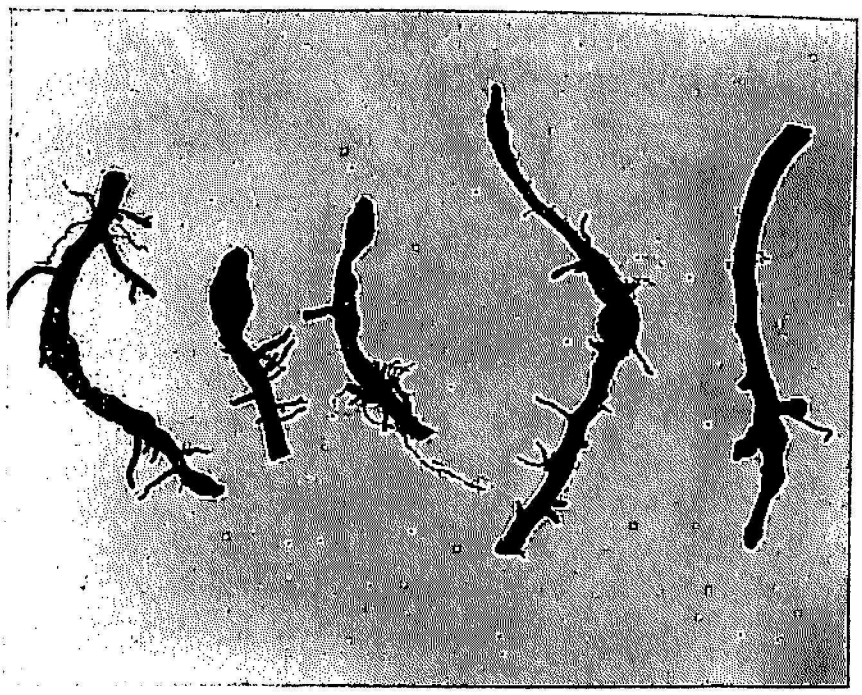


FIG. 4.—Large galls exhibited by root-knot nematode-infected sugarcane roots.

the root cap. In addition to penetration through the intact root cap, an instance was found in which a gallery left by a larva of *M. incognita incognita* in a secondary root connected with a break in the root cap (fig. 5).

No surface cell-damage was observed other than the entrance burrows made by the larvae. Direction of larval penetration in the four root-tip zones was found to be at different angles ranging up to about 90° with regard to the central axis of the root, but always oriented against the direction of root growth. Larvae were never seen oriented in the direction of root growth.

The galleries or burrows left by the infective larvae were composed of separated but intact cells as well as broken and empty cells. In these galleries there was no evidence of bacteria or fungi being present nor of damage caused by these organisms. It may be that the staining procedure used is

not suitable for revealing these other organisms. No discolorations or differences in staining were found in the tissues involved, nor was cell enlargement observed along these burrows (fig. 5).

These galleries were about the width of an infective larva and were nearly straight. In all cases only one larva was found to be associated with each burrow. In some instances portions of the burrows were found to be closed by unbroken cells. These cells may have been cells between which the nematodes had passed or, perhaps, they were altered in position by the process of embedding and sectioning.

Infective larvae and other stages were found within the vascular tissue.

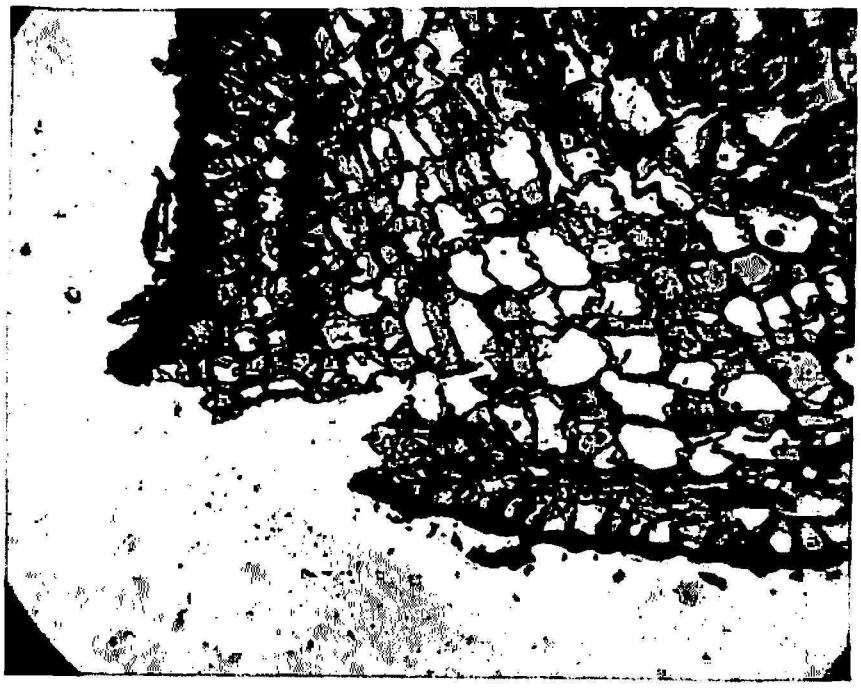


FIG. 5.—Gallery of a larva connected to a break in a sugarcane root cap.

However, since there was no evidence of a migration gallery through tissues of the vascular cylinder, it is possible that the nematodes had settled in this place before it became differentiated. Nematodes within the vascular cylinder, though scattered, were always oriented parallel to the walls of the cylinder with their heads directed away from the root tip.

Sites for Feeding and Maturation

The formation of giant cells and the production of egg masses were never observed in the root cap and meristematic zone. However, these occurred frequently in the zone of cell elongation. Egg masses and giant cells were observed in the older root tissues associated with nematodes lying entirely within the vascular cylinder or having their anterior portion inserted into the vascular tissue with the remainder of their bodies being in the cortex.

No females were found in the older, differentiated parts of the roots except in close proximity with the vascular cylinder. Only one instance was observed in which a female of *M. incognita incognita* in the cortical tissue did not have its head inside the vascular cylinder but did have giant cells around its anterior end (fig. 6).

Pathogenic Effects

The production of giant cells was the most important pathogenic effect caused by root-knot nematodes on the tissues of sugarcane roots. In order to get an understanding of the abnormal condition in the giant-cell area,

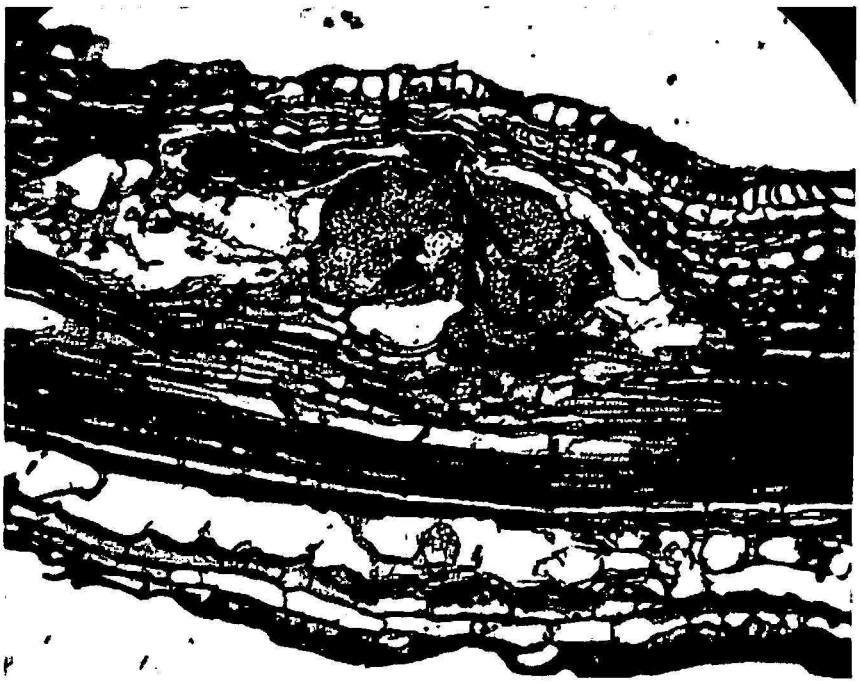


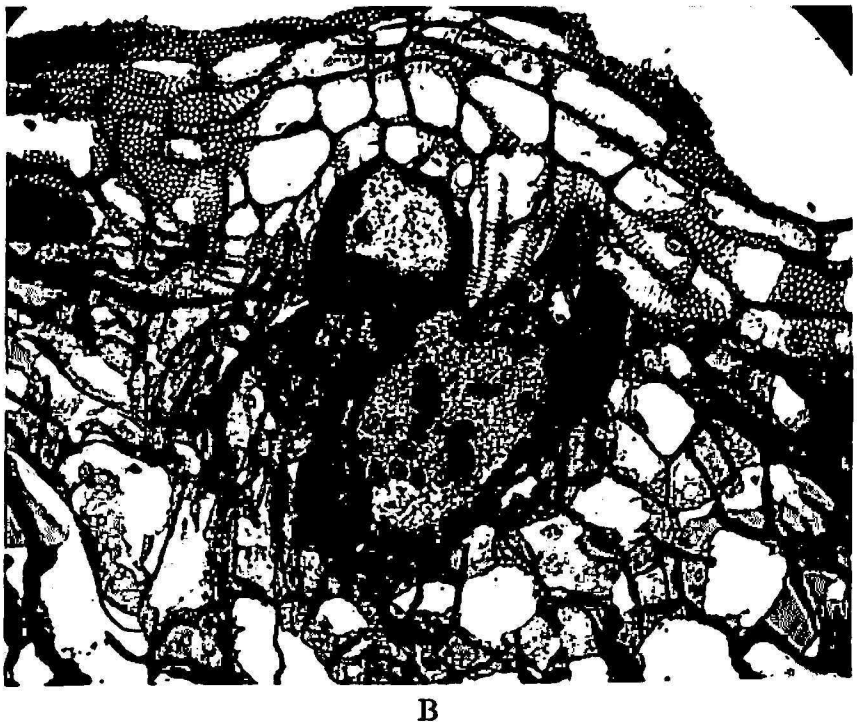
FIG. 6.—Female nematode with head not inserted into vascular tissue.

cells were studied first at the outermost part of the area and from there into the center where the nematode's head was located. The outermost cells had increased slightly in size, their cytoplasm and cell walls stained green as with all other unaffected cells, and their nuclei were very slightly enlarged and stained light red. Sometimes next to these cells, but closer to the center of the giant-cell area, a distinct hyperplastic zone was observed where the cells were numerous, small, and tightly packed together. Some of these had that part of their cell wall which adjoined the giant cells completely missing and represented cells in the process of coalescence. Mitotic figures were not observed in the giant cells although the staining technique did reveal them in the meristematic zones. The cells of the hyperplastic zone had walls and cytoplasm which stained the normal green with nuclei which were slightly enlarged but of the normal light-red color. There were a few cases

in which this hyperplastic zone was not detected even within the same root, although hyperplasia was generally present aroung the giant cells associated with mature females (fig. 7A).

Inside the giant-cell area, near the nematode's head, the cytoplasm of the cells became granular and stained light red. Cell walls stained green but

A



B

FIG. 7.— A, Hyperplasia around giant cells; B, fusion of giant-cell nuclei.

the cells and their nuclei were very much enlarged. These giant cells possessed many nuclei, each with conspicuous nucleoli which stained dark red. Groups of these nuclei fused together within the cells (fig. 7B).

In some of the cells around the nematode's head there was another type of nuclear fusion which perhaps followed the first fusion. Here the individual or previously fused groups of nuclei became flask-shaped and grouped together with their narrow ends pointing toward a central point (fig. 8A). This seemed to be followed by dissolution of the nuclear membranes and coalescence of their contents, resulting in a single amorphous mass of nucleoplasm (fig. 8B).

Cell-wall dissolution was very often seen in the giant cells (fig. 9A). This dissolution made possible the coalescence of cytoplasmic contents and resulted in the multinucleated condition of the giant cells. It is possible that the cell wall of the newly formed giant cells is composed of the undissolved portions from the other cells which joined in the coalescence.

The shape of the giant cell varied with respect to the tissue from which it was formed. Giant cells formed from cells which would give rise to pericycle, endodermis, or cortical tissue were found to be generally rounded or oval. Those formed from the tissue which would give rise to vascular tissue were generally elongated (fig. 9B). Some giant cells close to the vascular tissue had internal knoblike structures which were part of the internal lining of the cell and were perpendicular to it. These were small and stained the same green color as the cell wall (fig. 10).

The sites for feeding and maturation already mentioned (region of cell elongation, cortical tissue with nematode's head inside vascular tissue, and vascular cylinder) were the places where the pathogenic effects were most pronounced.

Nematodes feeding in the region of cell elongation caused differentiation of most of this zone into giant cells. Large numbers of root-knot nematodes feeding at the beginning of this zone caused severe damage. Generally, a large terminal gall was found in which the characteristic small and closely packed meristematic cells of the apical meristem had partly disappeared and was replaced by large nonmeristematic cells. This condition was accompanied by the absence of a root cap and the presence of a few vascular vessels which were so close to the root apex that the region of cell elongation appeared considerably reduced (fig. 11A).

Nematodes with their anterior portions embedded into the vascular cylinder and their remaining portions lying in the cortex, and nematodes completely inside the vascular cylinder were observed also to have a giant-cell area around their heads. In both cases vascular vessels frequently curved around the giant-cell area (fig. 9B).

An abnormality of the xylem was found associated with nematodes feed-

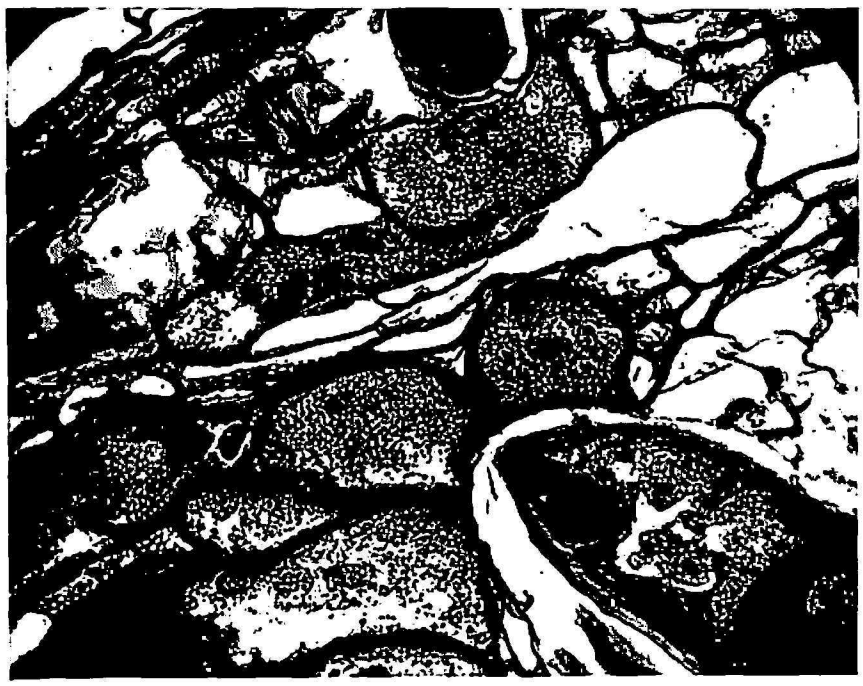
A

B

FIG. 8.—A, Fusion of flask-shaped nuclei; B, amorphous mass of nucleoplasm that appeared to result from fusion of the flask-shaped nuclei.

ing within the vascular cylinder. This abnormality which was characterized by the presence of xylem vessels disorderly arranged was found in the vicinity of the giant-cell area (fig. 11B).

The root-knot nematode not only caused damage to the plant tissue



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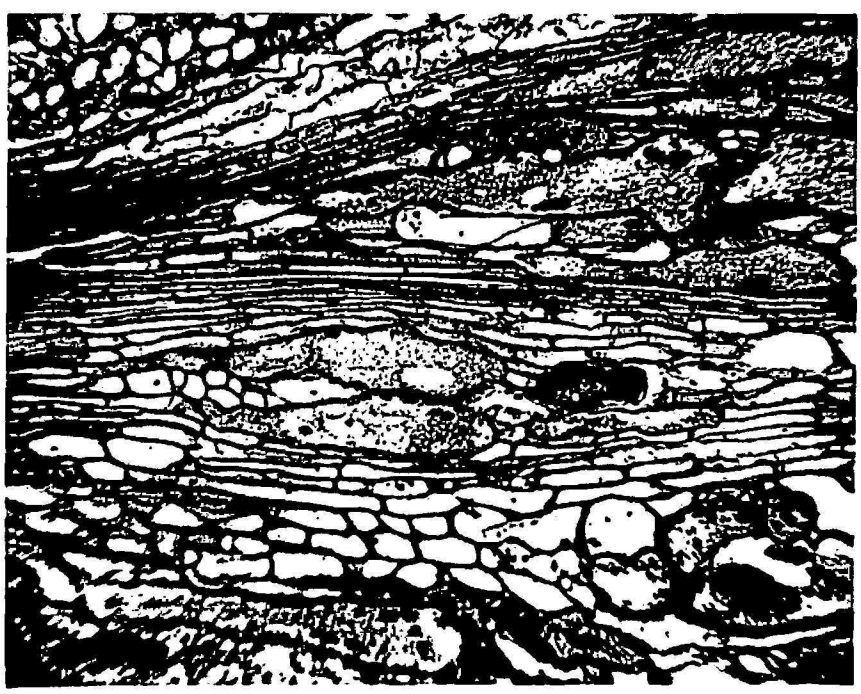

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FIG. 9.—A, Cell-wall dissolution between giant cells; B, elongated giant cells within the vascular tissue and curving of the vascular vessels.

surrounding the head of the nematode, but also damaged cells which surrounded the rest of the body. Since the nematode's body increases greatly in volume during growth, the cells which surrounded the swollen part of the body were pushed aside, and commonly a circle of crushed cells surrounded

this part of the nematode (fig. 12A). This condition also occurred around egg masses (fig. 12B) and giant-cell areas (fig. 12A).

Gall formation was found to be a result of a combination of different effects. These were the increased number and volume of the affected root cells and of the maturing nematodes and their egg masses. Not a single case of necrosis was found associated with gall formation.

Root Cavities

Cortical cavities or pits were found which penetrated into the vascular tissue (fig. 13). Some penetrated straight into the central core, maintaining

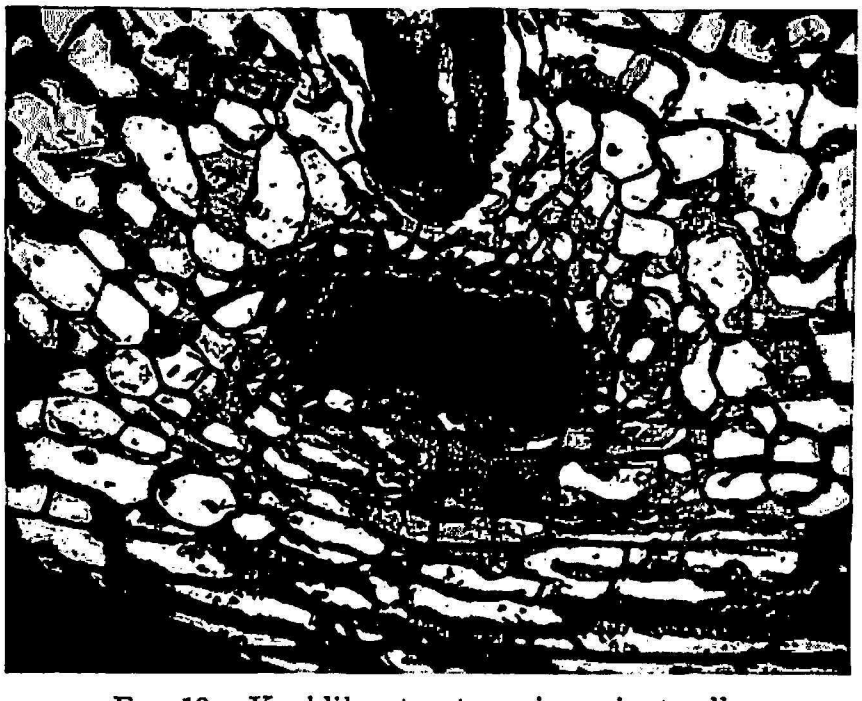
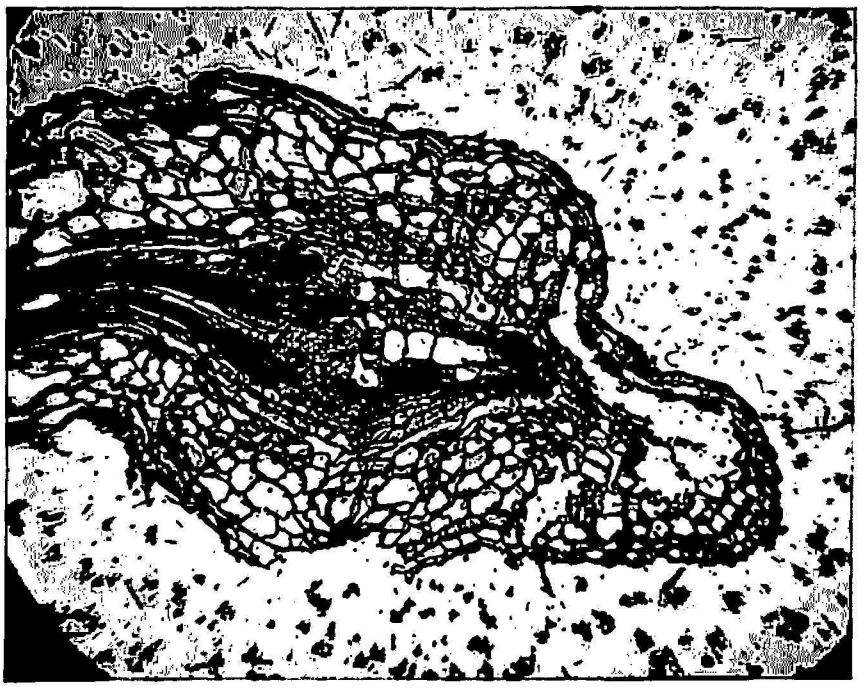


FIG. 10.—Knoblike structures in a giant cell.

a uniform diameter equal to that of the opening at the surface. Others expanded internally having a diameter slightly greater than that of the opening. Neither necrosis nor giant cells were seen in the surrounding tissues of these cavities.

SUITABILITY OF P.R. 980 SUGARCANE AS A HOST

The percentage of mature females to the total number of nematodes within the roots was used as a criterion of host suitability for reproduction of each of the five nematode isolates. The following percentages were calculated on nematode data for the entire 7-cm. root lengths studied in the primary root samples (tables 1–5), where sufficient quantities of mature females were found. Sixteen and seven-tenths percent of all the *M. arenaria* nematodes had reached maturity at the time of root harvest, which was



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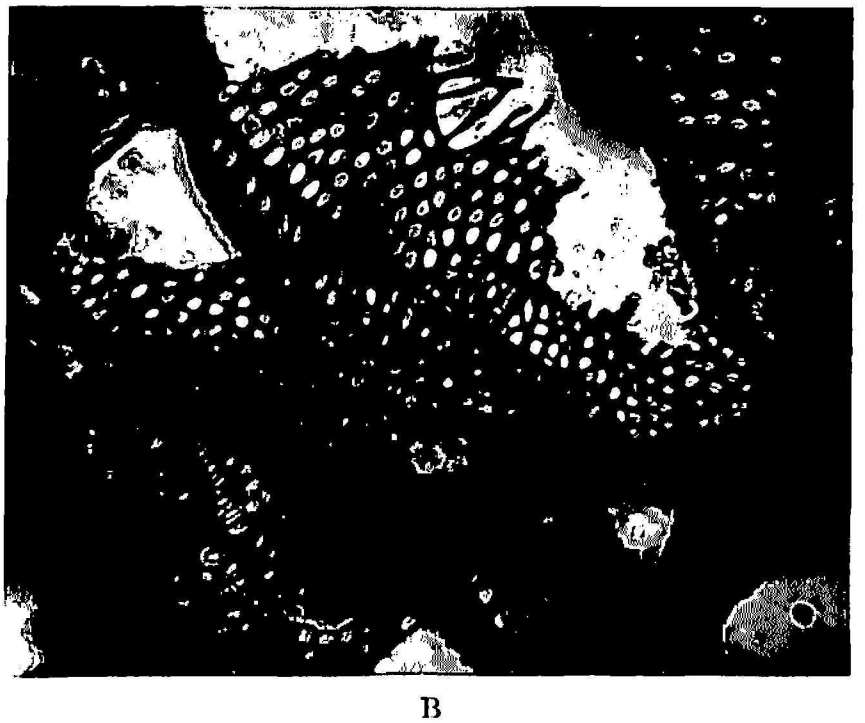

B

FIG. 11. A, Absence of apical meristem and root cap in a highly infected root tip; B, abnormality of the xylem associated with nematode injury.

5 months after inoculation. The percentage of *M. incognita incognita* that reached maturity was 6.6. Only 4.9 percent of the *M. incognita acrita* selection from pineapple reached maturity as compared to 26.3 percent of this subspecies selection from *E. foetidum* and 32.2 percent for the sub-

A

B

FIG. 12.—A, Mechanical injury to cells surrounding the posterior portion of female nematodes and the giant-cell area; B, mechanical injury to cells surrounding egg masses.

species from cucumber. Thus, there appeared to be distinct host-suitability differences for the three nematode species tested and especially for the *M. incognita acrita* selection from pineapple as compared to the other two selections of this subspecies. *M. incognita acrita* from pineapple differed

from the others also in its comparatively lower number of nematodes infecting the sugarcane (table 7). There were no previous indications of declining infectivity or viability in any of the selections during the course of culturing and at the time of inoculation of the sugarcane.

The low host-suitability rating for *M. incognita incognita* appeared to be due to a slow rate of development rather than to a low amount of infection, as the numbers of young females in the primary roots were quite high (table 2).

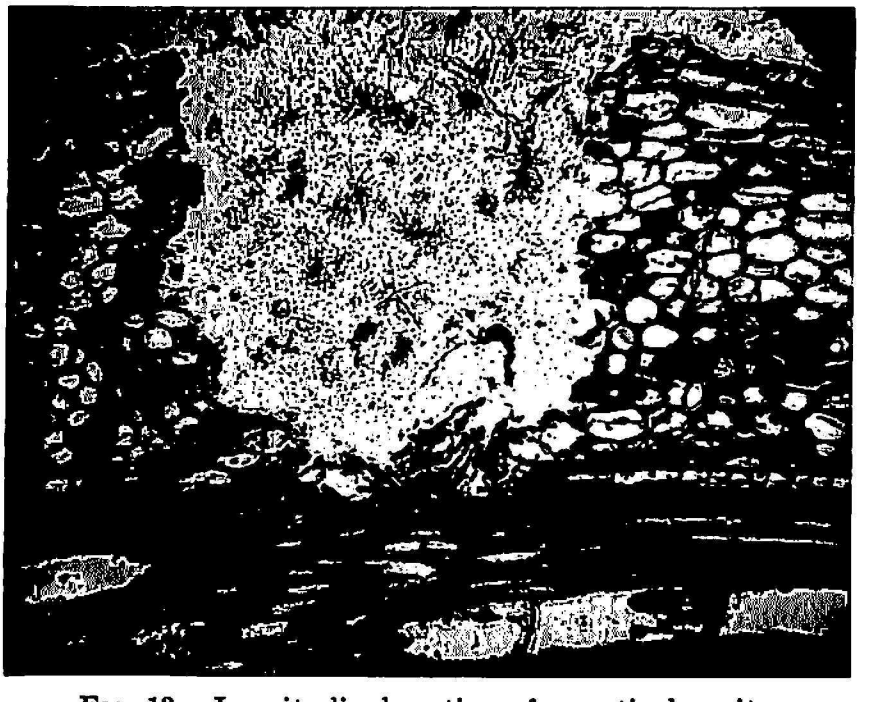

FIG. 13.—Longitudinal section of a cortical cavity.

DISCUSSION

Roots inoculated with each of the five root-knot nematode isolates developed distinct curvatures. In comparison, the noninoculated control roots were found to be straight or with a very slight wavy appearance. The internal cause of this curvature was not established. Perhaps it can serve as an additional useful field symptom for detecting the disease.

Nematodes were not solely responsible for the superficial, brownish-black lesions found on the roots, since these lesions were also found on the supernatant treated and control roots. Although the frequency of lesions was slightly higher on the roots infected with nematodes, differences in lesion appearances were not established. It is remarkable that other microorganisms were not present in these lesions, although it could be that the staining technique used would not reveal them. Since the soil was not checked for other casual agents, the presence of other soil organisms, like arthropods, was possible.

The internal lesions or cortical cavities of the roots were not solely dependent upon the presence of the nematodes. Although a definite increase in the number of cavities was found in the nematode treatments, their appearances did not differ from cavities in the control roots. It was noted that no cavities contained nematodes or the remains of their bodies, nor were giant cells formed in the immediate vicinity. This was carefully checked because Treub (*21*) had reported that cavities resulted where female root-knot nematodes had been and that giant cells could be found. Thus, these new data indicate that the cortical cavities or pits can be caused in other, as yet, unexplained ways. Loss of lateral roots was one possibility considered. However, no traces were found in the main vascular cylinder suggestive of the previous existence of lateral roots. These cavities by themselves are possibly of little harm to the roots as they do not interfere much with the vascular tissues. Perhaps, they are more significant as portals of entry for various organisms.

The observation that large numbers of nematodes in all stages of development were present in the first centimeter of the roots can be explained as the result of retardation of root growth in length, since infection did not occur in portions of the roots where differentiation was already completed. The internal study of roots having galls at the apex showed that the apical meristem and the root cap were either very much reduced in size or completely absent. Such roots would be expected to grow slowly or not at all. This effect may also be caused by the penetration of large numbers of larvae through the apical meristem, and, even though a gall is not found present at the tip, root growth is expected to be retarded or stopped.

Retardation or stoppage of root growth could be an important factor to the plant since short roots furnish less support for the plant and less surface area for contact with the mineral elements and moisture in the soil. In addition to this, a gall in the root may represent a partial or complete disruption of the vascular tissue. These effects could account for symptoms such as chlorosis, dwarfing, wilting, and reduction of yield.

The larger the number of nematodes and the longer they have been present in a gall, the larger the size of the gall. The spheroidal and ellipsoidal gall shapes were found to be due to the nematode distribution inside the gall. Nematodes tightly packed in a common place in the root produced a spheroidal gall. On the other hand, if these nematodes were more dispersed, but still in a small region, an ellipsoidal gall resulted. Proliferation of lateral roots in the vicinity of the galls was not a regular occurrence.

The internal study of the infected roots showed that the typical giant-cell formation occurred in sugarcane in response to all five isolates of root-knot nematodes feeding on the root tissues. In sugarcane, the giant cell is a syncytium resulting from cell enlargement, cell-wall dissolution, coales-

cence of cytoplasmic contents, and cell division. These results show partial agreement with the reports of Christie (*4*) for tomato roots, but are completely different from what Kostoff and Kendal (*11*) found for tobacco roots. The fusion of the pear-shaped nuclei of the giant cell and the presence of the knoblike structures reported by Krusberg and Nielsen (*12*) in sweetpotato roots, were also found to occur in sugarcane roots. These knoblike projections were found on the inner surfaces of giant cells in the vicinity of the vascular cylinder. The staining reaction of these projections was the same as for the walls of the giant cells, but different from the lignified xylem elements which stained red.

In this study, in which only one host was used, the giant-cell and galling responses of the host to the five root-knot nematode isolates were identical. The evidence indicated that giant-cell formation occurred in cells which had not yet become differentiated. There was no conclusive evidence that cells which have already differentiated can become transformed into giant cells. The finding of giant cells in already differentiated tissues is no proof that these cells had developed as a result of later infection. Giant-cell formation was first detectable at the beginning of the region of cell elongation. As a result of root development, this area, in which the nematodes had become sedentary and in which giant cells continued to develop, eventually became incorporated into the zone of differentiation. This explains why the nematodes may be found either completely inside or with their heads inserted into the vascular tissue. This also explains what appears to be the interruption of the normal differentiation of the vascular elements, pericycle, and endodermis by the presence of giant cells and nematodes. Thus, the position taken by the nematodes in the region of cell elongation ultimately decides which tissues are subsequently to be affected by the nematodes.

The disorderly arrangement of the xylem elements which was always found around the outside of the giant-cell area seemed also to be a response to the nematodes. It may be that these cells were partly disorganized and failed to form normal xylem tissue, although able to continue to differentiate. Cells of the root tissues were also affected mechanically by the growth of the nematode's body and by the increase in volume of the egg masses and giant cells.

The sugarcane variety P.R. 980 may be considered a host with variable suitability for the nematodes tested. This variety was unsuitable for *M. incognita acrita* from pineapple which differed markedly from the other two selections of this subspecies both in infectivity and the ability to complete maturation. This suggests the existence of strain differences in this subspecies group. The variety P.R. 980 was also of low suitability for the reproduction of *M. incognita incognita*, but this was due to a slower matur-

ation rate. These differences in host suitability may have practical value in terms of rotation practices used in growing sugarcane, but yield tests will be necessary to determine whether differences with respect to the root-knot nematode species and their possible strains will be of importance to the sugarcane industry.

SUMMARY

An experiment was conducted to determine the nature of the pathogenic effects of five isolates of root-knot nematodes on the roots of the promising commercial sugarcane variety, Puerto Rico 980, the suitability of this sugarcane as a host for the five nematodes, and the possibility of the existence of strains in the species *M. incognita acrita.*

The five root-knot nematodes, which included three species, were isolated as single egg masses from different kinds of host plants. The isolates included the species *Meloidogyne arenaria* from pepper, *M. incognita incognita* from tobacco, and three selections of *M. incognita acrita* from pineapple, *Eryngium foetidum,* and cucumber. Treatments consisted of inoculating the roots of P.R. 980, grown in steam-sterilized soil, with suspensions of 5,000 root-knot nematode larvae and, as a separate treatment, with the supernatant water from about the nematodes. Control plants were not inoculated. After a period of 5 months the roots were harvested and fixed in formalin-aceto-alcohol. The roots were studied to determine the gross effects of the nematodes on the roots, the number of nematodes present and their life cycle stages within the roots, and their pathogenic effects.

Larvae penetrated mostly through the apical meristem of the root tip and migrated to the central part of the root, leaving galleries or burrows composed of broken and separated cells.

The internal response of the root tissues to the five nematodes was mainly the formation of giant cells. No necrosis was observed. The first indication of the formation of giant cells was a slight increase in size of the cells and of their nuclei. This apparently was followed by cell-wall dissolution and coalescence of cytoplasmic contents resulting in a multinucleate syncytium known as a giant cell. Groups of small numbers of nuclei within the giant cell fused together. Cell division occurred in the cells adjacent to the giant cells but the walls of these cells also became dissolved and their contents were gradually incorporated by the giant cells. Groups and individual nuclei then became flask-shaped and aggregated with their narrow ends oriented toward a central point. Finally, their membranes dissolved and their contents coalesced.

Giant cells were first detected at the beginning of the region of cell elongation and apparently were formed only from cells which had not differentiated. However, as a result of root development, giant cells were

also found in and around the vascular cylinder and gave the impression that the nematodes had modified already differentiated tissues.

Xylem elements, which probably had already begun differentiation at the time of infection, completed development but failed to form a normal tissue in proximity to the developing nematodes.

Mechanical damage also occurred in the root cells as a result of pressure from expansion in volume on the giant cells, maturing nematodes, and egg masses. The galled areas of the roots also resulted from these expansions. Spheroidal galls resulted when the nematodes within them were close together, while ellipsoidal galls formed when the nematodes were more widely dispersed.

Meristems and root caps of highly infected roots were much reduced or absent, resulting in a high percentage of galls and nematodes at or close to the apexes. In general, the highest number of nematodes per centimeter of root occurred in the first centimeter from the root apex.

Lateral root proliferation was not found to be consistently associated with gall formation. Pronounced root curvature was associated with nematode infection. Superficial lesions on the roots and cortical cavities were not produced solely by the nematodes, and although their frequency was higher on nematode-infected roots, differences in appearance to those on the control were not found. No cavities contained nematodes, remains of their bodies, nor were giant cells formed in the immediate vicinity.

This sugarcane variety, P.R. 980, was an unsuitable host for one *M. incognita acrita* selection, which differed enough in its infectivity and maturation as to suggest a strain difference. Reproduction of *M. incognita incognita* was also low, but evidently due to a slow maturation rate as its infection rate was high. This nematode would probably increase in population during the long growing period of sugarcane in the field. P.R. 980 was a suitable host permitting large population increases for *M. arenaria* and for two of the *M. incognita acrita* selections. Pathogenic relationships of the five nematode isolates were similar in other aspects in this host.

RESUMEN

Se llevó a cabo un experimento con el fin de determinar la naturaleza de los efectos patogénicos de cinco tipos diferentes de nematodos de agalla a las raíces de la prometedora variedad comercial de caña Puerto Rico 980; para estudiar la adaptabilidad de la mencionada variedad de caña como planta hospedadora de estos tipos de nematodos; y para conseguir evidencia de que hubieran distintas razas de la especie *M. incognita acrita.*

A los cinco tipos de nematodos, de tres especies, procedentes de diferentes plantas hospedadoras, se les separaron sus sacos de huevos. Estos tipos incluyeron las siguientes especies: *Meloidogyne arenaria* del pimiento, *M.*

incognita incognita del tabaco y tres selecciones de *M. incognita acrita* de piña, de culantro del monte (*Eryngium foetidum*) y de pepinillo.

Los tratamientos consistieron en inocular las raíces de la caña P.R. 980, desarrolladas en suelo esterilizado a vapor, con suspensiones de 5,000 larvas de nematodos de agalla y también como un tratamiento aparte, aplicándoles el agua que flotaba alrededor de los nematodos. El tercer tratamiento era el testigo. Después de cinco meses, las raíces se extrajeron del suelo y se fijaron en una solución de formalina, ácido acético y alcohol. Luego se estudiaron para determinar cómo las afectaban los nematodos, cuántos de éstos había presente y en qué etapa en su ciclo de vida se hallaban.

Se observó que las larvas de los nematodos penetraron principalmente a través del meristemo apical de la raíz y migraron a la parte central de ésta, dejando galerías compuestas de células rotas y separadas.

Se descubrieron células gigantes donde empezaba la región de alargamiento celular y, aparentemente, éstas se formaron de células que no se habían diferenciado. Sin embargo, como resultado del crecimiento radical, también se encontraron células gigantes dentro y alrededor del cilindro vascular, lo cual daba la impresión de que los nematodos habían modificado el tejido ya diferenciado.

La reacción de los tejidos internos radicales a la presencia de los cinco tipos de nematodos fue principalmente la formación de células gigantes y no se observó necrosis alguna. La primera indicación de la formación de células gigantes fue que hubo un pequeño aumento en el tamaño de las células y sus núcleos. Esto aparentemente fue seguido por la disolución de las paredes celulares y la coalescencia de los contenidos citoplásmicos, de lo cual resultó un sincitio multinucleado conocido como célula gigante. Algunos grupos pequeños de núcleos, dentro de las células gigantes, se unieron. Ocurrió división celular en las células adyacentes a las células gigantes, pero las paredes de éstas también fueron disueltas y sus contenidos fueron gradualmente incorporados por las células gigantes. Otros grupos de núcleos y núcleos individuales luego se tornaron piriformes y se agruparon poniendo sus extremos más angostos orientados hacia un punto central. Finalmente, sus membranas se disolvieron y sus contenidos se fundieron.

Los componentes del xilema, que probablemente habían ya empezado a diferenciarse al tiempo de la infección, completaron su desarrollo; pero no formaron un tejido normal en las inmediaciones de los nematodos según se desarrollaban.

Ocurrió un daño mecánico a las células radicales como resultado de la presión ejercida por la expansión en volumen de las células gigantes, de los nematodos que estaban desarrollándose y de la de los sacos de huevos. También se formaron áreas hipertrofiadas en las raíces como resultado de

estas expansiones. Además, se formaron agallas esferoidales cuando los nematodos dentro de éstas se encontraban juntos, mientras que por otra parte, se desarrollaron agallas elipsoidales cuando los nematodos se encontraban dispersos.

Los meristemos y las pilorrizas de raíces altamente infectadas se redujeron considerablemente o desaparecieron, lo que resultó en una alta acumulación de agallas y nematodos en, o cerca de los ápices. En general, se observó que el número más alto de nematodos por centímetro de raíz se concentró en el primer centímetro apical.

La proliferación de raíces laterales no estaba asociada consistentemente a la formación de agallas. La distorción de las raíces se encontró asociada a la infección de los nematodos. Las lesiones superficiales en las raíces y cavidades corticales no las produjeron únicamente los nematodos y aunque esta condición abundaba más en las raíces infectadas, no surgieron diferencias al compararse con las que se observaron en los testigos. Tampoco se observaron nematodos, ni residuos de sus cuerpos en las cavidades, ni tampoco se formaron células gigantes en sus alrededores.

La variedad de caña de azúcar P.R. 980, no es una planta hospedadora propicia para una de las selecciones de *M. incognita acrita*, porque difería lo suficiente en su infectividad y desarrollo como para sugerir una raza diferente. La reproducción de *M. incognita incognita* fue también poca, evidentemente debido a un lento proceso de madurez, toda vez que su grado de infeción era considerable. Este nematodo probablemente pudo aumentar en número durante el largo período de crecimiento de la caña de azúcar en el campo. La variedad P.R. 980 fue una planta hospedadora propicia, permitiendo grandes aumentos en número de *M. arenaria* y dos selecciones de *M. incognita acrita*. Las relaciones patógenicas de los cinco tipos de nematodos fueron similares en otros aspectos en cuanto a esta planta hospedadora.

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