HISTOPATHOLOGY OF RESISTANT AND SUSCEPTIBLE PEANUTS INFECTED WITH *MELOIDOGYNE HAPLA*

By

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HISTOPATHOLOGY OF RESISTANT AND SUSCEPTIBLE PEANUTS INFECTED WITH MELOIDOGYNE HAPLA

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Nematode surveys conducted in the peanut (Arachis hypogaea L.) growing areas in Oklahoma indicated that the northern root-knot nematode, Meloidogyne hapla Chitwood, 1949 is a major peanut production problem in the infested areas of the state (36). In many areas an average peanut yield reduction of 50 percent was attributed to this nematode and in some heavily infested fields as high as 89 percent production loss was estimated.

At present, control procedures recommended for plant-parasitic nematode include dry fallowing, flooding, crop rotation and chemical control. The danger of wind erosion makes the use of dry fallowing unfeasible especially in the peanut growing areas in the state, while flooding is prevented by insufficient water and topographical factors. Crop rotation, on the other hand, due to the government allotment system, may be economically unfavorable to the grower because he may not have alternate land upon which he can grow the crop. The persistence of the root-knot nematode in the soil also makes this method less effective. Chemical control, although relatively efficient, is limited by the high cost of chemicals and governmental restrictions.

The use of resistant varieties is potentially the most economical and effective method of controlling plant parasitic nematodes. Although their use may be limited by the presence of resistant-breaking

biotypes, resistant plants, especially if they have quality comparable to the susceptible standard varieties, will entail no extra cost to the grower and no alteration in cultural farm practices.

It was with this knowledge that a peanut breeding program for root-knot nematode resistance was initiated at Oklahoma State University. Since there was little information regarding root-knot nematode resistant peanuts, an initial screening program was conducted by Castillo (4), to search for resistant germplasm which could be used as a basis for breeding. He reported high degree of resistance in some wild Arachis lines and in two cultivated lines, F416 and NC4X.

To facilitate breeding nematode resistant varieties, the nature of mechanism of such resistance must be well understood. Rohde (31) defined resistance to nematodes as a set of characteristics of the host plant which act more or less to the detriment of the parasite. This may range from the plant being tolerant, allowing the parasite to develop and reproduce, through the plant being immune, prohibiting the nematode from entering or feeding. Hence, a gradation of resistance exists ranging from slight to complete, and the resistance may be due to one or a combination of several mechanisms. Knowledge of the nature of plant resistance to nematodes therefore, is important in determining the mechanisms that can be incorporated into the more desirable commercial variety. In his subsequent study on the nature of resistance, Castillo (4) observed less nematode penetration in the wild peanut line P246 (PI262286-USDA plant introduction number) than in the susceptible variety Spantex. He also observed delayed nematode development and a decrease in population with time in the resistant plant. Histological comparison of root galls in the susceptible and resistant

plants showed no apparent difference. However, the histopathological examination was limited to a single period in the nematode development. Except for Castillo's study, there is no other information available on the histological response of peanut to root-knot nematode infection.

This study was initiated to furnish additional information on the histopathological responses of resistant and susceptible peanuts to M. hapla infection, to determine the time of penetration of the nematode and to test and screen other commercial varieties and plant introduction lines for possible resistance. Hopefully this information will provide a better understanding of the nature of root-knot nematode resistance in peanuts and facilitate the breeding for resistant varieties.

CHAPTER II

REVIEW OF LITERATURE

Resistance to nematodes has been found in many crops and to many genera of nematodes. Several reviews have presented extensive listings of studies conducted in this area (2, 12, 31, 32). These studies have provided valuable information towards the understanding of the nature of plant resistance to nematodes.

Howard (12) suggested three types of nematode resistance: first, a resistance to invasion; secondly, a resistance after invasion; and thirdly, a tolerance to invasion. In some instances toxic plant secretions as in asparagus and margiold have been postulated to be the primary cause for the failure of nematodes to penetrate (23, 30). Lack of attraction in some plants has also been reported (4, 25). Shepherd (35) however, reported no correlation between resistance to attack by a given cyst nematode species to the production of root diffusate.

Tolerant plants, on the other hand, although invaded by the nematode, show relatively little loss of yield. Tyler (37) defined tolerance to root-knot nematodes as the ability of a plant to continue productive growth even with heavy and increasing infestations. This type of reaction was attributed by Howard (12) to plants being drought resistant or having a strong root system.

Resistance after nematode invasion, especially to root-knot and cyst nematodes, appears to be the most common (12, 32), although resistance to penetration of root-knot nematode larvae has been reported by Goplen and Stanford on lucerne (11), Peacock on tomato (25), and Castillo on peanut (4).

Rohde (32) postulated three conditions leading to this post-infection type of resistance: nutritional incompatibility, lack of host response to infection and hypersensitivity. Therefore, larvae may enter roots of resistant plants as readily as those of susceptible plants, but little or no development or reproduction occurs. This type of resistant reaction to root-knot and cyst nematodes is usually indicated by the plant cell response to the parasite and in the degree of nematode development.

In their host suitability studies with Heterodera trifolii, Mankau and Linford (6) indicated that nematode development was closely related to the developmental rate and size of the syncytium. Similar correlation of root-knot nematode development and giant cell formation was reported by Crittenden (6), Dropkin (8) and Dropkin and Nelson (9) in resistant soybeans. Endo (10) observed gradual giant cell degeneration and collapse in soybeans resistant to H. glycines. Working on resistant peach rootstocks to M. javanica, Malo (19) observed suberin-like materials around walls of giant cells eight to 10 days after infection. "Walling-off" increased with time until nematode development stopped.

Hypersensitive reaction of host tissues due to nematode infection has been reported by many workers. Dean (7) observed root necrosis in resistant tomato and sweet potato causing the death of M. incognita

larvae. Similar observations were reported by Riggs and Winstead (29) on resistant tomato infected with two species of root-knot nematodes. Bergman, as cited by Endo (10), found plants resistant to H. shactii exhibited root necrosis and observed subsequent degeneration of the larvae, however, in some plants, a few larvae developed beyond the second stage and were associated with restricted giant cells. Tissue necrosis has also been attributed for nematode resistance observed in crops such as soybeans (6, 8, 9), cotton (3), tobacco (27), chrysanthemum (39) and citrus (38).

CHAPTER III

MATERIALS AND METHODS

General Methods

Cuttings of the susceptible variety, Spantex and the resistant lines F416 and P246 were allowed to root in a mist chamber for 30 days after which they were transplanted to glass-fronted observation boxes (15) (Figure 1A, 1B). After two weeks, individual roots were inoculated by pipetting an aliquot suspension of 100 second stage Well's isolate (4) M. hapla larvae directly onto the root tip area. The roots were then covered with a sterile fine soil-sand mixture. A small sheet of plastic film was placed under each root tip region prior to inoculation to prevent the larvae from being washed into the surrounding soil. Subsequent examination was conducted according to the study involved.

Larval Penetration Studies

In the M. hapla larval penetration study, six roots of each line were collected and washed at 6-, 12-, 24-, 48-, 72-, and 92 hours after inoculation. The roots, approximately 40 mm in length, were fixed immediately upon collection in Craf III (33) and held until they were stained in acid-fuchsin lactophenol and stored in pure lactophenol (21). Nematodes were examined and counted under a binocular stereomicroscope by pressing the roots between two glass slides.

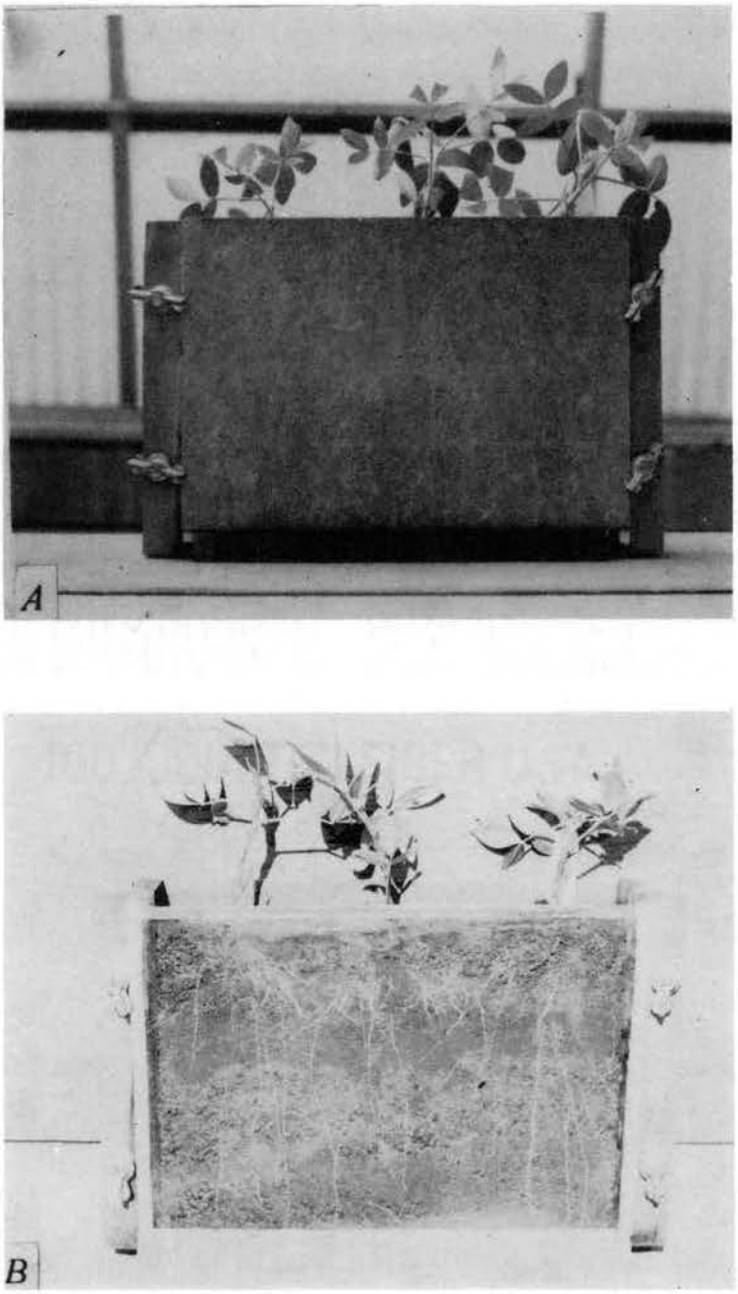

Figure 1. A. Modified Root Observation Box with Transite Cover in Place. B. Box with Cover Removed for Observation. Note: Roots Behind Glass.

Histopathological Studies

In this study, all roots were washed 24 hours after inoculation. Root samples from each plant were taken daily for seven days and then at three-day interval up to 19 days, and on the 25th day. A final sample was taken from P246 at 35 days for comparison of cellular responses during the egg laying period. The roots were fixed immediately upon collection in Craf III and held for at least 24 hours. The root samples were cut into pieces of about 10 mm in length and then dehydrated with a graded series of tertiary butyl alcohol and infiltrated with paraffin. Longitudinal and cross sections of approximately 12 microns were made and then stained with safranin and fast green. The dehydration and staining process was according to Sass (33). The cross-sectional area of giant cells was computed by tracing cell outlines from photomicrograph negatives projected by a darkroom enlarger. Individual cut-outs of the tracings of cells were then weighed and transformed to square micron units.

Screening for Resistance to M. hapla

The procedure of Castillo (4) was adopted in the screening trials. Infested soil was prepared by mixing one gram of chopped, galled roots of tomato which had been infected for at least three months, into a sterile soil-sand mixture in 12-cm pots. Seeds or rooted cuttings were sown directly in the infested soil and grown in the greenhouse at 22 to 29 C. The tests were replicated four times with the susceptible Spantex variety as control. After 30 days, the roots were washed and classified according to degree of root galling. Galling was rated on a 1 to 5 severity scale (Figure 2) with: 1 = none; 2 = trace;

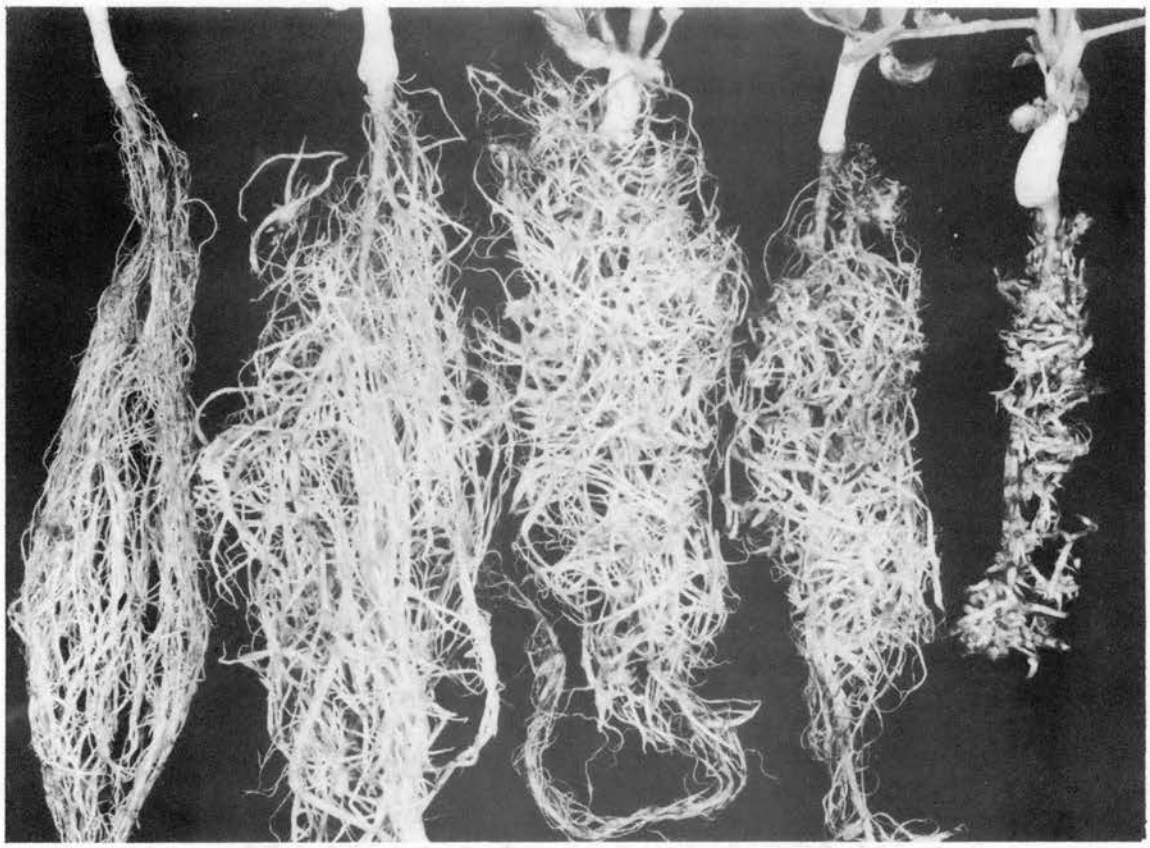

Figure 2. Gall Index. Left to Right: 1, None; 2, Trace; 3, Moderate; 4, Severe; 5, Very Severe. (After Castillo, 1969).

3 = moderate; 4 = severe; and 5 = very severe. Plants with an average root gall index of 1 or 2 were regarded as resistant; 3, moderately resistant; 4 or 5, susceptible.

CHAPTER IV

RESULTS

Larval Penetration Studies

Table I shows the average number of second stage larvae of M. hapla found in each infected root at different time exposures. Relatively few larvae were recovered from any plant. Results revealed that larval penetration of roots of resistant and susceptible peanuts occurred as early as six hours after inoculation. Although not statistically significant, fewer larvae were recovered in the resistant plant P246 than in the intermediately resistant F416 and the susceptible variety Spantex. There was no significant increase in per root larval penetration in any plant with increase in time of exposure.

Percent root infection in each plant is presented in Table II. Percent root infection was significantly lower in the resistant P246 than in the susceptible Spantex. There was no significant difference between the intermediately resistant F416 and Spantex or P246. Results, however, showed F416 with lower percent of infection than Spantex. An increasing percent in root infection with time was observed in all plants, although an apparent decrease was noted in F416 and Spantex from 48 to 96 hours.

Penetration of larvae was observed to be primarily around the root tip region. Most nematodes were found in this area although some nematodes especially in Spantex, were located in the cortical region

TABLE I

AVERAGE NUMBER OF M. HAPLA LARVAE RECOVERED PER ROOT IN RESISTANT AND SUSCEPTIBLE PEANUTS[1]

Time (hours)	Average number of larvae recovered[2]		
	P246	F416	Spantex
6	1.0	2.0	1.5
12	3.0	1.3	4.0
24	1.3	2.0	1.6
36	1.3	3.0	2.0
48	1.3	2.5	2.0
72	1.0	1.3	3.0
96	1.3	1.2	2.0
Mean	1.4	1.9	2.3

[1]Based on six root samples.

[2]P number assigned by the Oklahoma Agricultural Experiment Station.

TABLE II

PERCENT ROOT INFECTION OF RESISTANT AND SUSCEPTIBLE PEANUTS[1]

Time (hours)	Percent roots infected[2]		
	P246	F416	Spantex
6	16.6	16.6	50.0
12	16.6	50.0	33.3
24	33.3	33.3	75.0
36	33.3	87.5	80.0
48	42.8	40.0	50.0
72	42.8	50.0	40.0
96	50.0	50.0	60.0
Mean	33.6	46.7	56.9

LSD 5% = 16.5, 1% = 23.2.

[1]Based on six root samples.

[2]P number assigned by the Oklahoma Agricultural Experiment Station.

well behind the root tip. A few larvae were found as much as three mm from the root apex.

Observation of acid-fuchsin stained roots revealed gall formation in Spantex within 48 hours and at later periods some of the nematodes recovered were noticeably larger. A similar observation in F416 was encountered at 72 hours. No gall formation was noticed in the resistant P246. No apparent extensive necrosis was observed during this time, although some very darkly-stained root tips were observed in P246 and F416.

Comparison of Giant Cells in Resistant and Susceptible Peanuts

Presented on Table III are cross-sectional areas of giant cells of resistant and susceptible peanuts at 25 and 35 days after nematode inoculation. Results showed that average giant cell area of the susceptible Spantex and the intermediately resistant F416 was statistically larger than those of the resistant wild line P246 at any period. Giant cells of Spantex and F416 had a mean area of 127.6 and 128.9 sq. microns, respectively, compared to 58.8 and 88.8 sq. microns of P246 at 25 and 35 days, respectively. An increase in size and number of nuclei of giant cells with nematode development was observed in all plants.

Histopathology of *Arachis hypogaea* Spantex

Twenty-four hours after inoculation, root-knot nematode larvae were found in various regions in the root. Some larvae were in the cortex with their heads oriented toward the vascular region (Figure 3A).

TABLE III

CROSS-SECTIONAL AREA OF GIANT CELLS OF RESISTANT AND SUSCEPTIBLE PEANUTS IN 25 AND 35 DAY OLD INFECTIONS[1]

Cell No.	Area[2]			
	Spantex[3]	F416[3]	P246[3]	P246[4]
1	125.3	85.2	62.3	59.6
2	112.7	89.4	66.3	64.1
3	115.0	115.6	58.8	70.9
4	115.0	100.9	60.1	129.8
5	112.8	193.1	63.0	116.1
6	184.9	189.6	42.6	92.6
Mean	127.6	128.9	58.8	88.8

LSD: 5% = 38.893; 1% = 53.045.

[1]Area in sq. microns.

[2]P number assigned by the Oklahoma Agricultural Experiment Station.

[3]25 days after inoculation.

[4]35 days after inoculation.

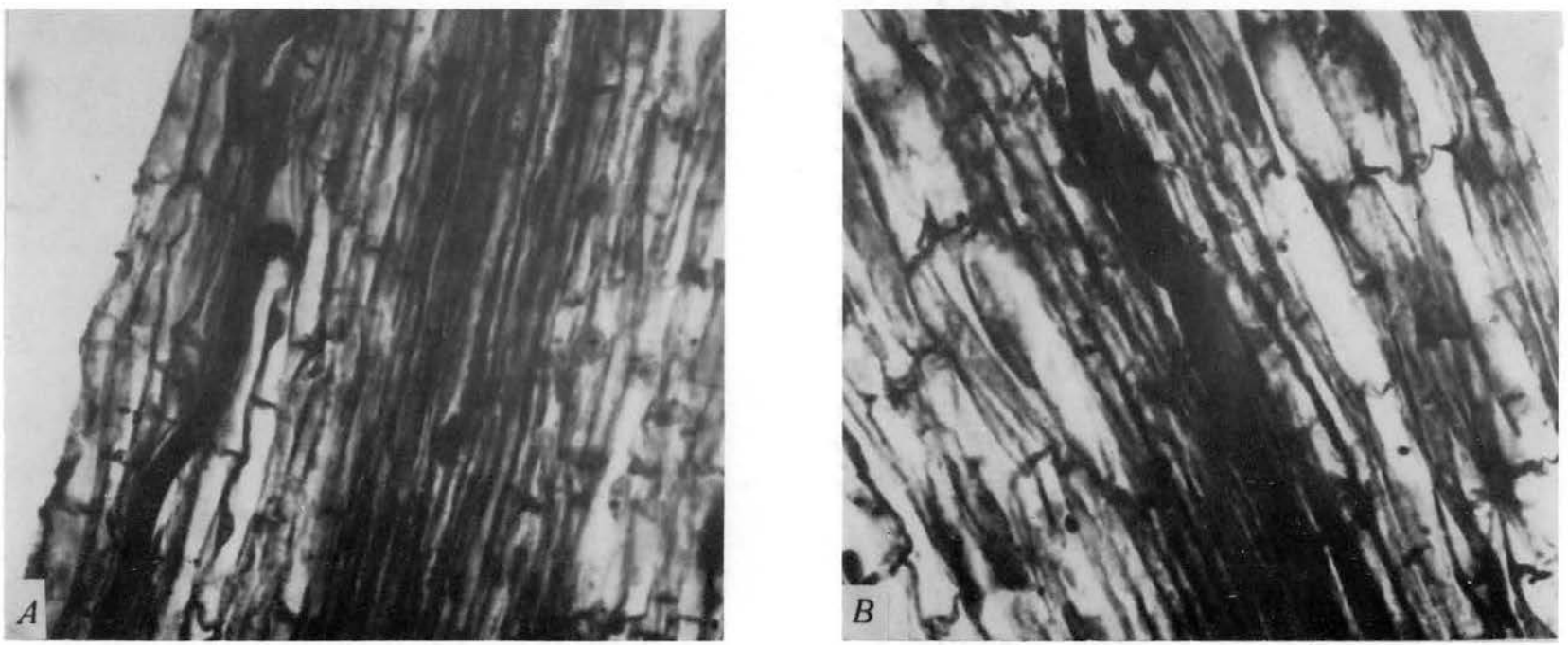

Figure 3. Longitudinal Sections of Spantex Roots One Day after Inoculation. A. Larvae in Cortex (X 340). B. Larvae in Vascular Cylinder (X 340).

Others, however, were already feeding in the vascular region (Figure 3B). Vacuolation of the cytoplasm and enlargement of the nucleus of the vascular parenchyma near the nematode head was noted at 24 hours (Figure 4A). In the root tip, initial giant cell formation was observed in the region of maturation. Cells in immediate proximity to the nematode head exhibited very granular cytoplasm and were somewhat enlarged (Figure 4B). Intercellular and intracellular nematode movement in the cortex, vascular region and root tip was suggested by their position. Cellular destruction along the nematode path was also noted in the stele and in the root tip where multiple infection occurred (Figure 5A).

Two and three days after inoculation, further giant cell development was observed both at the root tip and in the vascular bundle. Cells in front of the nematode exhibited an increase in size and dense granular cytoplasm (Figure 5B). Nuclei of the vascular parenchyma were enlarged and brightly stained with safranin (Figure 6A). Slight cellular hypertrophy and hyperplasia was noted in the region of maturation. Galls were already visible at this period in several roots.

Four days after inoculation, vascular parenchyma became more dense. The wall of the adjacent vessel appeared to dissolve (Figure 6B). Coalescense of the two cells near the nematode head was apparent.

At seven days, growth of the infected root was stopped and galling was very pronounced (Figure 7A). Cellular elongation and differentiation at the apex was not observed. The nematode infection site could be seen as a stained area at the center of the root tip. A small mitotic area in the pericycle, probably a developing lateral root, could also be observed. Lateral roots were later found in most root

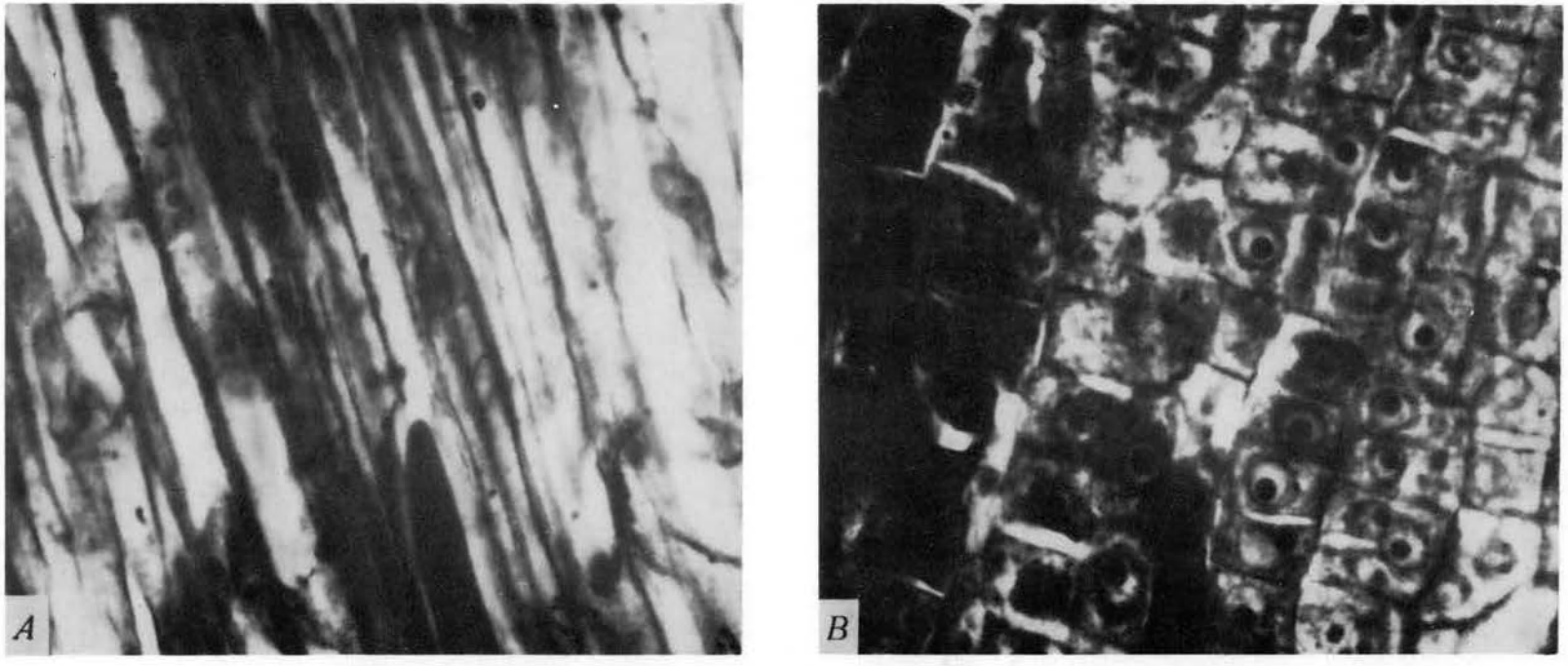

Figure 4. Longitudinal Sections of Spantex Roots One Day after Inoculation. A. Larva in Stele (X 832). B. Larva in Root Tip Initiating Giant Cell Formation. Note: Cell Wall Dissolution (X 832).

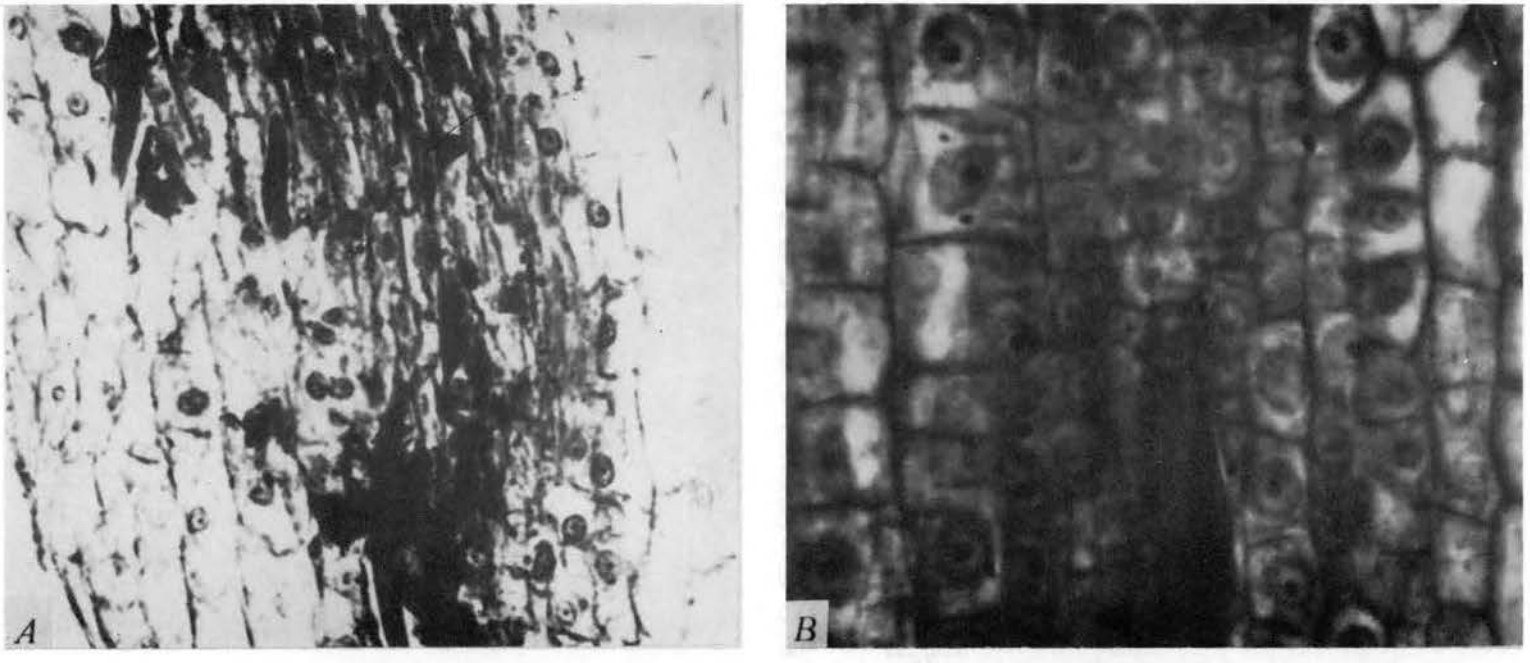

Figure 5. A. Longitudinal Section of Spantex Root One Day after Inoculation. Note: Multiple Infection at Root Tip (X 340). B. Longitudinal Section of Spantex Root Two Days After Inoculation. Note: Giant Cell Initiation Near Nematode Head (X 832).

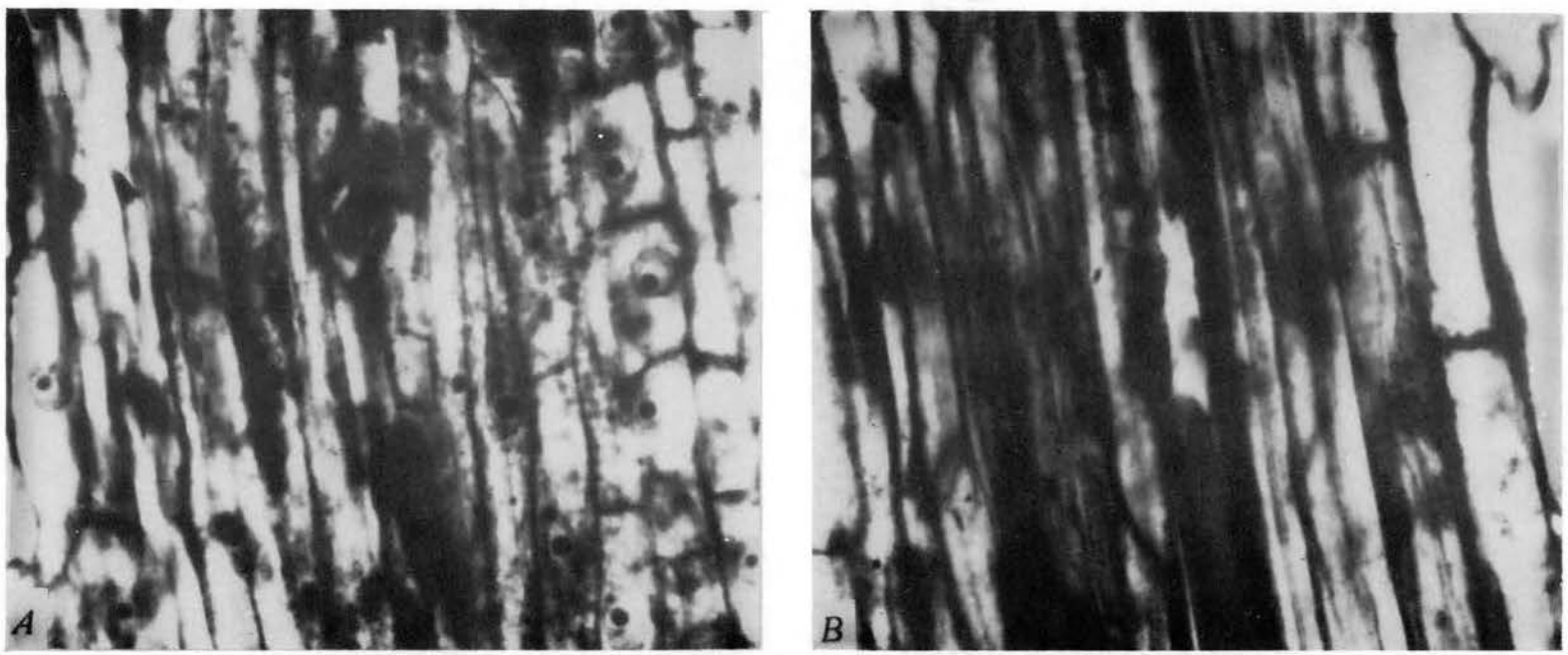

Figure 6. A. Longitudinal Section of Spantex Root Three Days after Inoculation. Note: Nematode Head in Vascular Cylinder (X 832). B. Longitudinal Section of Spantex Root Four Days after Inoculation (X 832).

galls examined. A nematode could be seen among the abnormal cells of the vascular bundle (Figure 7B). Continuity of the vascular elements was blocked by the nematode and giant cells. Growth in size of the nematode, probably in the third stage, was noticed. Cellular hyperplasia in the stele and hypertrophy of cortical cells were pronounced. Most of the giant cells were located in the vascular region but some were found near the pericycle and in the cortical area (Figure 8A). Giant cells were brightly stained and exhibited dense, granular cytoplasm.

In 10 day old infections, considerable enlargement of the gall and giant cells was evident (Figures 8B, 9A). Nematodes feeding on some giant cells were positioned perpedicular to the longitudinal axis of the root with their bodies in the cortex and heads oriented toward the giant cells in the vascular bundle (Figure 9B). There was extensive cellular hyperplasia in the pericycle and stele around the nematodes and the giant cells.

Cross sections of roots at 16 and 20 days after inoculation showed growth of the gall and giant cells (Figures 10A, 10B, 11A, 11B). Around the greatly enlarged giant cells in the vascular bundle, were small, compact cells indicating their high hyperplastic activity (Figures 10B, 11B). The cells were apparently pericyclic in origin. Parenchyma cells in the vascular bundle contained numerous starch grains (Figure 12A).

Twenty-five days after infection, egg masses were observed in galled roots. Mature, saccate-shaped females were found with their rounded body in the cortex and oriented perpendicular to the stele. Breaks in the cortical tissue resulted with the posterior end of the

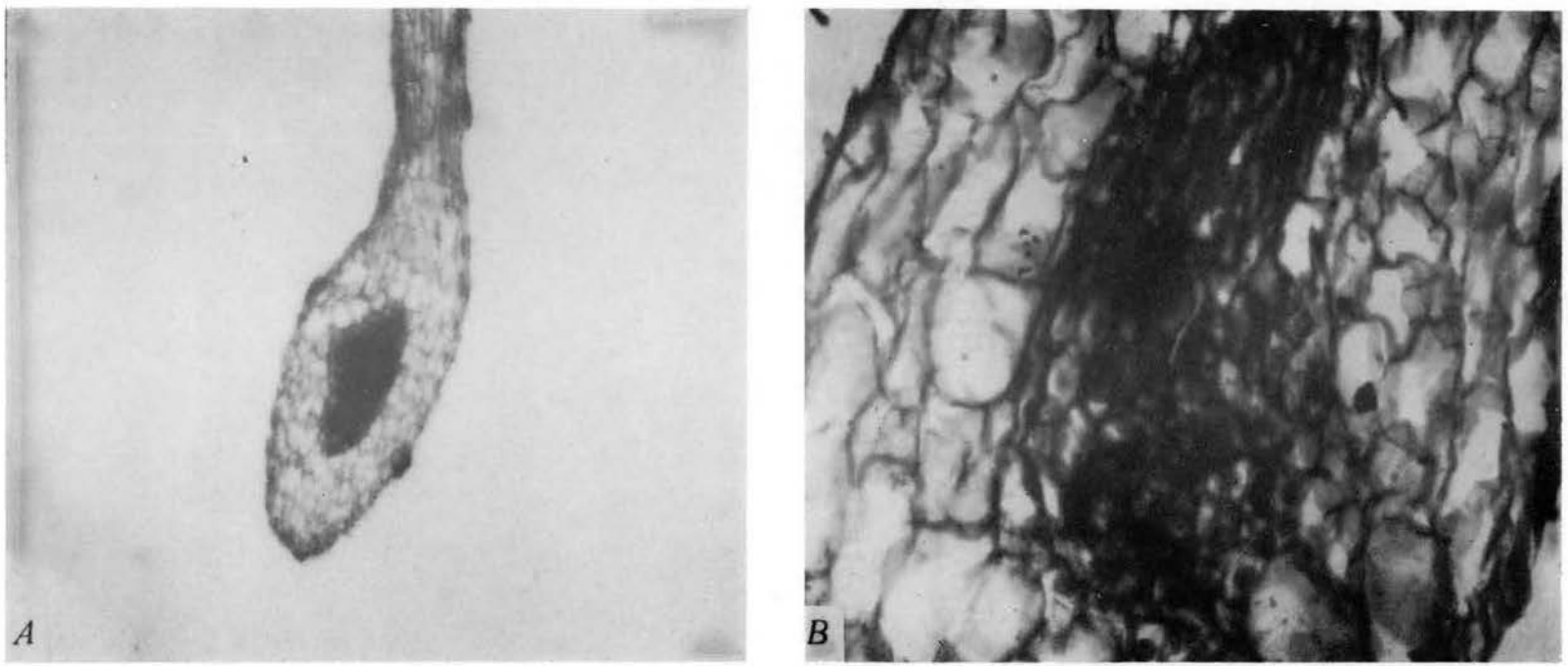

Figure 7. A. Longitudinal Section of Galled Spantex Root Seven Days after Inoculation (X 90). B. Enlarged Portion of Gall. Note: Nematode in Vascular Bundle (X 360).

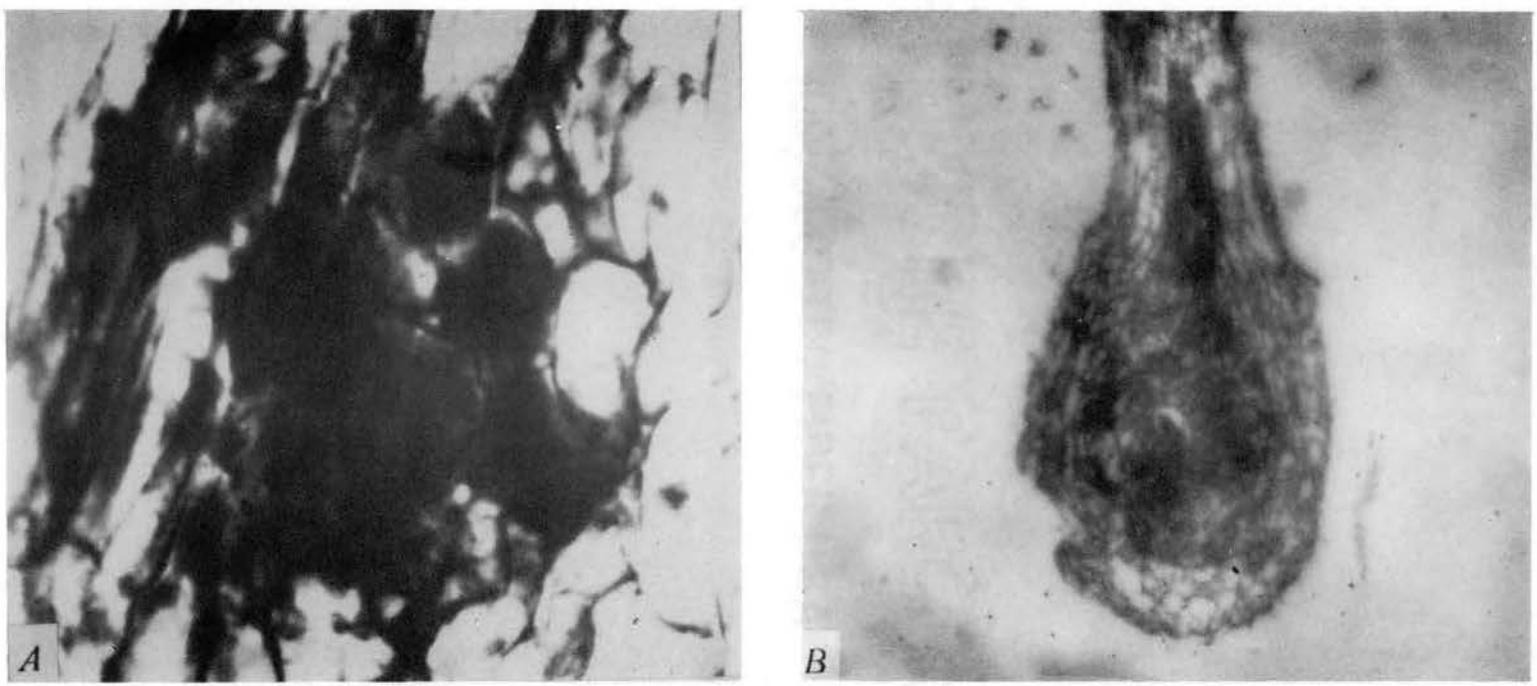

Figure 8. A. Longitudinal Section of Giant Cells in a Seven-day Old Infection of Spantex (X 832). B. Cross Section of a Galled Root of Spantex Ten Days after Inoculation (X 90).

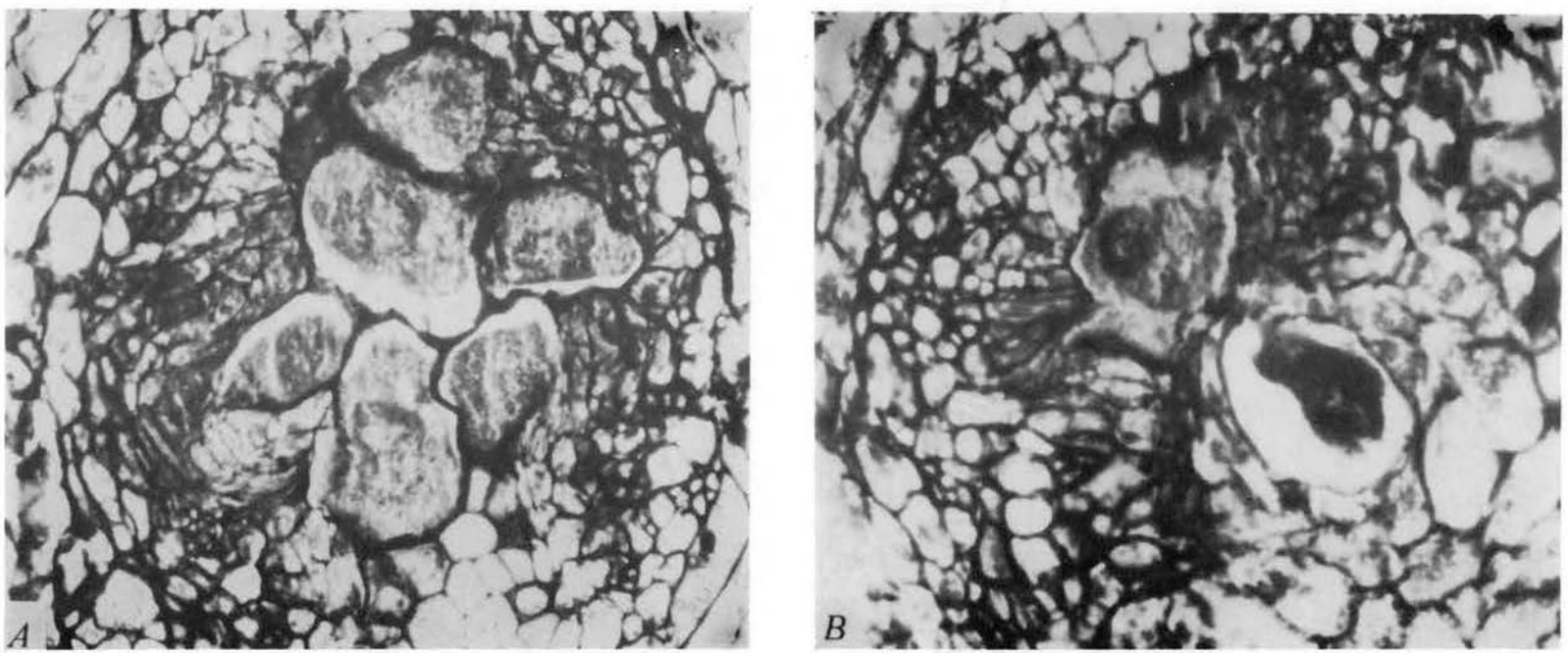

Figure 9. Cross Sections of Galled Spantex Root Ten Days after Inoculation. A. Giant Cells Surrounded by Hyperplastic Cells (X 390). B. Enlarged Nematode Feeding on Giant Cell (X 330).

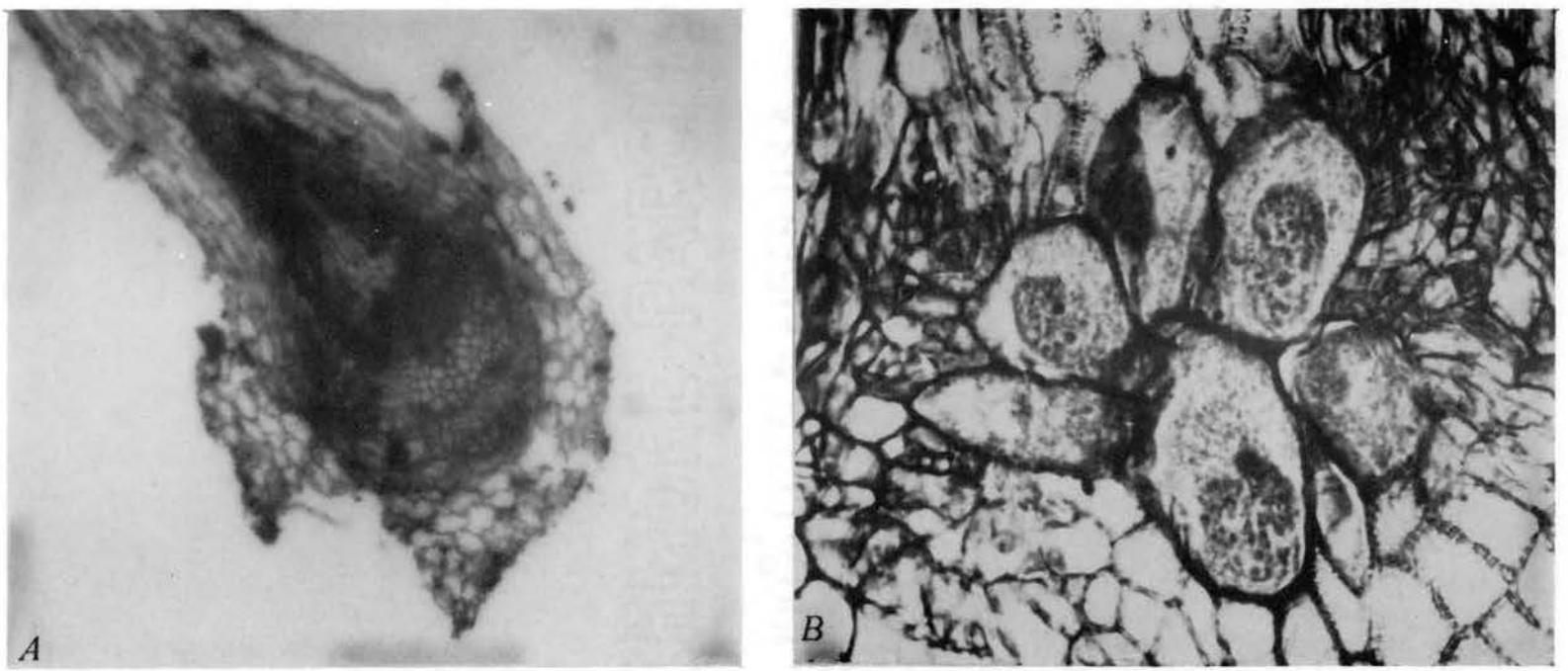

Figure 10. Cross Sections of Galled Spantex Root 16 Days after Inoculation. A. Gall with Lateral Root (x 90). B. Enlarged Portion Showing Multinucleate Giant Cells (X 390).

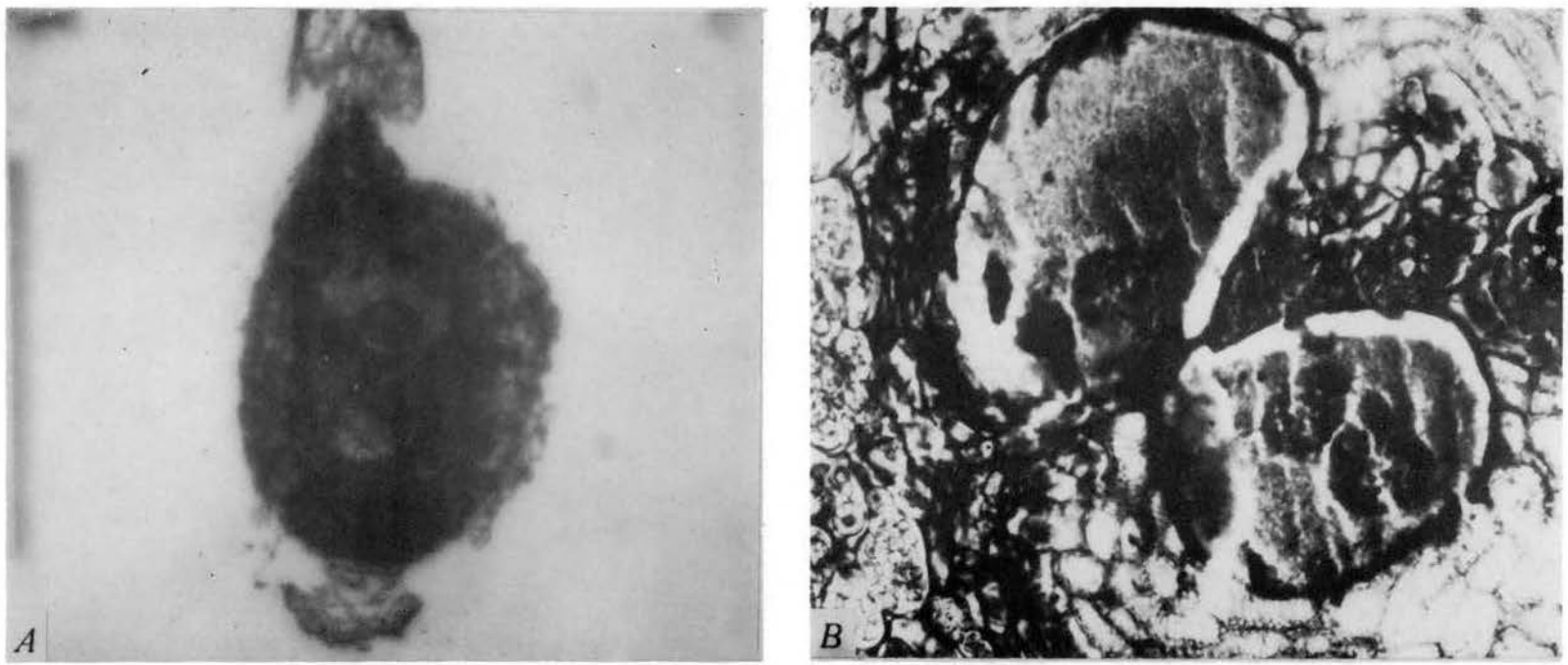

Figure 11. Cross Sections of Galled Spantex Root 20 Days after Inoculation. A. Galled Root with Giant Cells (X 90). B. Enlarged View of Giant Cells in the Vascular Cylinder (X 390).

nematode protruding from the root or just below the outside layer of cells (Figure 12B). Some second stage larvae were also found in several galled roots indicating infection by second generation larvae. Giant cells were greatly enlarged and in many cases extended as much as one-half the diameter of the stele (Figure 13A, 13B). The number of nuclei varied but as many as 50 were counted in one giant cell. Grouping of nuclei in syncytia was also noticed. The shape of giant cells varied and sometimes several giant cells were observed to coalesce forming a much larger giant cell.

Histopathology of *Arachis* sp. F416

Within 24 hours after inoculation, root-knot larvae were found in the cortex (Figure 14A, 14B). Passage through the tissue was both intercellular and intracellular. The larvae were variously oriented. Some were lying intercellularly parallel to the longitudinal axis of the root, while others were oriented perpendicular to the vascular region. Intracellular penetration caused cell destruction in the cortex.

On the second day after inoculation, larvae were found inside the vascular bundle (Figure 15A, 15B). Intracellular nematode migration through the vascular bundle killed cells and damaged tissue. Hypertrophy and hyperplasia were not observed during this period.

Longitudinal sections of root tips three days after inoculation showed necrotic areas near nematode head (Figure 16A). The cells in these necrotic areas were collapsed and brightly stained with safranin. Similar cell reaction on the fourth day was observed in some roots while others exhibited no such necrotic response.

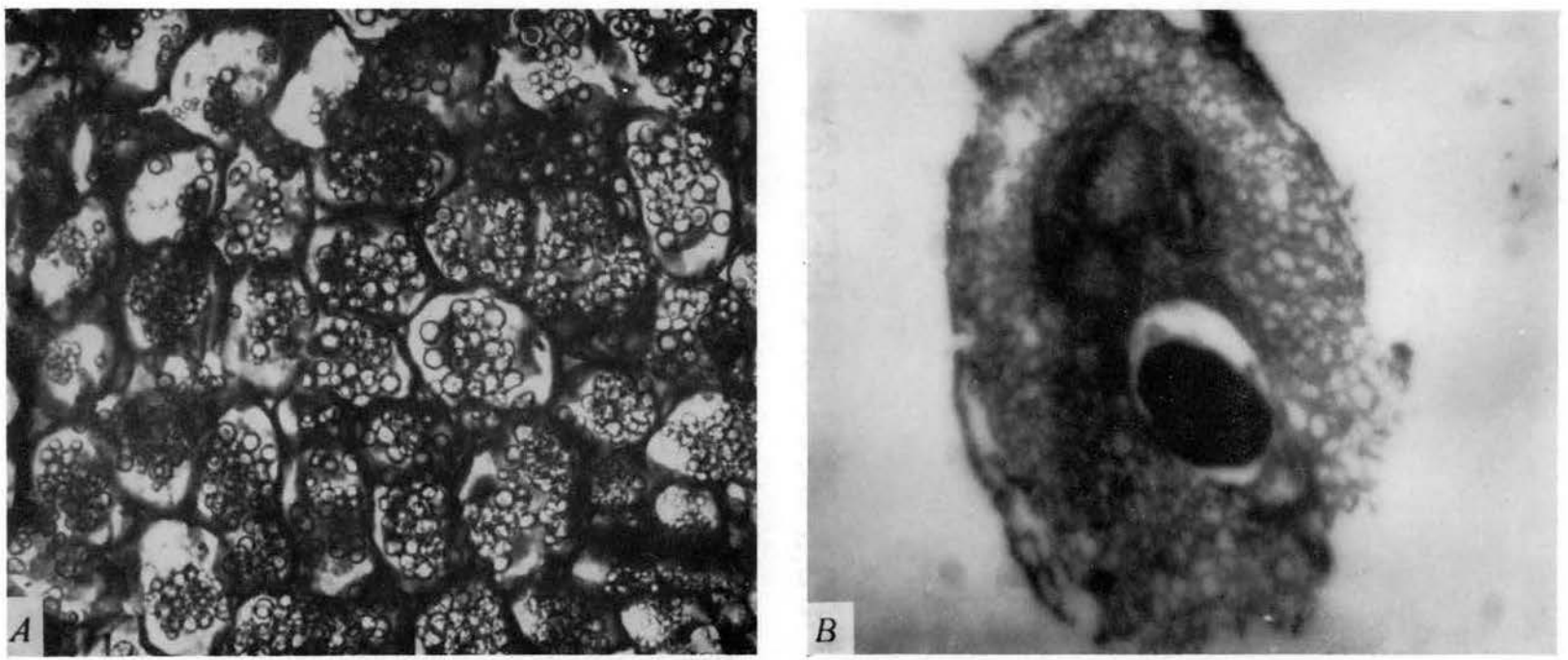

Figure 12. A. Cross Section of Galled Spantex Root. Note: Starch Grains in Vascular Parenchyma Cells (X 340). B. Cross Section of Galled Spantex Root 25 Days after Inoculation with Egg-laying Female (X 80).

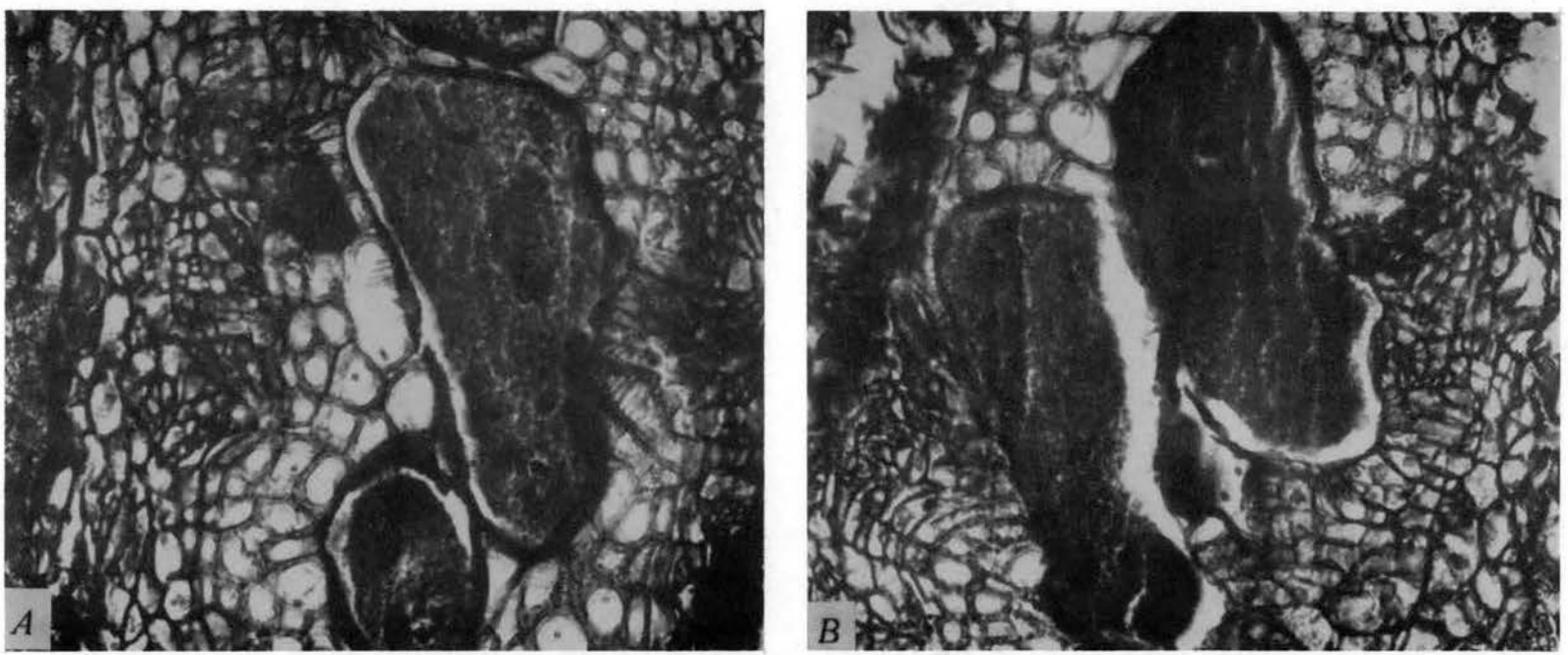

Figure 13. Cross Sections of Giant Cells in Galled Spantex Root 25 Days after Inoculation. A. Giant Cells of Different Shape and Size (X 330). B. Same Giant Cells at a Different Plane (X 330).

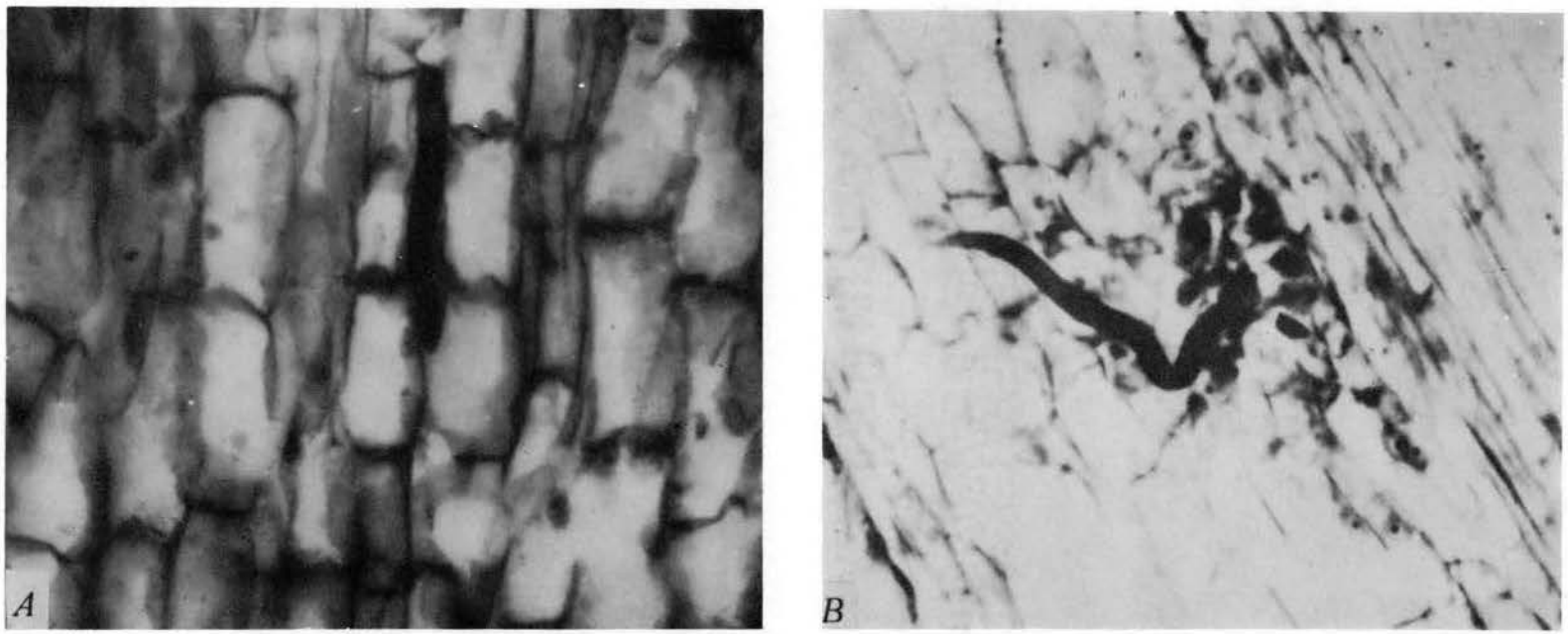

Figure 14. Longitudinal Sections of F416 Roots One Day after Inoculation. A. Intercellular Position of Larva in Cortex (X 350). B. Intracellular Penetration of Larva (X 350).

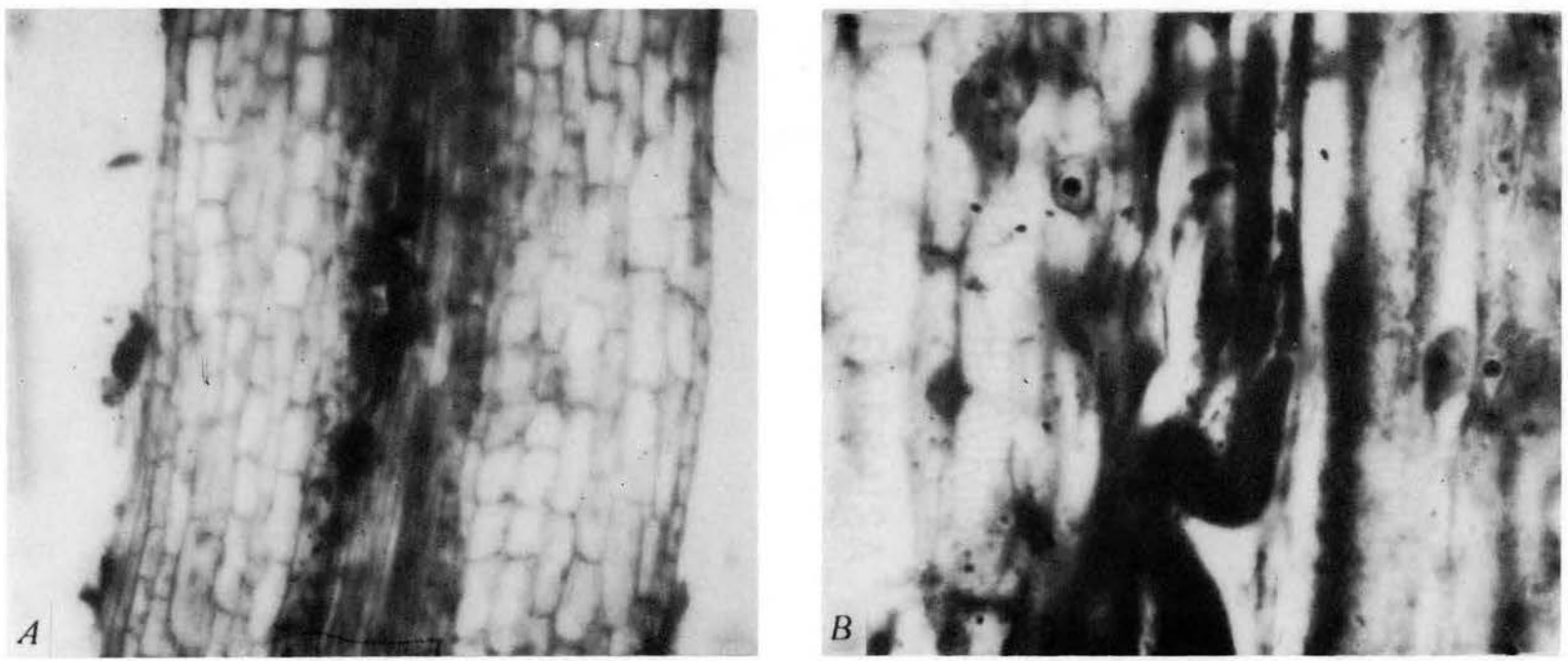

Figure 15. Longitudinal Sections of F416 Roots One Day after Inoculation. A. Larva in Vascular Cylinder (X 350). B. Enlarged View. Note: Cell Damage (X 742).

In five day old infections, root galling was very distinct (Figure 16B). Root elongation was arrested and cellular hypertrophy in the cortex and hyperplasia in the vascular region was observed. A distinct mitotic area arising from the pericycle could be observed. This area would probably give rise to a lateral root. Early giant cell formation at the root tip was observed (Figure 17A, 17B). The giant cells, even at the early stage of development, were distinctly different from the normal cells due to their very dense cytoplasm, multinucleate condition and larger size. Closer examination showed apparent coalescense of the adjacent normal cells with the giant cells. In one syncytium, four nuclei were counted.

No infected roots were collected at six, seven, 10 and 13 days after inoculation. This was probably due to the limited larval penetration. However at 16 and 20 days after inoculation, galled roots were collected. Growth of the nematode and giant cell enlargement were observed (Figures 18A, 18B, 19A, 19B). Giant cells were located in the vascular bundle and were surrounded by hyperplastic cells. As many as 32 nuclei were counted in one giant cell. Vascular parenchyma cells contained numerous starch grains and cortical cells exhibited hypertrophy. The nematodes were enlarged and in their typical feeding position, with their body in the cortex and the head reaching to the syncytia in the stele. Cells around the nematode's body were flattened and thin, suggesting pressure exerted by nematode growth.

Egg laying females were observed at 25 days after inoculation (Figure 20A). Egg masses were found in most of the galled roots collected. Giant cells were distinctly larger (Figure 20B) and their nuclei were often observed grouped in darkly-stained areas in the

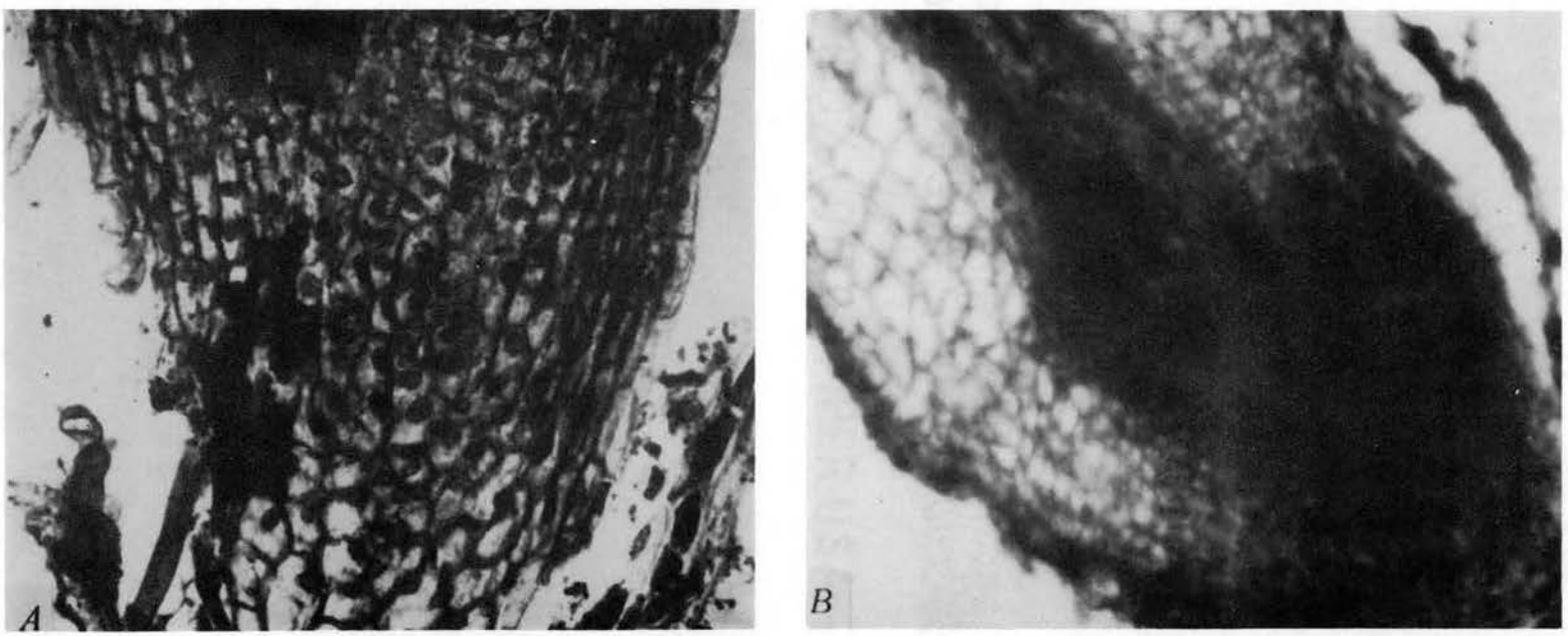

Figure 16. A. Longitudinal Section of F416 Root Three Days after Inoculation. Note: Necrotic Area Near Nematode Head (X 340). B. Longitudinal Section of a Galled F416 Root Five Days after Inoculation. Note: Lateral Mitotic Area (X 150).

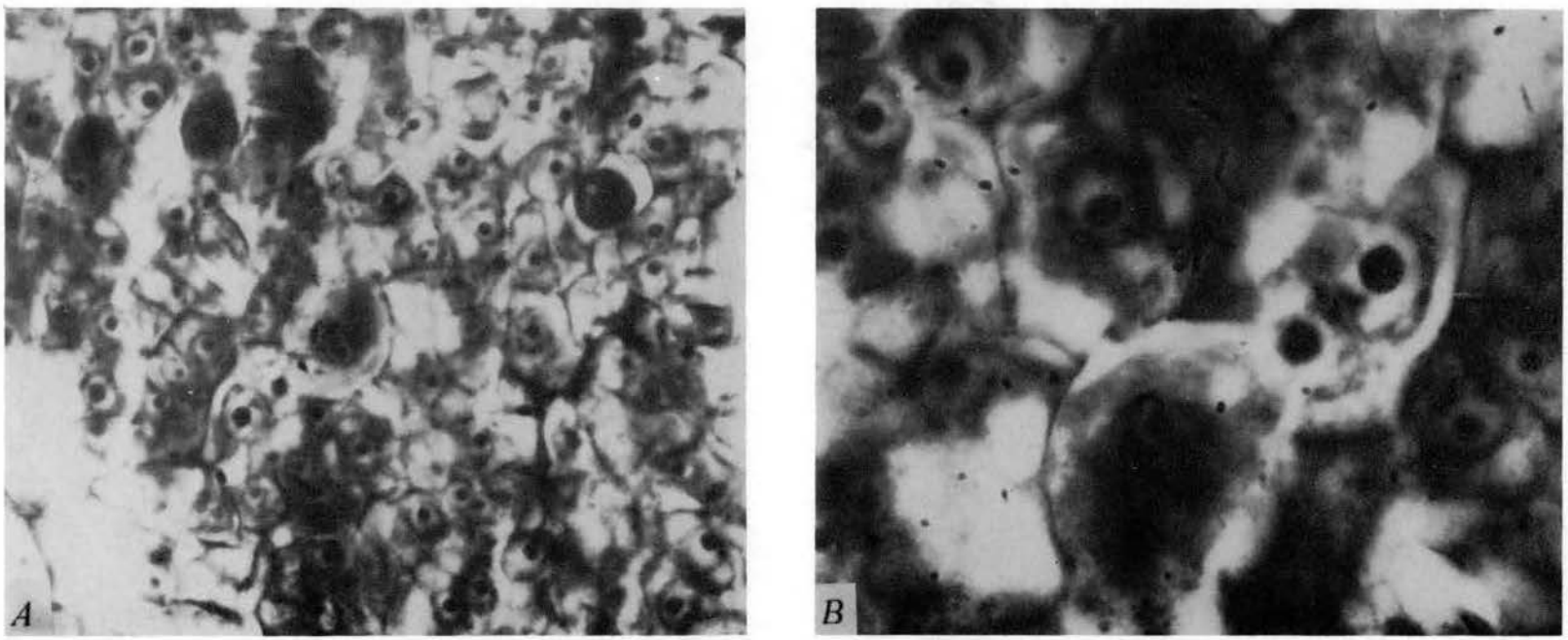

Figure 17. A. Cross Section of Galled F416 Root Five Days after Inoculation Showing Early Stage of Giant Cell Development (X 765). B. Enlarged View. Note: Coalescence of Giant Cell with Adjacent Normal Cells (X 1700).

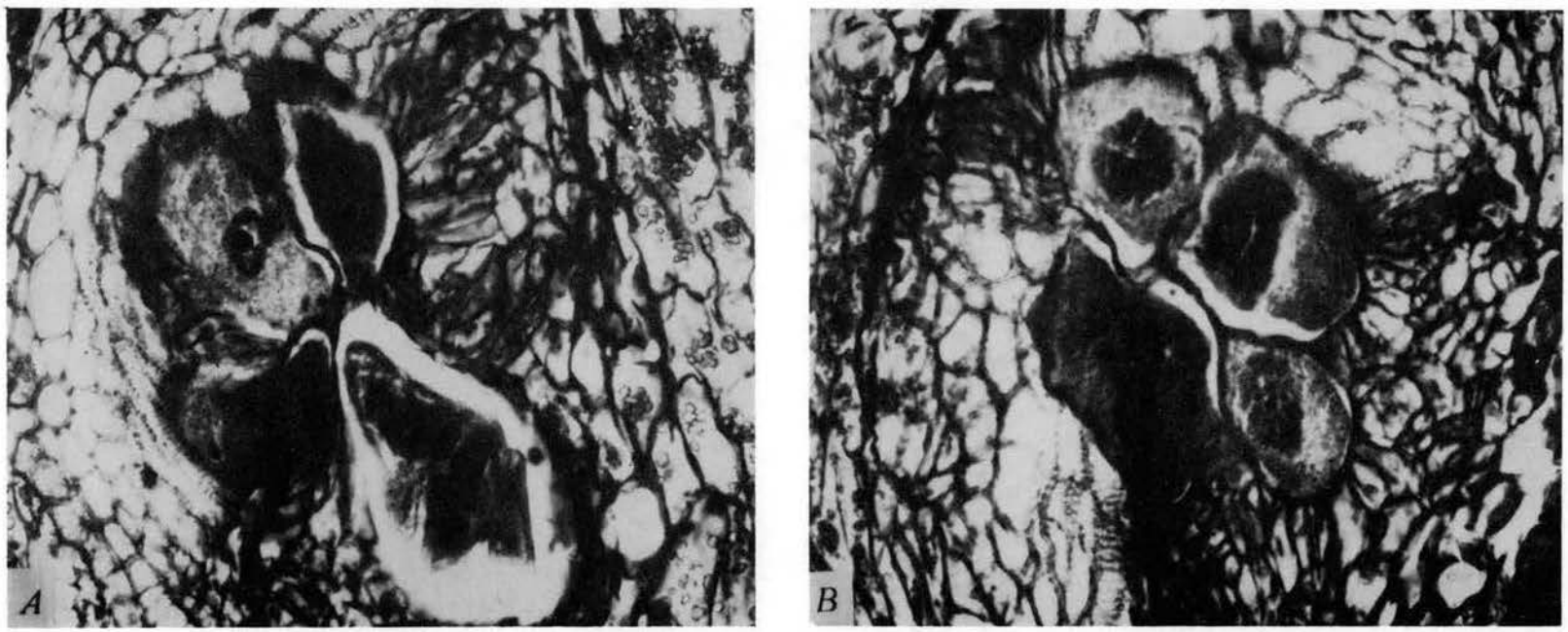

Figure 18. Cross Sections of Galled F416 Roots 16 Days after Inoculation. A. Nematode Feeding on Giant Cells (X 330). B. Multinucleate Giant Cells in Vascular Cylinder (X 330).

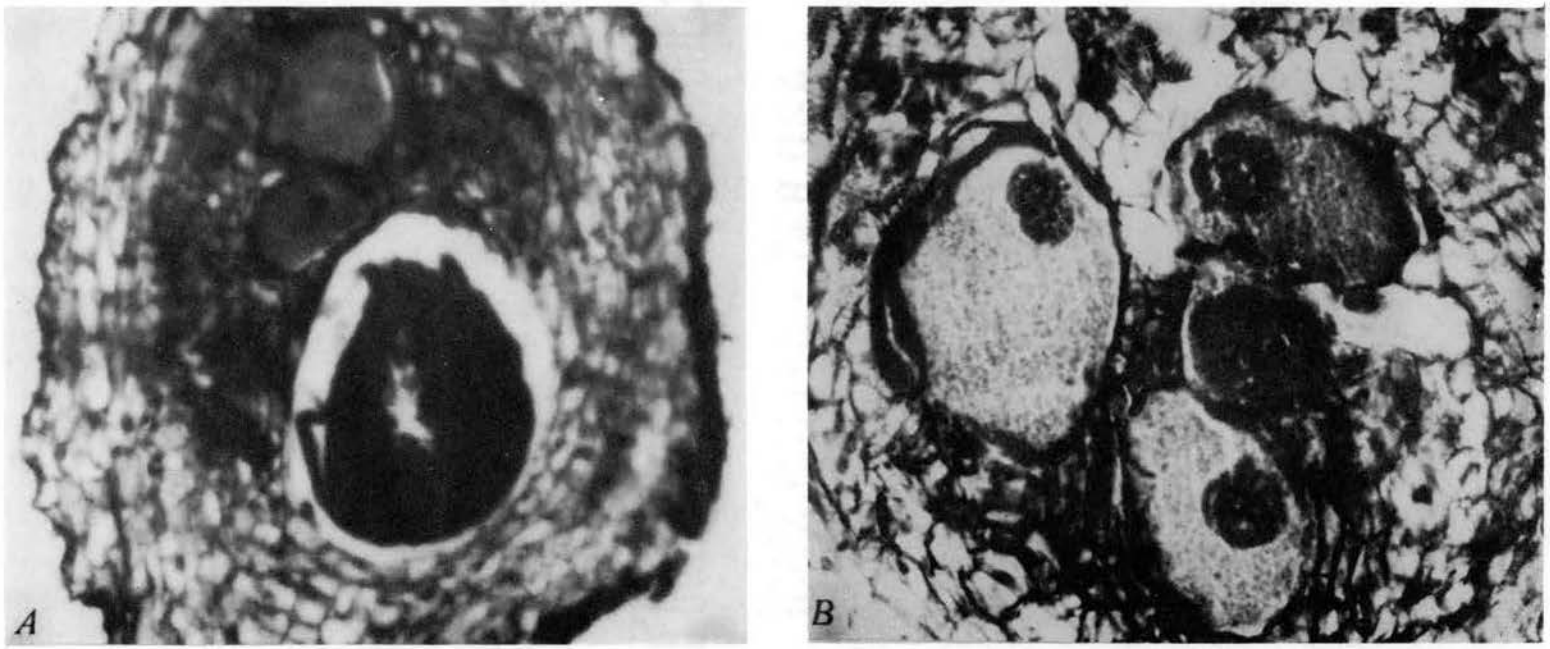

Figure 19. Cross Sections of Galled F416 Root 20 Days after Inoculation. A. Gall Showing Relative Size of Nematode (X 165). B. Giant Cells in Vascular Cylinder (X 330).

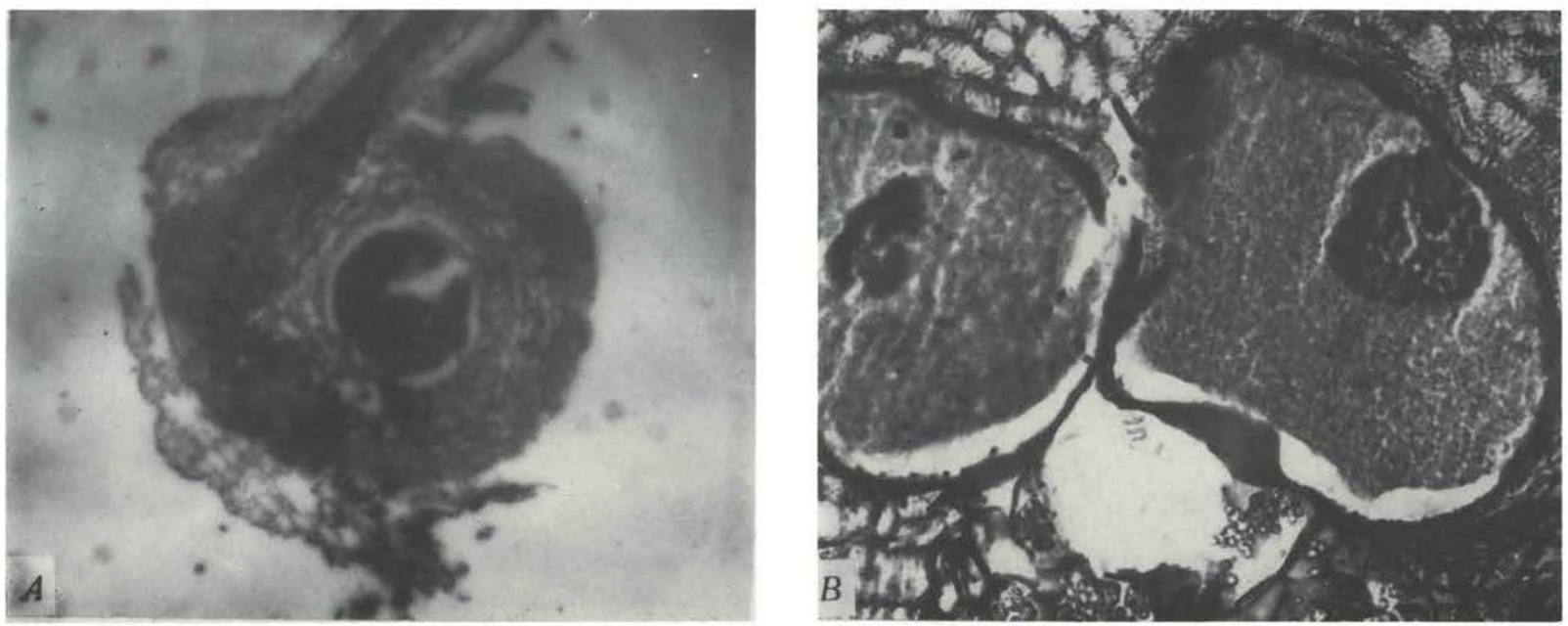

Figure 20. A. Longitudinal Section of Galled F416 Root 25 Days after Inoculation (X 66).
B. Cross Section of Giant Cells in Galled F416 Root 25 Days after Inoculation (X 330).

cytoplasm. In some giant cells, several such groups of nuclei were observed. The size of the nuclei in each syncytium varied.

Histopathology of *Arachis* sp. P246

Due to the apparent limited penetration of nematode larvae, few roots were found to have been infected. Observations were therefore restricted to periods in which the roots collected were successfully penetrated.

One day after inoculation, larvae were found in the cortex lying intracellularly (Figure 21A). Some root tips exhibited necrosis following multiple larval penetration (Figure 21B). Dark portions in the root tip indicated necrotic areas where cells had collapsed and were brightly stained with safranin. No nematodes were found in the vascular bundle.

A three day old infection showed larvae inside the vascular bundle (Figure 22A). Nematode position and extensive cellular damage along their path indicated intracellular migration. Penetration occurred at the root tip and the path of larval migration was traced upward through the vascular bundle.

A longitudinal section of a root, six days after inoculation showed a vermiform larva with its anterior portion in the vascular region (Figure 22B). The necrotic area near the head of the nematode was composed of collapsed pericycle cells which were brightly stained with safranin. Intracellular migration of the nematode in the cortex caused cellular destruction and the formation of a tunnel along its path (Figure 23A). Cellular reaction such as giant cell formation, hypertrophy, or hyperplasia was not observed.

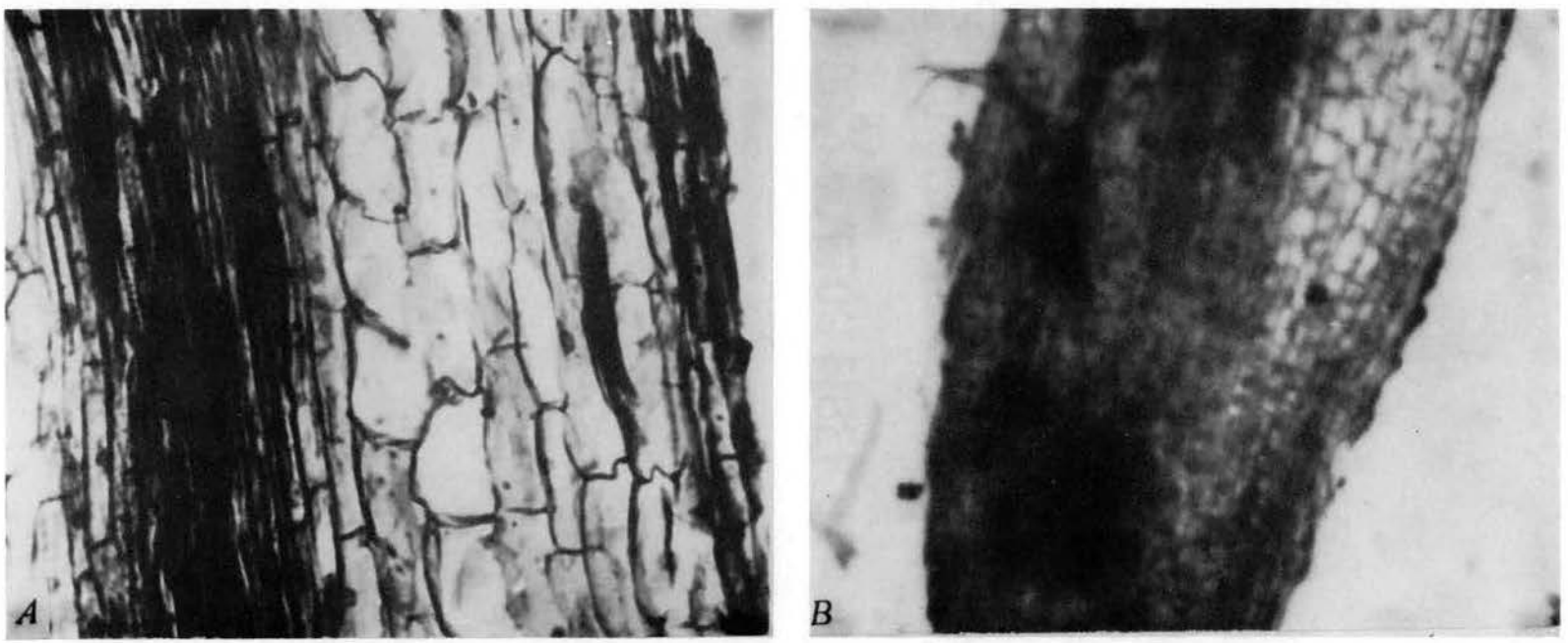

Figure 21. Longitudinal Sections of P246 Roots One Day after Inoculation. A. Intercellular Position of Larva in Cortex (X 330). B. Necrotic Areas in Root Tip (X 155).

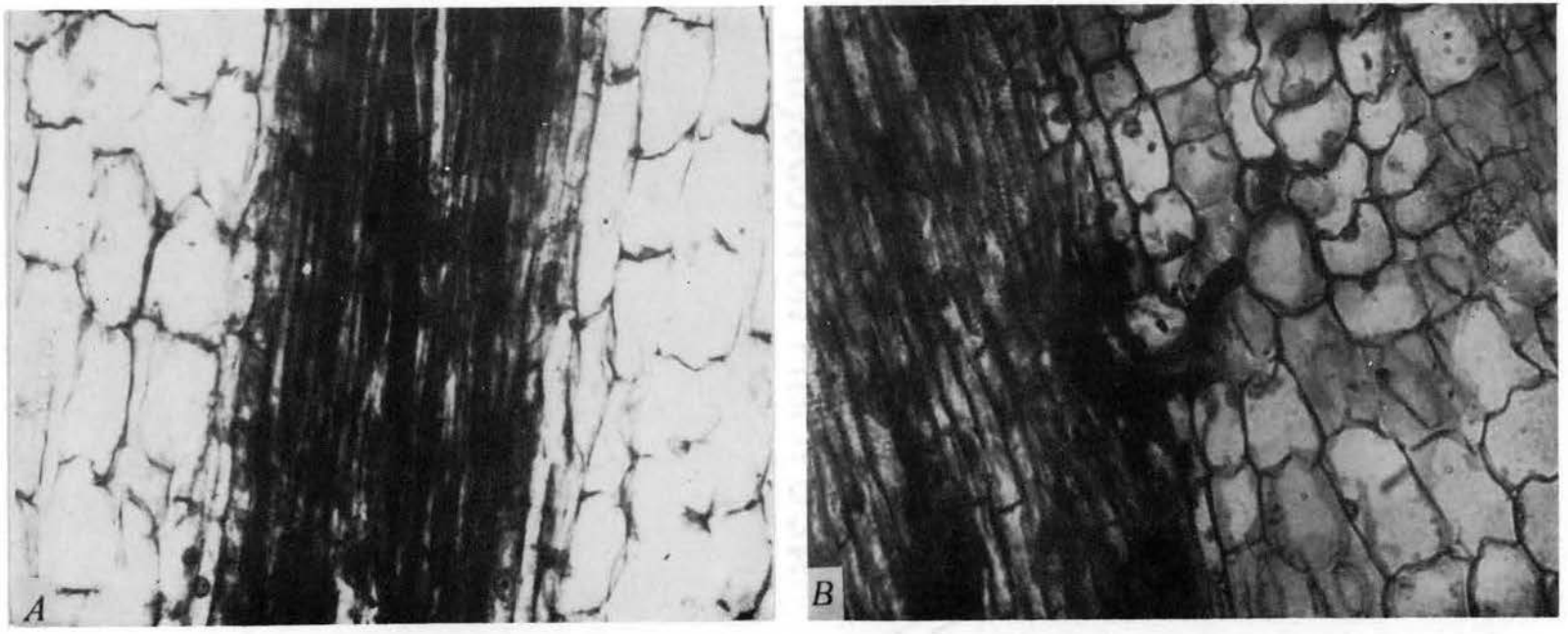

Figure 22. A. Longitudinal Section of P246 Root One Day after Inoculation. Note: Nematode in Vascular Cylinder (X 330). B. Longitudinal Section of P246 Root Six Days after Inoculation (X 330).

A galled root collected at 13 days after infection, showed a larva feeding on some giant cells in the vascular bundle (Figure 23B). The nematode was only slightly enlarged and probably was in the third larval stage.

In 25 day old infections, enlargement of both the nematode and its giant cells was observed (Figure 24A, 24B). Vascular parenchyma were also noted to contain numerous starch grains. No egg mass, however, was found in the roots sampled.

Thirty-five days after inoculation, an egg mass was observed in a galled root with the mature female nematode. Further enlargement of the giant cells was evident (Figure 25A, 25B). Syncytia were located in the stele, surrounded by vascular elements and hyperplastic cells. They were multinucleate with dense cytoplasm. Hypertrophic cortical cells were also observed.

Screening for Resistance in Peanut to M. hapla

Varieties, plant introductions and hybrids of A. hypogaea tested for resistance to M. hapla are shown in Tables IV and V. The hybrids were F_2 progenies of crosses made among the intermediate resistant lines F416, NC4X and PI288151.

A total of 245 plants were screened for resistance to M. hapla. Of these, only two plants showed moderate degree of galling. In two separate trials, the plant introduction line PI315617, showed a mean root gall index of 2.2. In a single trial, one of the progeny of the hybrid 68-266B, exhibited a gall index of 3.0. Further screening is needed to determine the stability of the low gall indices observed in these plants.

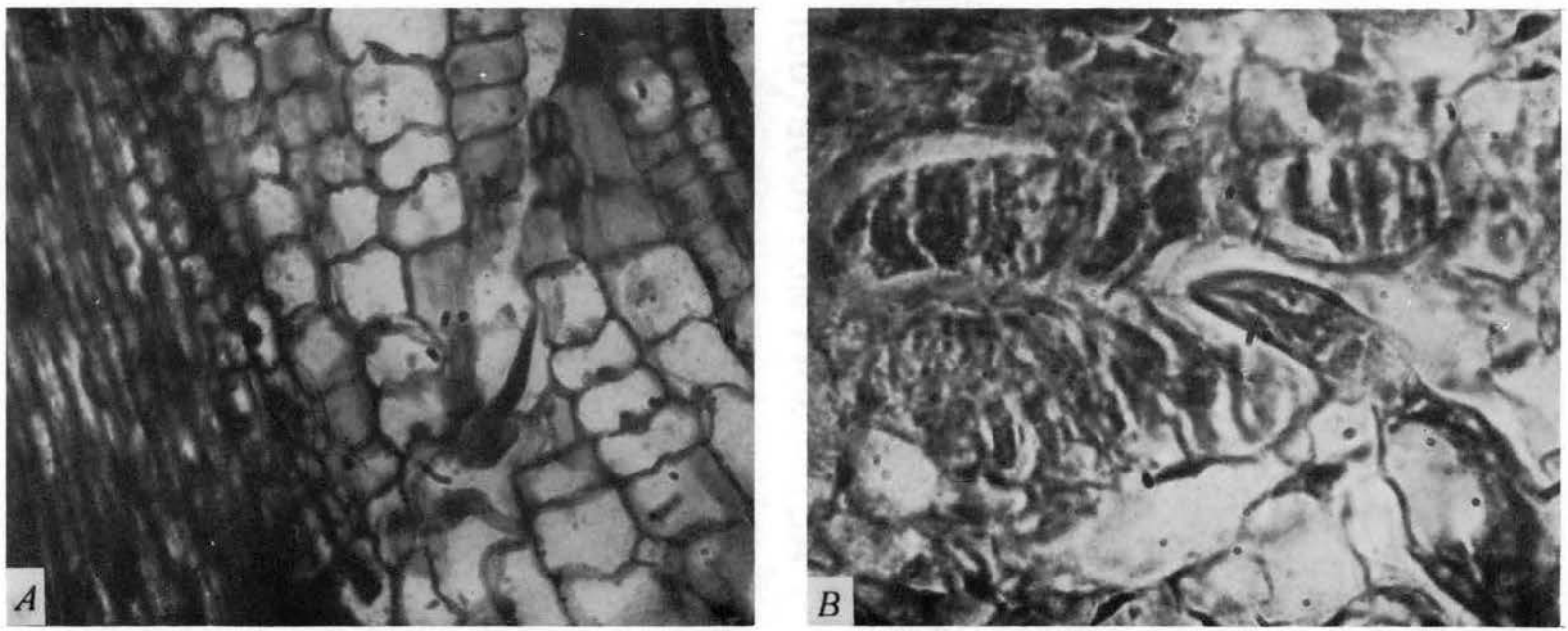

Figure 23. A. Longitudinal Section of P246 Root Six Days after Inoculation. Note: Tissue Damage Along Nematode Path (X 330). B. Cross Section of Galled P246 Root 13 Days after Inoculation (X 742).

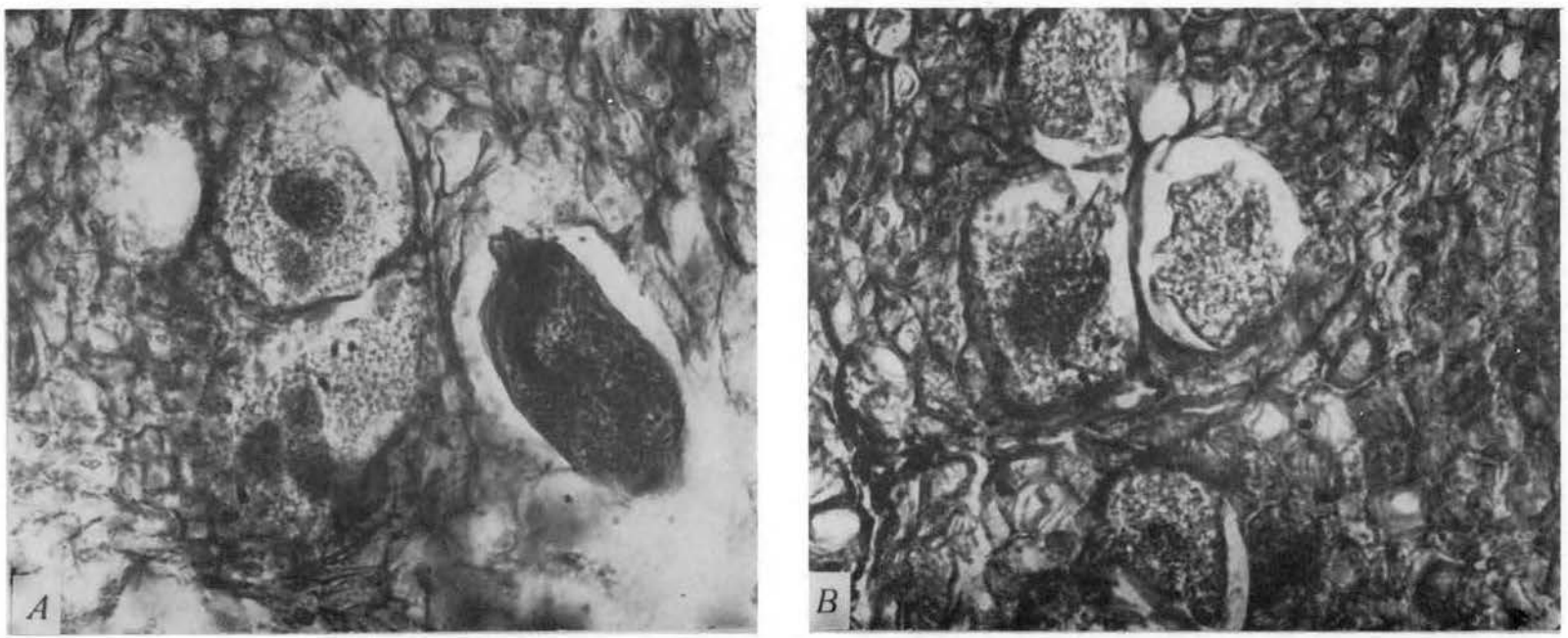

Figure 24. Cross Sections of Galled P246 Roots 25 Days after Inoculation. A. Nematode Feeding on Giant Cells (X 330). B. Giant Cells in Vascular Cylinder (X 330).

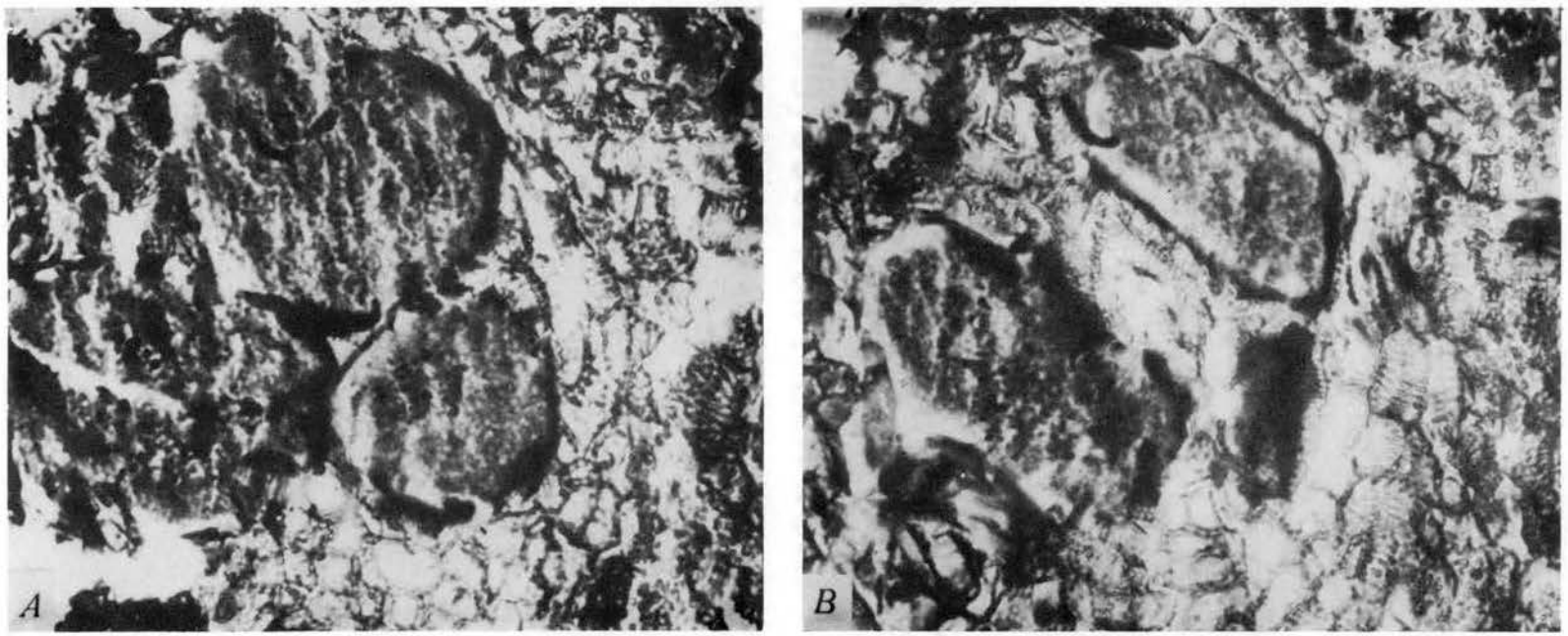

Figure 25. Cross Sections of Galled P246 Root 35 Days after Inoculation. A. Coalescence of Giant Cells (X 330). B. Two Separate Giant Cells in Vascular Cylinder (X 330).

TABLE IV

LIST OF PLANT INTRODUCTION LINES AND VARIETIES OF *A. HYPOGAEA* SCREENED FOR RESISTANCE TO *M. HAPLA*[1]

PI 295190S	PI 315618	PI 323235L	NC 301H68
295193	315623	323236	343H68
295195	315629	323238	6339
295201	315632	323239	10211
295205	315637	323240	10211R
295210	316126	323241	10219F
295214	316127A	323241L	10219R
295215	316127B	323266	10219RB
295223	316135	323267	10223
295743	316136	323583	10242
295752	316138	323505	10243
295981	316139	325083L	10243B
298844RS	316441	326587	10277Y
298854R	318465	326588	10446
300239RI	318731A	326591	10446R
300242	318731B	326592	10446YB
311003S	318733	326593	10449
313122	318735	329224	10449R
313131	318736	329225	10452
313147	318737	329226	10453
313148	318738	329227	10459
313157	318739	330643FS	10464F
313175	318740	330643PM	10464R
313191	318741	330644	10468
313192	318741S	330646F	15726
314894	318742	330646R	15731
314898S	318742S	330647F	15732
315605	318743	330647P	15736
315607	323234	330648FS	15739
315608	323235	330648P	15740
315617	323235H68		

[1]PI numbers assigned by the New Crops Research Branch of ARS, USDA.

TABLE V

LIST OF PEANUT HYBRIDS WITH THE PARENT VARIETIES AND THE NUMBER OF PROGENIES SCREENED FOR RESISTANCE TO M. HAPLA[1]

Hybrid	Parents[2]		No. of progeny
	Female	Male	
68-194	F416	NC4X	17
68-266A	NC4X	F416	11
68-266B	NC4X	F416	16
68-266LS	NC4X	F416	3
68-17A	PI288151	F416	18
68-17B	PI288151	F416	2
68-17LS	PI288151	F416	3
68-184	F416	PI288151	16
68-236A	F416	NC4X	8
68-236B	F416	NC4X	11
68-236LS	F416	NC4X	11

[1]Crosses made by Dr. D. J. Banks, Dept. of Agronomy, OSU and ARS, USDA.

[2]PI numbers assigned by the New Crops Research Branch of ARS, USDA.

CHAPTER V

DISCUSSION

The larval penetration study showed nematode entry of roots of the susceptible Spantex, intermediate F416 and resistant P246 within six hours after inoculation. There was, however, an apparent inhibition of penetration in the resistant P246 as indicated by the fewer larvae recovered than in the intermediately F416 and susceptible Spantex. Since, in the present study, the nematodes were pipetted directly onto the root tip, it was assumed that effects of any differences of root attraction among the plants would be less a factor. This suggests therefore, the existence of a barrier in the resistant P246 that affects nematode invasion. Similar observations were also reported by Castillo (4) in an earlier study in which he indicated a pre-infection type of resistance.

Although resistance to root-knot nematodes is usually expressed after nematode penetration of roots (12, 32), several studies have indicated that some plants exhibited resistance to root-knot nematode penetration. Sasser and Taylor (34) suggested that resistance in plants to root-knot nematodes may be caused in part by failure of the larvae to enter the roots or entry of reduced number with little or no development. Goplen and Stanford (11) attributed the resistance of white clover to the failure of M. hapla larvae to penetrate. Working with M. incognita on resistant tomato, Peacock (25) found lesser larval

penetration in resistant than in susceptible plants. He also observed that fewer larvae were attracted to resistant roots. Castillo (4) reported similar observations on M. hapla resistant peanut and further suggested the existence of toxic root substances that resulted in the decrease of nematode population with time.

Post-infection resistance is believed to be the most common type of resistance to root-knot nematodes (12, 32). This type of resistance is usually indicated by the plant cell response to the parasite and in the degree of nematode development. Results of the present study indicated differential size and development of giant cells of the resistant P246, intermediate F416 and susceptible Spantex. Mean cross-sectional area of giant cells in P246 was significanctly less than those in Spantex and F416. It should be noted, however, that the number of observations was limited and that this area does not represent the actual size of the cell since the syncytium varies in dimensions along the length of the root.

Delayed giant cell development in the resistant P246 was also observed. Initiation of giant cell formation in Spantex and F416 was observed within one to two days after penetration, while in P246, no apparent syncytial formation was observed even six days after inoculation. These cellular reactions suggest the lack of favorable plant response in P246 and is apparently correlated with the delayed nematode development. Many investigators have reported similar observations. In their host suitability studies with H. trifolii, Mankau and Linford (20) indicated that nematode development was closely related to the developmental rate and size of the syncytium. A similar correlation of root-knot nematode development and giant cell formation and size was

reported by Crittenden (6), Dropkin (8) and Dropkin and Nelson (9).

An apparent necrotic reaction in the intermediate resistant F416 was observed within three days after inoculation. In the resistant P246, a nematode with necrotic cells near its head was observed only at six days after inoculation. Although cellular destruction and necrosis, especially at the root tip, were observed in the susceptible Spantex, no collapsed and brightly-stained cells were located around the nematode head.

Root-knot nematode resistance observed in various plants has been attributed to the hypersensitive reaction of plant tissues to the nematode. Riggs and Winstead (29) found dead root-knot larvae in resistant tomato as early as 24 hours after infection, while Dean (7) observed necrosis in resistant sweet potato within 48 hours. In the present study, observations during the early periods of infection were limited and made from slide-mounted root sections. Therefore effect of necrotic tissues on nematode survival was not ascertained. It is interesting to note that although F416 exhibited the most necrotic cellular reaction, it did not show significant differences in syncytial development and size from the susceptible Spantex. The time required for nematode development was apparently similar since egg laying females were observed in both plants at 25 days after inoculation. Larval penetration and percent root infection in F416, although not statistically significant, were less than that obtained for Spantex. On the basis of these observations it appears that the nature of resistance in F416 is different from that in P246.

Intercellular and intracellular migration of M. hapla larvae in the cortex, vascular cylinder and root apical meristem was similar to

that reported by other workers (5, 14, 16, 17). Extensive cellular destruction due to intracellular penetration and migration of the larva was observed in all plants. Tunnels in tissues formed along the nematode pathway may serve as ideal ports of entry for other pathogens (18, 28). Typical cellular and tissue reactions in root-knot nematode infection, such as cell wall lysis and subsequent formation of multinucleate giant cells with dense, granular cytoplasm, abnormal cellular hyperplasia and hypertrophy and root galling were also observed in this study.

Similar observations in giant cell formation by root-knot nematodes have been reported by earlier workers (5, 9). In addition, Owens and Sprecht (24) and Littrell (17) observed mitosis without cell division in giant cells. However, Huang and Maggenti (13, 14) recently reported, on the basis of chromosome counts, that giant cells are formed exclusively by mitosis without cytokinesis and further concluded that cell wall dissolution plays no part in giant cell formation. Furthermore, early stages in lateral root formation, a characteristic of M. hapla galls was noted. Starch grain formations in the vascular parenchyma cells are apparently associated with root development in peanut. Badami (1) and Yarbrough (40) also observed numerous starch grains in the vascular bundle during the secondary growth of the primary root in A. hypogaea.

Results and observations in the present study confirmed the presence of pre-infection resistance in P246 to M. hapla penetration. Further, post-infection resistance was indicated by lack of favorable plant response. Slow developmental rate and small size of giant cells were probably correlated with delayed nematode development. Findings

also indicate that the apparent resistance in F416 may be of a different nature from that of P246. Although limited observations indicate a necrotic reaction to larval penetration in F416, the cellular response in most roots was similar to that observed in the susceptible Spantex. Further study is needed to determine more precisely the nature of resistance of F416 to M. hapla. This cultivated line presently provides the primary source of resistant germplasm in the breeding program because of failure to achieve crosses between the resistant wild line P246 and the susceptible cultivated lines (personal communication with Dr. D. J. Banks, Oklahoma State University-USDA, ARS).

Results in the screening trials for root-knot nematode resistance, showed very few of the plants tested including hybrids of the intermediate resistant lines exhibited even a moderate degree of resistance. This suggests the need for continued and more intensified testing of plant materials for nematode resistance.

CHAPTER VI

SUMMARY

Results of experiments conducted to determine the histopathological response of resistant and susceptttible peanuts to M. hapla infection indicated the following:

1. Penetration of both resistant and susceptible roots by M. hapla larvae occurred within six hours after inoculation.

2. Percent root infection was lower and larval penetration was less in the resistant P246 than in the susceptible Spantex. This indicates the existence of pre-infection resistance in P246.

3. Post-infection resistance was characterized by lack of favorable plant cell response. There was a slower developmental rate and smaller giant cells in P246 than in the intermediately resistant F416 and susceptible Spantex.

4. There was an apparent hypersensitive reaction to larval penetration in F416. In some roots, however, cellular response was similar to that observed in the susceptible Spantex.

5. Intracellular and intercellular penetration and migration of M. hapla larvae caused extensive tissue damage in both resistant and susceptible peanuts.

6. Very few of the additional plants tested for nematode resistance, including hybrids of intermediate resistant lines, exhibited

even a moderate degree of resistance. This indicates the need for continued and more intensified testing of plant materials for nematode resistance.

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