HOST-PARASITE RELATIONSHIPS WITH DEFINITION OF PEANUT RESISTANCE TO THE NORTHERN ROOT-KNOT NEMATODE, *MELOIDOGYNE HAPLA*

By

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INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are important parasites of peanut. The association of these nematodes with peanut was first reported in 1889 when Neal (52) observed masses of knotty roots in Florida. Following Chitwood's (14) revision of the genus *Meloidogyne* Goeldi, root-knot nematodes attacking peanut have been assigned the names *M. arenaria* (Neal) Chitwood and *M. hapla* Chitwood. The peanut nematode, *M. arenaria*, has been reported on peanut in Georgia (21, 43, 71), Alabama (21, 71) Virginia (47, 71) and Texas (3). The northern root-knot nematode, *M. hapla*, has been reported in Georgia (71), Alabama (21, 71), Virginia (21, 47, 71), Delaware (31), North Carolina (21), Florida (21), Oklahoma (3), and Texas (3). *Meloidogyne hapla* appears to be the most prevalent root-knot nematode on peanut in the northeastern States (43).

Early surveys conducted over a three-year period in Caddo County, Oklahoma, indicated that *M. hapla* was responsible for an average annual yield reduction of 52% in infested soils (68). More recently, yield reduction in excess of 89% was noted in infested areas, accompanied by a reduction of sound mature kernels of more than 50% (6).

Resistance to root-knot nematodes has been reported in many plants (37). Apparently, this resistance can be divided into two phases. The first, or pre-infection phase, is based on the resistance of plant roots to nematode invasion. This resistance may be due to the absence of an attracting root diffusate, presence of a repellent substance, thick root cell walls and/or root cell walls chemically resistant to nematode

enzymes. The second, or post-infection phase, involves resistance which is exerted after infection and results in the failure of the larvae to develop normally and reproduce. The basis of this resistance might be the presence of chemical inhibitors or toxic substances in the plant cells, absence of favorable response by the plant, specifically, lack of giant cell formation, absence of necessary nutrients required for nematode development, and/or hypersensitivity of plant cells to nematode enzymes.

The other type of reaction by which plants withstand nematode attack is tolerance. Although invaded by larvae which develop normally, tolerant plants show relatively little loss of yield. Such a reaction is probably due, at least in part, to either having a vigorous root system or being drought-resistant.

Most reports on M. hapla on peanut have been based only on association and very few studies have dealt with the determination of the interactions between the host and the parasite under controlled conditions. Also, resistance in peanut to this nematode has not yet been found. The present investigation was therefore designed to study the host-parasite relationships between peanut and M. hapla, to search for resistance or tolerance in peanut and to define the nature of any resistant reactions encountered.

REVIEW OF LITERATURE

The northern root-knot nematode, Meloidogyne hapla, is widely distributed, having been found in the northern parts of Europe, Canada, Australia, also in South and Central Africa (29). According to Chitwood (14), there is evidence for the existence of M. hapla in North America at least as far back as 1917. He stated that the parasite could have been introduced from Europe on nursery stock or taken to Northern Europe from here in the early colonial days. Raski (55) found the nematode in California laurel and salt rush, which are native to California, and suggested that it may also be native to that State.

Tarjan (70) stated that M. hapla was originally found in Green Mountain var. of potato (Solanum tuberosum L.). Since then it has been observed attacking other plants and at least 350 were recorded as hosts, including beets, Brassica spp., clovers, peppers, Chenopodium spp. legumes, Nicotiana spp., Phaseolus spp., and Vicia spp. (29). In Ontario, Canada, 41 species of weeds belonging to 20 families and 43 genera were found to be hosts of the nematode (74), suggesting a diversified host range.

The great similarities in morphology and life cycle make it possible to discuss the root-knot nematodes as a group. The pioneering report of Christie (18) upon which most of the succeeding discussions are based, has been very useful in the study of the nematodes' development and feeding habits. It is generally accepted that the first molt occurs inside the egg before the larva has attained its maximum length. The

first stage is, therefore, spent within the egg. After the first molt, further larval growth occurs. Upon eclosion, the second stage larva migrates to and invades the root, usually in the region of elongation immediately behind the root cap. Penetration of the epidermis is effected by repeated and rapid thrusts of the stylet into the cells. The larva may remain outside, feeding on epidermal cells for as long as 24 hr. Following initial penetration, the larva forces its way into and migrates through the tissue until it becomes sedentary, usually near the stele. When the larva assumes its final position, it feeds only on cells within the reach of its stylet. It is in this position that the second, third and fourth molts take place, after which the adult stage is reached. There is controversy concerning the intervals between the parasitic molts, but it is generally agreed that these molts are completed within a few days. Following the fourth molt, males egress from the larval cuticles as motile vermiform nematodes. Males are not necessary for reproduction, since females can lay viable eggs without mating by the process of parthenogenesis (76). However, occasional cross-fertilization was demonstrated in M. graminicola Golden and Birchfield (75) suggesting that males of root-knot nematodes play a limited role in reproduction. The adult females remain sedentary and increase greatly in length and width and gradually become pear-shaped. Upon maturation, a gelatinous matrix is extruded through the anus (45). Oviposition begins and eggs are expelled through the vulva and accumulate within the matrix. The egg mass usually ruptures the cortex and the epidermis and it can usually be seen as a whitish to yellowish brown mass on the surface of the root.

Few workers have studied the development of M. hapla. At 20.4 C,

Tarjan (69) observed that on snapdragon oviposition and infection by second generation larvae occurred in 30 and 68 days, respectively, whereas the same stages occurred in 39 and 63 days after inoculation to tomato. Bird (9) reported that at temperatures ranging from a nightly minimum of 11.1 C to a daily maximum of 40.5 C, the onset of parasitic molts of M. javanica (Treub) Chitwood and M. hapla occurred as early as the 14th day after inoculation to tomato. He believed that the three parasitic molts occurred in about three days and oviposition started on the 29th day.

It is now apparent that the development of M. hapla is affected by the suitability of the host and the environment, especially temperature. This species is believed to be favored by low temperatures and it can withstand freezing temperatures while others are less able to do so (38). When nematode egg masses were exposed to various soil temperatures, Daulton and Nusbaum (22) found that at -2 C, the eggs of M. hapla survived longer than those of M. javanica. They also observed that the eggs of the former were tolerant to -2 C and less tolerant to 33 C than those of the latter. Thomason (73) reported that nematode reproduction on tomato was extremely limited at 35 C. Wuest and Bloom (82) found that eggs hatched optimally at about 21 C after 30 days incubation in vitro, whereas about 27 C was the optimum for hatching during the initial stages of incubation and the rate of egg hatch increased with lower temperatures throughout the incubation period. More recently, Bird and Wallace (10) reported that optimum temperatures for hatching, mobility, invasion and growth were 25 C, 15 C to 20 C and 20 C to 25 C, respectively.

The histological changes in root-knot nematode-infected roots are

believed to be due to proteolytic enzymes secreted by the nematode (38). This secretion is injected into root cells during periods of stylet activity prior to ingestion of cell contents. Cells of the host located around the head of the parasite do not develop into xylem, phloem and other elements of the central cylinder (21). Instead, the nematode's esophageal secretions stimulate dissolution of cell walls resulting in the formation of giant cells. Christie (21) characterized these giant cells as naked masses of protoplasm which serve as a source of food for the parasite. Hypertrophy of cortical tissues around the nematode and its feeding site results in swellings or galls (29). Mountain (51) suggested that accumulation of indole-acetic acid (IAA) in infection sites stimulates growth of root tissue and results in gall formation. He proposed that IAA is released by proteolytic enzymes, such as chymotrypsin, secreted by the nematode. He further postulated that the enzyme splits the peptide bonds of the protein chain releasing a number of amino-acids, including tryptophan. Tryptophan, an immediate precursor of IAA, is metabolized by the host to IAA.

The histopathology of M. hapla infection in soybean (65), gardenia (23), rose (24), onion (66), and garden balsam (53) have been reported. Sections of infected roots of these plants showed characteristic giant cells blocking and disrupting the vascular tissues presumably resulting in a reduction in efficiency of translocation of water and nutrients through the roots and could account for much of the injury to infected plants.

Galls caused by M. hapla are often smaller than those caused by other Meloidogyne spp. However, Townshend and Davidson (74) observed variation in the size of galls depending on the thickness of the root

of the host. They further noted abnormally large galls on a few weed species caused by multiple infections by the nematode. Development of lateral roots above and below the galls is also a characteristic symptom of M. hapla infection (29). The presence of the parasite seems to stimulate mitotic activity in the pericycle resulting in the formation of a layer of small-celled parenchyma tissue where the increased number of lateral roots have their origin (18). In some infected root tips of rose (24) and soybean (65), the presence of the parasite either suppressed or ceased mitotic activity in the apical meristem and growth was often retarded.

As with most plant parasitic nematodes, the other symptoms of M. hapla infection in roots of the host are those resulting either from root destruction (under heavy infection) or from blocking of translocation in the vascular cylinder of the root. These include foliage yellowing (68) or browning (43), retarded growth or stunting (13, 38, 43), and death (13, 43). Consequently, reduction in plant yield usually results (13, 30, 39). Chitwood (15) observed that the amount of damage to tomato, onions and lima beans increased as the level of inoculum was increased. Chapman (13) reported similar observations in alfalfa and red clover.

In soybeans, M. hapla has been implicated in two disease complexes. Taylor and Wyllie (72) demonstrated that the incidence of pre-emergence damping-off caused by Rhizoctonia solani Kuehn was greatly increased by the presence of the nematode. More recently, they (83) reported that inoculation with Phytophthora sojae Kaufman and Gerdemann and M. hapla caused more severe symptoms of root rot than either pathogen alone.

The associations of M. hapla with peanut damage in the field have

been reported (30, 43, 47). The characteristic symptoms incited by this nematode in other hosts were likewise observed. The nematode caused galling on all underground parts of the plant, including roots, pegs, pods, and pod stems. Machmer (43) noted that early infection of the peg was detrimental to the seed embryos and galled plants frequently exhibited many necrotic pegs and only a few mature peanuts. He further noted that infected pods were warty and their stems were easily severed. Garren (30) observed reduction in the size and number of kernels and pods which were sometimes disfigured. In addition to galling on pegs and pods, Miller and Duke (47) noted poor nodulation and appearance of rootlets on the pegs.

Sasser (62) demonstrated the susceptibility of peanut to M. hapla under greenhouse conditions. Despite the need for a more detailed investigation of host-parasite relationships, no reports of this nature could be found in the literature.

Plant-nematode interactions may fluctuate widely under different conditions. Studies on host-parasite relationships and determination of resistance require the selection and standardization of the least variable and most efficient techniques so that treatment effects can be accurately determined. Mountain (50) reviewed the techniques which have been used in studying the role of nematodes in plant disease development. Many of the techniques reviewed are applicable to the study of host-parasite relationships involving root-knot nematodes.

Various inoculation techniques for infecting plant roots with root-knot nematodes have been employed. Sasser (60) used small gelatine capsules, each containing a single egg mass and a little moist sterile soil to insure getting the inoculum into the root zone. Studying larval

penetration of roots, Dropkin (26) applied nematodes to the roots in a drop of water on a cover glass. The nematodes were then covered with moist sand and the cover glass removed. Godfrey (32) suggested that more rapid testing of nematode resistance in plants could be accomplished by inoculating with infective larvae. Bird (9) inoculated seedlings by pipetting larvae in a water suspension around the root tips on 0.6% agar in Petri dishes, or more effectively, by planting seedlings in perlite containing infective larvae. Dropkin and Boone (27) infected intact seedlings and excised roots of tomato cultured in test tubes containing White's (80) medium (0.15% agar) by pipetting single larva onto each root tip. When large numbers of plants are to be inoculated with nematodes and the necessity of reducing the opportunity for plants to escape infection is important, Barrons (7) advocated the use of galled roots cut into pieces applied in the planting furrows in benches. Bailey (4) tested thousands of tomato seedlings for root-knot resistance by Barrons (7) procedure, but he preferred to use pots. Other methods of inoculating plants with nematodes were discussed by Cairns (12).

Evaluation of plant-nematode relationships and resistance is usually based on the plant's reaction to the nematode, as well as on the effects of the plant on nematode development and reproduction, the latter being determined by in situ staining (12).

The ways by which plants survive nematode attack include tolerance and resistance. Tyler (77) defined tolerance, as applied to root-knot nematodes, as the ability of a plant to continue productive growth even while it is subject to a heavy and increasing infection. The productiveness or absence of above-ground symptoms in the presence of the parasite has sometimes been interpreted as resistance (37). The practi-

cal difference between the two is that tolerant plants are invaded by nematodes, which develop normally, and therefore contribute to the increase in population of the parasite, whereas resistant plants may or may not be invaded by nematodes. If invaded, resistant plants either will not support or will greatly reduce nematode reproduction and thus reduce the population level. Howard (37) attributed tolerance in plants either to a strong root system or to the ability to withstand water stress. Few studies on tolerance to root-knot nematodes have been conducted.

Resistance to root-knot nematodes has been extensively investigated. Tyler (77) originally defined resistance as the ability of the plant to obstruct nematode invasion. Barrons (8), however, found that just as many larvae entered the roots of the resistant Crotalaria as entered the roots of the susceptible tomato. For the 24 resistant plants with which he worked, resistance was manifested not through failure of larvae to enter the roots, but through failure of larvae to survive after entering. Resistance before and after invasion was further evidenced by subsequent reports. Sasser (62) reported different types of interaction between Meloidogyne spp. and various resistant plants, including failure of larvae to penetrate roots, invasion by few larvae with no development and invasion by many larvae with only few developing. Death of root-knot larvae after entering roots of resistant plants has been reported by Riggs and Winstead (56) and others. Christie (20) found more nematodes invading alfalfa roots than those of Lantana and suggested that some plants are easily invaded by nematodes while others are not. He further suggested that all resistant plants are not necessarily resistant for the same reason, but stated that most resistant plants fall in

the same category as those with which Barrons (8) worked. Tyler (77) proposed that resistance may depend to some extent on plant vigor, which in turn depends on climate, plant nutrition, and other environmental conditions and, therefore, no absolute evaluation of resistance can be made for all conditions. The observations that certain old plants were either more or less resistant than young plants (57) further indicated that variable mechanisms of resistance to root-knot nematodes exist in plants.

Variations in host specificity and reaction within the genus Meloidogyne are also apparent, since plants resistant to one species are not necessarily resistant to another species. Thus, Stanford et al. (67) found alfalfa varieties resistant to M. javanica and to M. incognita acrita Chitwood which were susceptible to M. hapla. Other examples of host specificity were noted by Tarjan (70), Sasser (61) and others. Similarly, physiological variations within species of root-knot nematodes have been reported and reviewed (63).

The causes of resistance in plants to root-knot nematodes are not yet clearly known, but deductions have been made based on available information. For instance, resistance to invasion is sometimes attributed to lack of root attractiveness to the nematode (16) based on Linfords (40) original observation that root-knot larvae congregated around the growing point of Portulaca roots. Repellents or poisons from plants have also been suspected to be responsible for resistance to root-knot nematode invasion, although evidence to this is limited. With lesion and stunt nematodes, Oostenbrink et al. (54) showed that toxic secretions of marigold reduced nematode populations in the soil. Similarly, Rhode and Jenkins (58) reported a water-soluble glycoside from asparagus

roots which caused mortality of several species of nematodes. They attributed this to an unknown compound present in the rhizosphere of asparagus. "Something" in rutabagas (57) and an unknown chemical in millet (42) were noted to be associated with resistance to invasion of burrowing and sheath nematodes, respectively. Other probable causes of resistance to root-knot nematode invasion include thick root cell walls or root cell walls chemically resistant to nematode enzymes (16).

Resistance after nematode invasion was attributed by Christie (18) to the failure of resistant hosts to respond favorably to the stimulus of infection. He pointed out that root-knot nematodes are sedentary parasites as adults and are able to feed only upon a few cells that are within the reach of their stylets. If the esophageal secretions of the nematode fail to change the normal development and differentiation of the surrounding cells, the parasite is soon surrounded by cells that are either too thick to be penetrated by the stylet or so highly vacuolated as to be of little value as a food source. Barrons (8) suggested that since giant cells are necessary to furnish the developing nematodes with food, resistance may be due to certain chemicals within the resistant plant that counteract or neutralize the giant cell-inducing effect of the esophageal secretions. He believed that various degrees of resistance would be due to differences in the ability of plants to synthesize these chemicals. Hypersensitivity of plant cells as a mechanism of resistance to root-knot nematodes has been reviewed (57). Dean (25) observed extensive root necrosis in resistant tomato and sweet potato as a consequence of M. incognita (Kofoid and White) Chitwood infection and found that larvae either failed to develop or died in the necrotic tissues. Similar observations on cotton (48) and soybean (28) were

reported. Toxic chemicals responsible for inhibiting development or death of root-knot nematodes inside the host tissues have not yet been identified, but high amounts of chlorogenic acid were found in browned leaves of chrysanthemum varieties resistant to the foliar nematode (78). Little evidence supporting the hypothesis that some essential nutrients are being withheld from the parasite by resistant plants is available (79).

Several plants, including cereals and other grasses are unsuitable hosts for M. hapla (29, 47, 62, 74). However, only few reports on resistance in varieties or species of a genus which are normally susceptible to the nematode were found. Allison (2) observed the resistance of a few varieties and accessions of alfalfa to M. halpa. Later, Stanford et al. (67) working with many varieties and foreign plant introductions of alfalfa and Medicago spp., noted individual plants of the variety Vernal and the common strain Hilmar that were also resistant to the parasite. Progeny tests showed transmission of resistance to the offspring. It was later found that resistance was determined by two different but closely linked dominant genes (34) and that resistance in one alfalfa stock was due to complete failure of larvae to penetrate the roots (33). Winstead and Sasser (81) found 50 cucumber varieties, breeding lines and plant introductions which are resistant to M. hapla, but susceptible to the four other Meloidogyne species tested. Brucher (11) observed field resistance in some primitive and wild potatoes to four Meloidogyne spp., including M. hapla. He also observed that, in the greenhouse, the tetraploid wild potato was more resistant than the diploid wild potato.

Tyler (77) listed peanut as "highly resistant" to the root-knot

nematode, Heterodera marioni (Cornu) Goodey. It became apparent, after the host range studies which followed Chitwood's (14) revision that Tyler (77) was referring to a Meloidogyne spp. other than M. hapla or M. arenaria. Miller and Duke (47) reported that peanut of "a foreign introduction with a purple skin" showed good resistance to M. arenaria. Resistance to M. hapla in peanut has not yet been found. A search for this resistance was initiated in Virginia in 1955, but results have not been very promising (47).

MATERIALS AND METHODS

General Methods

The nematode isolates used in this study were recovered from peanut roots collected from various localities in Oklahoma. All isolates were identified as *M. hapla* on the basis of their perineal patterns and were further designated based on the landowner of their collection site. Greenhouse populations of all isolates were maintained on tomato, *Lycopersicon esculentum* Merr. var. Rutgers. After five months, stock colonies of Wells, Barger and Butler isolates were established by pooling 12 egg masses from greenhouse populations. Stock colonies of all other isolates were established directly from field populations. Tomato was used as the host plant in all stock colonies as early trials indicated a higher infectivity of tomato-reared inoculum than peanut-reared inoculum.

The cultivated lines, consisting of varieties, breeding lines and plant introductions, of *Arachis hypogaea* L. and the unidentified wild *Arachis* spp. used in this study were supplied by cooperating agencies of the Crop Research Division of the Agricultural Research Service. Since the wild peanuts did not produce seeds, they were propagated by cuttings. Unless otherwise specified, the cultivated peanuts were propagated by seeds due to space limitations and difficulty of handling cuttings.

The inoculation techniques employed consisted of either of the

following: a) chopped tomato roots which had been infected for at least two months either mixed thoroughly with methyl bromide-sterilized soil (mixture of three parts soil and one part sand) prior to planting or placed around the exposed roots of established plants, b) aliquot suspension containing known number of newly-hatched larvae poured on the exposed roots or c) pieces of infected roots containing known number of egg masses introduced in the same manner as the chopped infected tomato roots. When the isolate used was not specified, it was the Wells isolate.

Greenhouse tests were conducted at a temperature range of 22 C to 33 C. Unless otherwise stated, environment chambers used were maintained at 28 C 16-hr. day and 20 C night temperatures with a light intensity of 3000 to 4000 ft-c supplied by cool white fluorescent supplemented with incandescent lamps. Arachis hypogaea 'Spantex' was used as the susceptible control, with the exception of one test, when Arachis sp. P-983 was used.

Gall (Fig. 1) and necrosis indices were based on a one to five severity scale (1, none; 2, trace; 3, moderate; 4, severe; 5, very severe). Plant growth was determined by taking fresh root weight and top weight. To study nematode development, a whole root system or a randomly obtained root sample from each plant or pot receiving nematode inoculum was stained with acid fuchsin following the procedure of McBeth et al. (46). Pieces of stained roots were then crushed between two glass slides and the degree of nematode development was determined by microscopic examination. Whenever necessary, nematodes were dissected from the roots for more critical examination. The nematodes were placed in six developmental groups (Fig. 2) following Christie's (19) procedure,

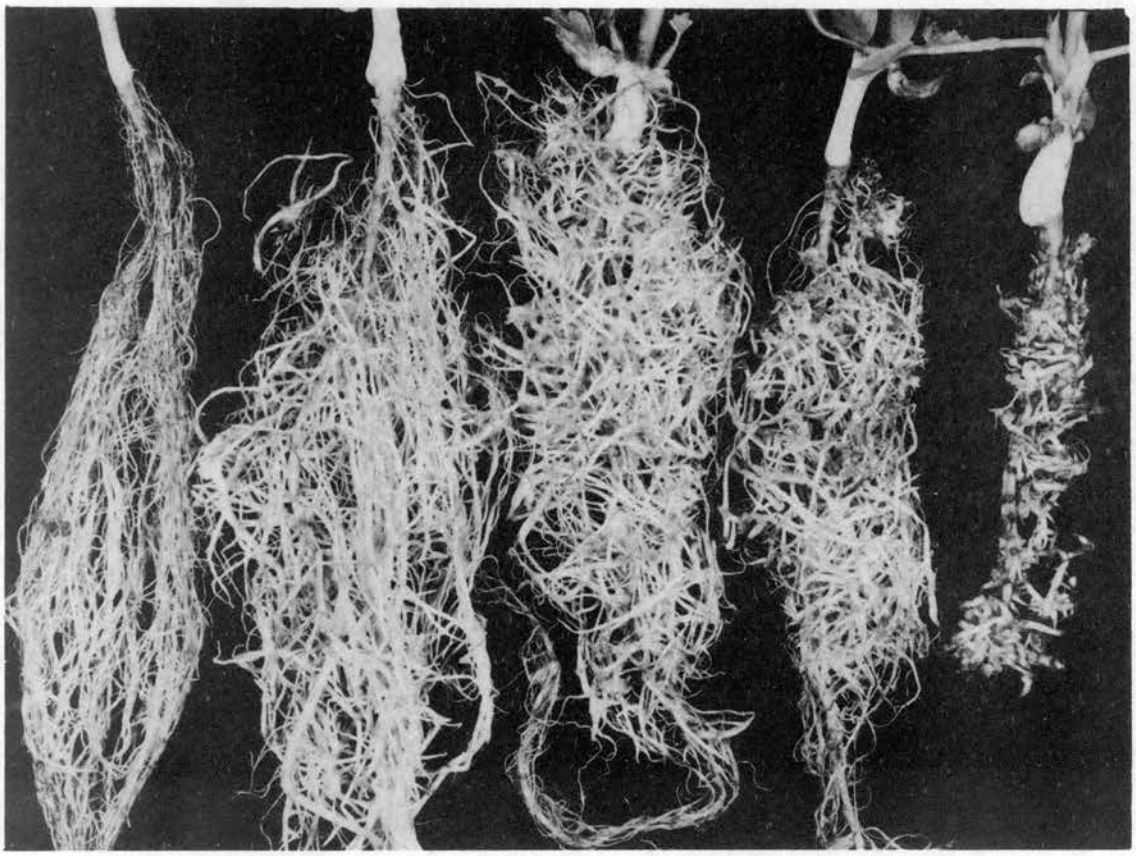

Figure 1. Gall Index. Left to Right: 1, None; 2, Trace; 3, Moderate; 4, Severe; 5, Very Severe.

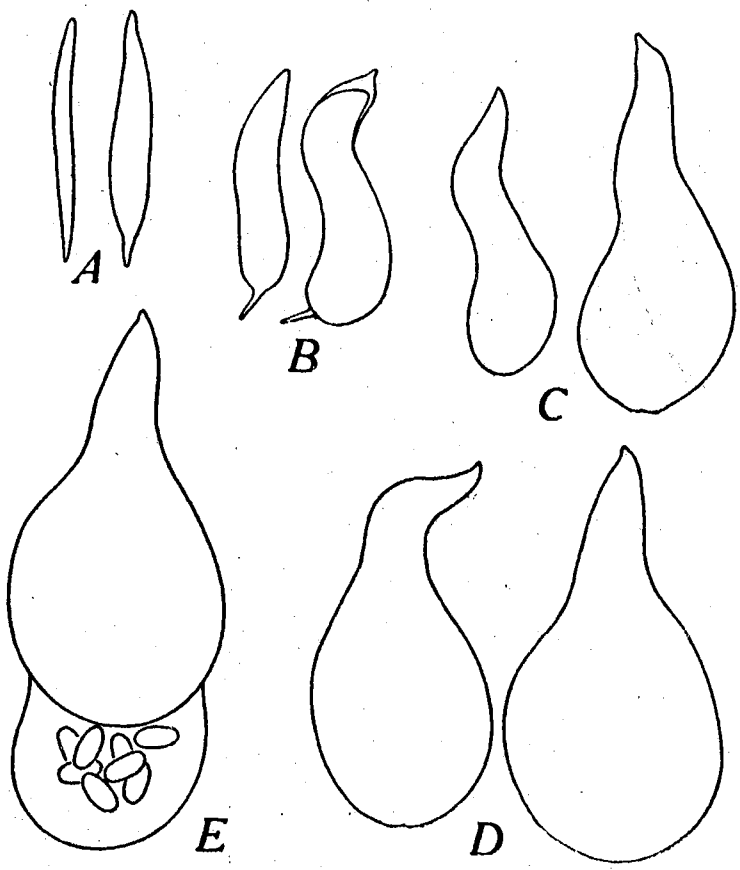

Figure 2. Root-Knot Nematode Developmental Groups (After Christie (19)).

with adult males included. To determine the presence of nematode larvae in the soil, the sieving-Baermann funnel technique of extraction was used.

Whenever necessary, experimental data were analyzed at 1% or 5% level of confidence using the analysis of variance technique. Differences among means were determined at 5% level of confidence using Duncan's multiple range test.

Laboratory Studies

To determine the suitability of growing media, seeds of A. hypogaea 'Spantex" were planted in 180-ml plastic cups containing either steam-sterilized soil, sand or perlite. Eight replicates of each treatment were placed in two lighted incubators kept at 24 C and 28 C. When the seedlings emerged, 40 ml of 20-20-20 fertilizer (30 ml in 3 gal) was applied to each cup. Larvae were surface sterilized with 0.1% streptomycin sulfate for 15 minutes and rinsed in three changes of sterile distilled water. Ten days after planting, 1000 larvae were pipetted onto the exposed roots of each plant. After 48 hr, plant growth and root galling were observed and the roots were stained to determine the presence of nematodes.

Attempts were also made to infect intact seedlings or excised root tips aseptically in 1.5-cm diameter test tubes or 9-cm diameter Petri dishes containing White's (80) medium. Agar concentrations in the medium of 0.15%, 0.3%, 0.5%, 0.7% and 1.0% were tested. Peanut seeds were surface sterilized with 2.0% sodium hypochlorite solution for 5 to 10 minutes and were germinated in Petri dishes containing 1.5% water agar. Each seedling or 5-mm root tip was transferred to a test tube agar slant

or Petri dish. Larvae were either picked singly or pipetted in groups of 10 from sterile distilled water and placed either adjacent to the root tip or in a small drop of sterile water on the root tip. Excised tomato root tips in Petri dishes treated in the same manner, were provided to check the feasibility of the culturing procedure. At least 10 replicates of each treatment were kept in 28 C incubator. After 15 days, plant roots were examined for galling and stained for nematode examination.

Emergence of Peanut in Nematode-Infested Soil

Fifty seeds of A. hypogaea 'Spantex' were planted in each metal pan containing 5 kg of soil mixed thoroughly with 7 g chopped infected tomato roots. This level of inoculum was approximately the same as 1 g chopped infected tomato roots per 10-cm diameter pot of soil. A pan containing sterile soil and the same number of seeds served as a control. Emergence counts were made 10 days after planting. The test was replicated 10 times in the greenhouse.

Effects of Inoculum Levels on Susceptible Peanut

Three separate tests were conducted using varying inoculum levels of chopped infected tomato roots, egg masses and larvae. Plants used as source of inoculum had been infected for about three months. To insure germination, two seeds of A. hypogaea 'Spantex' were planted in each 10-cm diameter pot. Upon germination of one seed, the other seed was removed from the soil. Per plant levels of inoculum were 0, 1 and 2 g for chopped infected tomato roots; 0, 5, 10, 20, and 40 for egg masses; and 0, 1000, 2000, 4000, and 6000 for larvae. Each level was

replicated four, four and three times for chopped infected tomato roots, egg masses and larvae, respectively. Chopped infected tomato roots and egg masses were introduced at planting time. Larvae were introduced 10 days after planting to insure survival of the larvae until sufficient roots were available. Thirty days after inoculation, top and root growths of the plants that survived inoculation with chopped infected tomato roots were observed and data on galling, root necrosis, root weight, and top weight were collected in the larvae and egg mass tests. The test on chopped infected tomato roots was conducted four times in the greenhouse. The tests on larvae and egg masses were conducted three and four times, respectively, in the controlled environment chamber.

Effects of Nematode Isolate, Temperature and Variety Interactions on Host-Parasite Relationships

A factorial experiment using three nematode isolates, Wells, Barger and Butler; two peanut varieties, Spantex and Dixie Spanish; and two air temperatures, 24 C and 28 C, was conducted in two controlled environment chambers both maintained at 20 C at night. Three plants were grown per 15-cm diameter pot containing 2 g chopped infected tomato roots mixed throughly with sterile soil. Each treatment was replicated four times. Uninoculated plants were provided in each treatment as controls. Data on galling, root necrosis, root weight, and top weight were collected 30 days after inoculation. The percentage of egg laying females was determined from stained 200-mg root sample randomly obtained from each pot receiving nematode inoculum. The experiment was conducted two times.

Histopathology of Nematode Infection

The roots of plants grown at 28 C for 30 days after inoculation

were washed and fixed in F.A.A. (formalin, 6 ml; 95% ethanol, 20 ml; glacial acetic acid, 1 ml; distilled water, 40 ml) for at least 24 hr. Samples of galled roots about 10 mm in length were dehydrated with a graded series of tertiary butyl alcohol concentrations and infiltrated with paraffin. Longitudinal sections, 12 μ thickness, were made and stained either with a triple stain, consisting of orange G, safranin and crystal violet, or a double stain, consisting of safranin and fast green. Dehydration and staining followed Sass' (59) technique.

Post-Infection Nematode Development

The susceptible A. hypogaea 'Spantex' was used in this study. Roots of two-week old plants grown singly in 180-ml plastic cups filled with sterile soil were inoculated with an aliquot suspension containing approximately 1000 larvae per plant. After one or two days in the controlled environment chamber, roots were washed to remove any larvae that had not penetrated and the plants were transplanted singly in 10-cm diameter pots containing sterile soil. Therefore, a two-day penetration period was allowed for all plants, except those in the one-day treatment. At daily intervals up to 40 days, one plant was removed to determine nematode development. The entire root systems of plants washed 1 to 10 days after inoculation were fixed in cold TAF (triethanolamine, 2 ml; formalin, 7 ml; distilled water, 91 ml) for at least 24 hr. and stained. Only the galled portions of roots washed after 10 days were stained. The experiment was conducted four times. The first trial included dissecting out of stained nematodes which had been inside the roots for 1 to 21 days. They were mounted in glycerine by Baker's (5) method and studied to determine molting periods. At least 10 nematodes

were examined for each interval, but only the most advanced stage was recorded.

Screening for Resistance and Tolerance in Peanut to M. hapla

Table I shows the 235 cultivated lines of A. hypogaea and 12 unidentified wild Arachis spp. tested for resistance or tolerance to M. hapla in the greenhouse. The wild and cultivated species were tested separately. Each plant was inoculated with 1 g chopped infected tomato roots. Four replicates of each line or species were tested. Arachis hypogaea 'Spantex' was included in each test as a susceptible control. Cuttings of the wild species were inoculated at the age of three to three and one-half months, whereas the cultivated lines were inoculated at planting. Plant growth was observed and the roots were rated for galling 30 days after inoculation.

The severely galled cultivated lines of A. hypogaea, which exhibited more vigorous growth than the others, were selected and tested for tolerance. Eight plants of each line were grown singly in 15-cm diameter pots in the greenhouse. Four of these plants were inoculated with 1 g chopped infected tomato roots and the other four were uninoculated. The roots were rated for galling and the plants were transplanted into pots containing sterile soil 30 days after inoculation. Three and one-half months later, yield data, consisting of fresh weights of pegs and pods, were obtained. The test was conducted three times.

The cultivated lines of A. hypogaea and the wild Arachis spp. that showed only trace to moderate galling were re-tested at least twice. Those that consistently showed less galling were further tested for resistance in the controlled environment chamber using 40 egg masses per

TABLE I

VARIETIES, BREEDING LINES AND PLANT INTRODUCTIONS OF A. HYPOGAEA AND WILD ARACHIS SPP. TESTED FOR RESISTANCE OR TOLERANCE TO M. HAPLA[1]

P.I. 161317	P.I. 292956	P.I. 311003	P.I. 313162
221068	294647	311262	313162S
248759	294652	311263	313163
259820	294654	311264	313165
259860	295169	311265	313166
268644	295171	311266	313166S
268684	295173	312141	313167
268689	295174	313118	313168
268771B	295185	313119	313169
268808	295188	313120	313170
288092	295190	313121	313171
288096	295191	313123	313171S
288106	295192	313124	313172
288122	295197	313125	313172S
288124	295198	313126	313173
288131	295199	313127	313176
288133	295202	313128	313177
288138	295220	313129	313178
288139	295244	313130	313179
288140	295245	313132	313180
288141	295268	313133	313181
288143	295269	313134	313182
288148	295735	313135	313183
288150	295736	313136	313184
288151	295737	313137	313185
288155	295743	313138	313186
288157	295974	313139	313187
288158	297389A	313140	313188
288159	298829R	313141	313189
288160	298834R	313142	313190
288161	298844R	313143	313191
288162	298857R	313144	313193
288167	298863R	313145	313194
288169	298873R	313146	313195
28817-	299468	313149	313196
288174	300239R_2	313150	313197
288177	300240R	313151	313198
288179	300586R_1	313153	313199
288180	300586R_2	313154	313200
288182	300590	313155	313200S
288188	302404	313156	313201
288191	304299	313158	313202
288200	305069	313159	313203
288209	306228	313160	313204
292692	306363	313161	314048

TABLE I (Continued)

P.I.	314048X	P.I.	315615	P.I.	315634	Va. 56R
	314817		315617		315635	Va. 61R
	314818		315618		315636	119-20
	314893		315619	Dixie giant C		186-28
	314895		315620	Dixie runner		P.I. 262286 (P-246)
	314896		315621	Dixie Spanish		P.I. 262794 (P-271)
	314897		315622	Early runner		P.I. 262801 (P-284)
	314898		315623	F393-7		P.I. 262814 (P-258)
	314899		315624	F416		P.I. 262819 (P-274)
	314900		315625	F439-16		P.I. 262827 (P-270)
	314980		315626	Florigiant		P.I. 262832 (P-273)
	315606		315627	NC2		P.I. 262841 (P-237)
	315609		315628	NC4X		P.I. 262842 (P-238)
	315611		315629	NC5		P.I. 262844 (P-250)
	315612		315630	Ser 56-15		P.I. 299474 (P-983)
	315613		315631	Spantex		F135 (P-940)
	315614		315633	Va. bunch 67		

[1]P.I. numbers are those assigned by the New Crops Research Branch of USDA, ARS; P numbers are those assigned by the Oklahoma Experiment Station for the wild Arachis spp.

plant as inoculum. This inoculum level was pre-determined as a sufficient level for determining plant susceptibility to the nematode. The treatment was replicated four times. The cultivated lines were inoculated 10 days after planting and the wild species were inoculated at the age of three to three and one-half months. Plant roots were observed for root necrosis and galling 30 days after inoculation. A 200-mg root sample per plant was stained to determine the presence of nematodes and the degree of nematode development. Resistance or susceptibility was based on average gall ratings (3-, resistant; 3 to 4, less susceptible; 4+, susceptible). Each test was conducted four times.

Effects of Increased Inoculum Levels on Resistance

Two separate experiments were conducted to determine whether or not the resistance in the wild peanuts would be lost at high inoculum levels. In the first experiment, three and one-half - month old cuttings of the wild Arachis spp. P-237, P-246, and P-258 with the variety Spantex as susceptible control were used. Per plant levels of inoculum were 0, 40, 80, and 160 egg masses, replicated 10 times. In the second experiment, the same wild peanuts were used, but because of lack of Spantex cuttings, the susceptible control used was Arachis sp. P-983. The cuttings were four months old when inoculated with 5 g chopped infected tomato roots each and the treatment was replicated eight times. Data on galling, root necrosis and root weight were obtained in the first experiment, but only galling data were collected in the second, 30 days after inoculation.

Effect of Nematode Isolates on Galling of Resistant Peanut

An experiment was designed to determine if resistance-breaking *M. hapla* isolates existed among the populations collected from different localities in Oklahoma. Three and one-half - to four- month old cuttings of *Arachis sp.* P-246 grown singly in 10-cm diameter pots were each inoculated with 40 egg masses of the different populations. Nine isolates from peanut, including Wells, and one from the common dandelion, *Taraxacum officinale* L., were used in the test. Each treatment was replicated four times and one cutting of Spantex was inoculated with each isolate as an inoculum check. The plants were kept in the controlled environment chamber for 30 days prior to rating their roots for galling.

Effect of Plant Age on Resistance

Observations from preliminary investigations indicated that young plants are more susceptible to galling than old plants. A test was, therefore, designed to compare reactions of young (one and one-half - month old) and old (three - month old) cuttings of the wild *Arachis* spp. P-237, P-246, P-258, and the cultivated variety Spantex. These plants were tested separately because of the difficulty of obtaining enough cuttings at the same time. Twelve cuttings of each age group were grown singly in 15-cm diameter pots and inoculated with 40 egg masses each. The plants were kept in the controlled environment chamber for 30 days prior to collection of data on galling, nematode recovery and development.

Nematode Penetration and Development in Young Resistant and Susceptible Peanuts

The result of the test on the effect of plant age on resistance led

to the design of an experiment which would determine time of galling and nematode penetration and development in young resistant and susceptible peanuts at selected periods. One - to one and one-half - month old cuttings of Arachis spp. P-246 and Spantex grown singly in 10-cm diameter pots were each inoculated with 1000 larvae. The plants were kept in the controlled environment chamber. At selected periods, the entire root systems of four plants each of the resistant and susceptible peanuts were stained to examine the nematodes that had penetrated. The presence of second generation larvae in the soil was determined 40 and 50 days after inoculation. The experiment was terminated after 50 days.

Nematode Reproduction in Resistant and Susceptible Peanuts

Ten two and one-half - month old cuttings each of Arachis sp. P-246 and Spantex grown singly in 15-cm diameter pots were inoculated with 1000 larvae per plant. Five days after inoculation, the plants were washed, transplanted into sterile soil and kept in the controlled environment chamber. The plants were re-washed and their roots stained 60 days after inoculation. Ten egg masses were randomly hand-picked from each root system of Spantex and as many as could be found were obtained from each root system of Arachis sp. P-246. Each egg mass was transferred to a drop of lactophenol on a glass slide, broken up and egg counts made with the aid of a microscope.

Histopathology of Nematode Infection in Resistant, Intermediate and Susceptible Peanuts

Four two and one-half - month old cuttings each of Arachis sp. P-246 (resistant), A. hypogaea (F416) (intermediate) and A. hypogaea

'Spantex' (susceptible) were each inoculated with 40 egg masses. The plants were washed, transplanted into sterile soil and kept in the controlled environment chamber five days after inoculation. Thirty days after inoculation, samples of galled roots from each plant were collected and processed in the same manner as in the previous histopathological study. The anatomy of galled roots of the resistant, intermediate and susceptible peanuts was compared.

Attractiveness of Resistant and Susceptible Peanut Roots to M. hapla

In an attempt to explain the difference in the degree of nematode invasion of resistant and susceptible peanuts, and experiment was designed to determine if there is a difference between the attraction of resistant and susceptible peanut roots to the nematode. This consisted, as one treatment, of growing singly three - month old cuttings of Arachis sp. P-246 (R = resistant) and Spantex (S = susceptible) in each half of a split 15-cm diameter pot containing sterile soil; a resistant plant on one side and susceptible plant on the other (R/S). The roots of these plants were separated by a plastic envelope containing 100 cc of sterile soil. The pot halves were held together by three 5-mm rubber bands and placed on a 20-cm diameter plastic saucer (Fig. 3A) in a controlled environment chamber. The other treatments were R/no plant, S/no plant and no plant/no plant (Fig. 3C). Water was supplied to the plants by adding it to the saucer as needed. After seven days, the plastic envelope was replaced by a folded piece of tulle containing 2 g chopped infected tomato roots mixed thoroughly with 100 cc of sterile soil. To obtain an even thickness of the infested soil within the tulle, replacement was accomplished by laying the tulle on a level glass on top

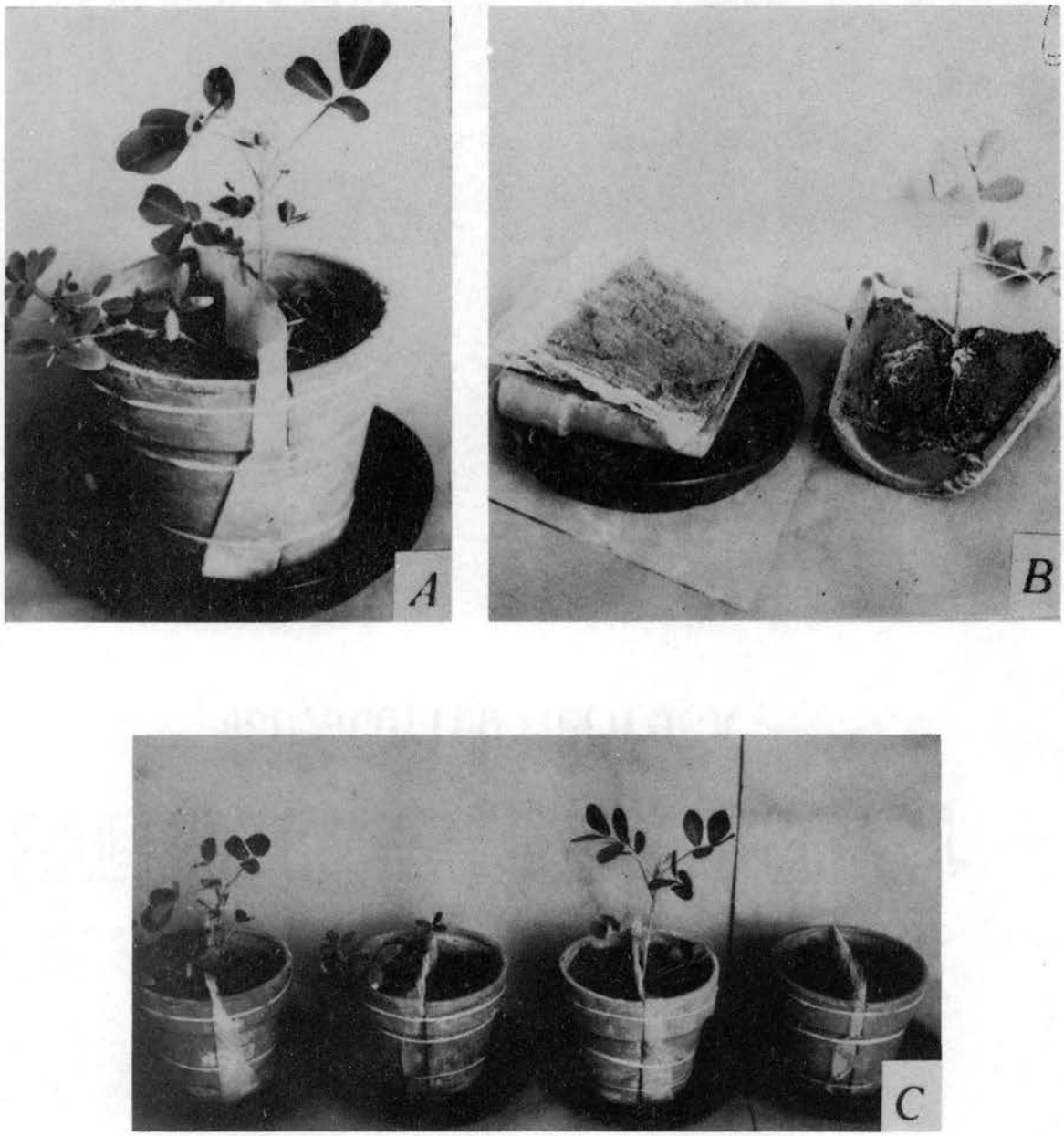

Figure 3. Split Pot Design Used in the Study of the Attractiveness of Peanut Roots to M. hapla. A, Soil-Filled Plastic Spacer Envelope Between Resistant and Susceptible Peanuts; B, Pot Halves Showing Glass Plate, Tulle Envelope Containing Nematode-Infested Soil and Plant Position at Inoculation; C, Treatments from Left to Right: R/S, R/No Plant, S/No Plant, and No Plant/No Plant.

of one of the pot halves (Fig. 3B) and the glass was then carefully removed, leaving the tulle behind. After seven days, nematodes in the soil from each half of the pot were extracted and the presence of nematodes in the roots was determined by staining the root systems. To be sure that only nematodes that migrated toward the roots would be counted the portions of the roots that penetrated the tulle were cut off prior to staining. The experiment was conducted three times.

RESULTS

Laboratory Studies

More vigorous plant growth was obtained using soil as a growing medium than either sand or perlite. Plant response indicated that 28 C is more nearly optimum for growth of peanut than 24 C. Recovery of one to five larvae per root system grown in sand or perlite occurred only rarely at either temperature. Per plant recovery of larvae from plants grown in soil at 24 C and 28 C ranged from 8 to 18 and 6 to 19, respectively. Three to seven swollen root tips per root system were observed in six plants grown in soil at 24 C and four to nine swollen root tips per root system were observed in five plants grown in soil at 28 C. One to three swollen root tips per root system were observed in five plants grown in either sand or perlite at either temperature.

Attempts to infect intact peanut seedlings or excised root tips grown in White's (80) medium in either Petri dishes or test tubes were unsuccessful. No intact seedlings or excised root tips in test tube agar slants were infected by nematodes. Among the 50 intact seedlings inoculated with 10 nematodes each in Petri dishes containing different agar concentrations, only one to three root systems became galled. One to three nematodes were found in each of these galled root systems. None of the excised root tips grew in the medium and no galls or nematodes were found in them. In contrast, excised tomato root tips grew in White's medium with agar concentrations of 0.15% and 0.3% and galls were

sometimes found even in root tips receiving single larva inoculations.

Emergence of Peanut in Nematode-Infested Soil

At the 1% level of confidence, a statistically significant reduction in emergence of peanut seedlings was observed in nematode-infested soil. The average decrease in emergence ranged from 12% to 48%, with an overall average of 33%.

Effects of Inoculum Levels on Susceptible Peanut

a) Chopped infected tomato root inoculum: Only about 62% of the plants inoculated with 2 g per plant survived for 30 days, compared to 100% survival of plants receiving 1 g and those uninoculated. The inoculated plants were all stunted and those inoculated with 2 g were more stunted than those inoculated with 1 g (Fig. 4). Plants inoculated with 1 g formed numerous small galls with excessive number of lateral roots emanating from them (Fig. 5). Plants inoculated with 2 g had fewer galls and lateral roots and very much reduced and necrotic root systems.

b) Egg mass inoculum: The responses of the susceptible peanut variety Spantex to the interactions involving five egg mass inoculum levels and two air temperatures are given in Table II. At the 5% level of confidence, statistically significant differences in galling, root necrosis, root weight, and top weight were obtained. The gall ratings of plants differed significantly between inoculum levels. Plants receiving one inoculum level had significantly lower gall rating than plants receiving the next higher level. No statistically significant difference in root necrosis was obtained between uninoculated plants and plants receiving 5 egg masses, but plants receiving 10, 20 or 40 egg

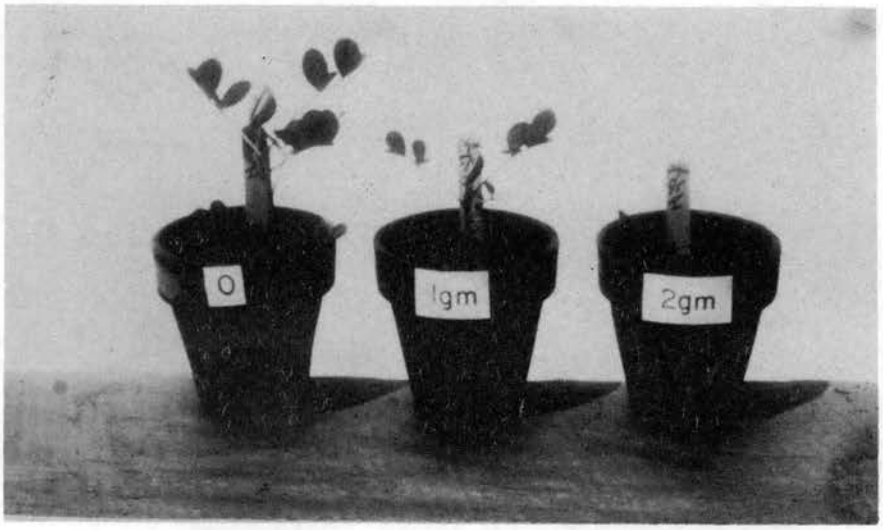

Figure 4. Effect of Chopped Tomato Root Inoculum Levels on Susceptible Peanut.

Figure 5. Lateral Root Proliferation from Galls. Note the Exposed White Mature Females.

TABLE II

EFFECTS OF EGG MASS INOCULUM LEVEL AND TEMPERATURE INTERACTIONS ON THE SUSCEPTIBLE PEANUT VARIETY SPANTEX[1]

No. of Egg Masses Per Plant	Plant Responses to Inoculum Levels (Data are Averages at Both 24 C and 28 C)			
	Gall Rating	Necrosis Rating	Root Weight (g)	Top Weight (g)
0	1.0a	1.4a	4.3b	3.4b
5	2.2b	1.5ab	5.1c	3.4b
10	2.9c	2.0bc	4.2b	2.7ab
20	3.7d	2.1c	3.9b	2.9ab
40	4.6e	2.5c	3.0a	2.2a
Temperature	**Plant Responses to Temperatures (Data are Averages at all Inoculum Levels)**			
	Gall Rating	Necrosis Rating	Root Weight (g)	Top Weight (g)
24 C	2.9a	1.9a	3.1a	2.4a
28 C	2.9a	1.8a	5.0b	3.4b

[1]Similar letters indicate no significant differences at P = 0.05 with Duncan's multiple range test.

masses had higher root necrosis rating than uninoculated plants. The root weights of uninoculated plants and plants receiving 10 and 20 egg masses were not significantly different. However, the root weight of plants receiving 5 egg masses was significantly higher than that of uninoculated plants and plants receiving 10, 20 or 40 egg masses. The root weight of plants receiving 40 egg masses was significantly lower than that of plants receiving the lower levels. No statistically significant differences in top weight were obtained between uninoculated plants and plants receiving 5, 10 or 20 egg masses, but the top weight of plants receiving 40 egg masses was significantly lower than that of uninoculated plants or plants receiving 5 egg masses.

There were no statistically significant differences in galling and root necrosis between plants at 24 C and plants at 28 C. However, the root weight and top weight of plants at 28 C were significantly higher than that of plants at 24 C.

c) Larval inoculum: Table III shows the responses of susceptible peanut to the interactions involving five larval inoculum levels and two air temperatures. At the 5% level of confidence, statistically significant differences in galling, root necrosis and root weight were obtained. The gall ratings of uninoculated plants and plants inoculated with 1000 larvae were not significantly different, nor were the gall ratings of plants inoculated with 2000 and 4000 larvae. Plants inoculated with 6000 larvae had significantly higher gall ratings than plants inoculated with the lower levels. The root necrosis rating of uninoculated plants was significantly lower than that of inoculated plants, but no significant differences in root necrosis were obtained between plants inoculated with 1000, 2000, 4000, and 6000 larvae. The root weight of uninoculated plants was not significantly different from that of plants inocu-

TABLE III

EFFECTS OF LARVAL INOCULUM LEVEL AND TEMPERATURE INTERACTIONS ON THE SUSCEPTIBLE PEANUT VARIETY SPANTEX[1]

	Plant Responses to Inoculum Levels (Data are Averages at Both 24 C and 28 C)			
No. of Larvae Per Plant	Gall Rating	Necrosis Rating	Root Weight (g)	Top Weight (g)
0	1.0a	1.4a	5.1a	4.8a
1000	2.6a	1.9b	5.3ab	4.8a
2000	3.7b	1.8b	5.8b	4.4a
4000	3.8b	2.0b	6.4c	4.6a
6000	4.3c	1.9b	5.6b	4.1a

	Plant Responses to Temperatures (Data are Averages at all Inoculum Levels)			
Temperature	Gall Rating	Necrosis Rating	Root Weight (g)	Top Weight (g)
24 C	3.1a	1.8a	5.1a	4.1a
28 C	3.1a	1.8a	6.1a	5.0a

[1]Similar letters indicate no significant differences at P = 0.05 with Duncan's multiple range test.

lated with 1000 larvae, but significantly lower than the root weight of plants receiving the higher levels. No statistically significant difference in root weight was obtained between plants inoculated with 1000, 2000 and 6000 larvae. Plants inoculated with 4000 larvae had significantly higher root weight than plants receiving the other levels. No significant differences in top weight were obtained between uninoculated plants and plants inoculated with 1000, 2000, 4000, and 6000 larvae.

Galling, root necrosis, root weight, and top weight between plants at 24 C and plants at 28 C were not significantly different.

Effects of Nematode Isolate, Temperature and Variety Interactions on Host-Parasite Relationships

Table IV shows the effects of nematode isolate, temperature and variety interactions on host-parasite relationships. Inoculated plants were significantly galled at the 5% level of confidence. The Butler isolate caused significantly less galling than either the Barger or the Wells. Statistically significant differences in top weight, root weight and root necrosis were also observed between inoculated and uninoculated plants. Plants receiving the Wells isolate had significantly higher top weight than those inoculated with either the Barger or the Butler isolate. Plants inoculated with the Butler isolate ahd significantly lower root weight than those receiving the Barger isolate. Per cent recovery of egg laying females from plants inoculated with the Barger isolate was significantly lower than from those receiving either the Butler or the Wells isolate.

Galling, root necrosis, root weight, and top weight of plants grown at 24 C and 28 C were not significantly different at the 5% level of confidence. Per cent egg laying females recovered at 24 C was signifi-

TABLE IV

EFFECTS OF NEMATODE ISOLATE, TEMPERATURE AND VARIETY INTERACTIONS ON HOST-PARASITE RELATIONSHIPS[1]

Nematode Isolate	Gall Rating	Necrosis Rating	Root Weight (g)	Top Weight (g)	Egg Laying Females Recovered (%)
	Effects of Isolate (Data are Averages of the Two Varieties at the Two Temperatures)				
Uninoculated	1.0a	1.0a	19.3c	12.6c	-----
Barger	4.2c	3.1b	11.6c	3.6a	8.3a
Butler	3.5b	2.9b	8.9a	3.7a	13.2b
Wells	4.1c	3.2b	9.6ab	4.8b	14.6b
	Effects of Temperature (Data are Averages of the Four Isolates on the Two Varieties)				
Temperature	Gall Rating	Necrosis Rating	Root Weight (g)	Top Weight (g)	Egg Laying Females Recovered (%)
24 C	3.4a	2.4a	11.0a	5.9a	14.3b
28 C	3.0a	2.7a	13.7a	6.5a	9.8a
	Effects of Variety (Data are Averages of the Four Isolates at the Two Temperatures)				
Variety	Gall Rating	Necrosis Rating	Root Weight (g)	Top Weight (g)	Egg Laying Females Recovered (%)
Spantex	3.1a	2.6a	12.0a	6.0a	12.1a
Dixie Spanish	3.2a	2.4a	12.7a	6.4a	12.0a

[1]Similar letters indicate no significant differences at P = 0.05 with Duncan's multiple range test.

cantly higher than at 28 C.

At the 5% level of confidence, no statistically significant differences in galling, root necrosis, root weight, top weight and per cent recovery of egg laying females between Spantex and Dixie Spanish were obtained.

Histopathology of Nematode Infection

Longitudinal sections of infected roots showed developing lateral roots originating from nematode infection sites. One nematode usually stimulated the formation of one lateral root (Fig. 6). Adult females were usually oriented with their heads inside the vascular cylinder and their bodies extending through the cortex toward the surface of the root, where egg masses could be observed at their posterior end (Fig. 7). Giant cells formed around nematode heads with their nuclei more deeply stained than nuclei of normal cells (Fig. 8). As many as 15 nuclei, which tended to aggregate at the center, were observed per giant cell. An average of five giant cells were observed per nematode. These giant cells were grouped together, usually separated only by their much thickened cell walls. Giant cells were generally inside the vascular cylinder, but cortical ones were not uncommon. In the stele, giant cells disrupted and blocked the vascular tissue (Fig. 9). Hypertrophy of cortical tissues resulting in swellings or galls accompanied many infections.

Post-Infection Nematode Development

a) Observations on mounted specimens: All larvae were vermiform until the fifth day. They began to enlarge on the sixth day and had

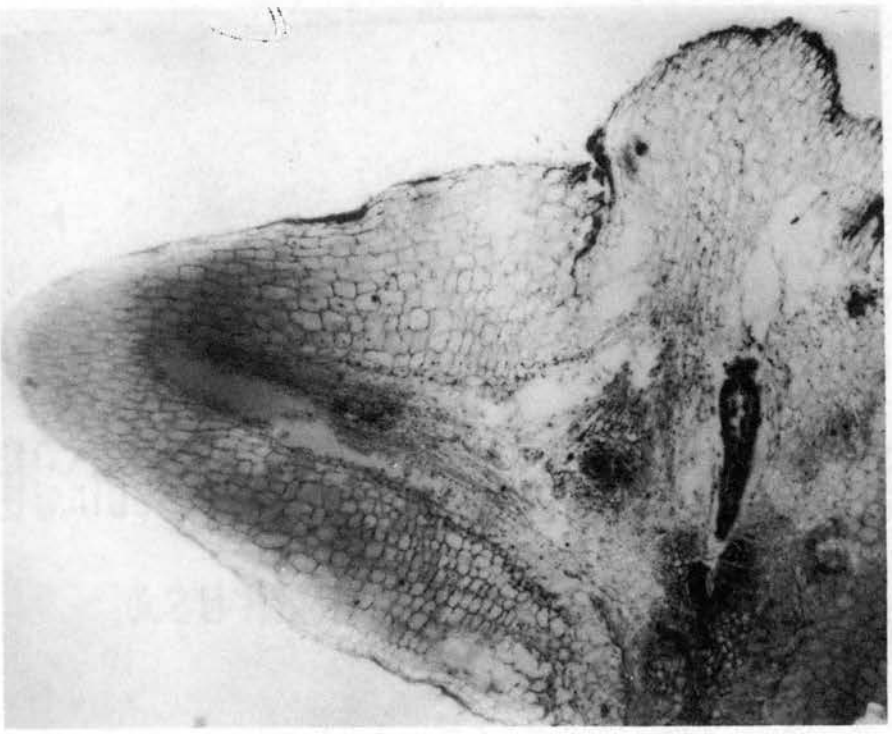

Figure 6. Longitudinal Section of a Root Tip of Susceptible Peanut Showing the Formation of a Lateral Root from a Nematode Infection Site.

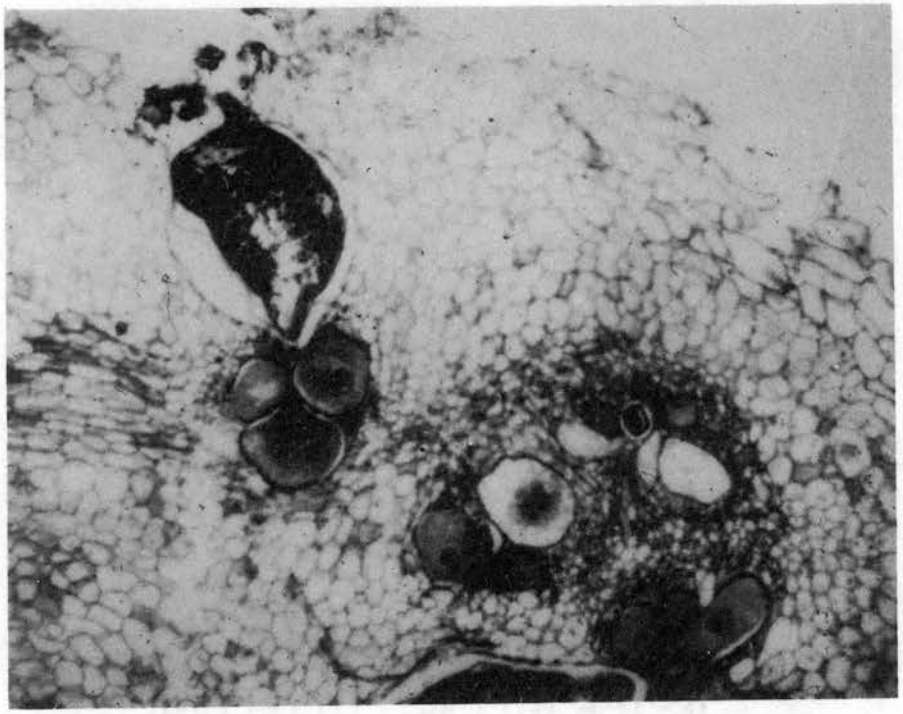

Figure 7. Longitudinal Section of a Galled Root Showing the Orientation of a Feeding Egg Laying Nematode

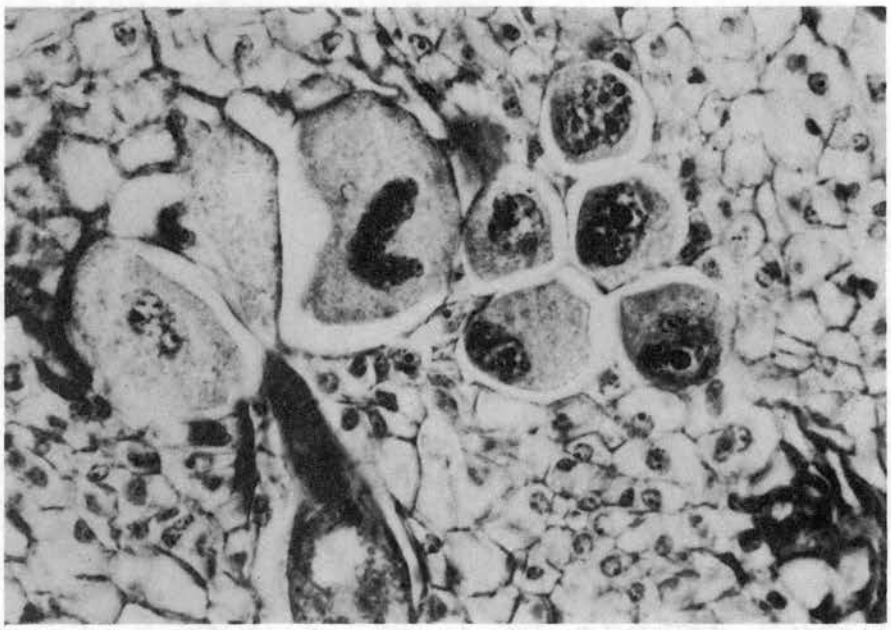

Figure 8. Longitudinal Section of a Galled Root Showing Giant Cells with Deeply Stained Nuclei Near the Head of a Nematode

Figure 9. Longitudinal Section of a Galled Root Showing a Giant Cell Blocking the Vascular Tissue

acquired a hemispherical posterior end, terminated by a spike, on the eighth day. The second (Fig. 10) and third (Fig. 11) molts of the most advanced nematodes were observed on the 11th and 12th day, respectively. The spiked tail was lost after the second molt (Fig. 10B), except in one larva (Fig. 10C). A few nematodes had already completed the fourth molt and had shed the molted cuticles on the 13th day.

b) Observations on nematodes in stained roots: Periods during which the developmental groups (Fig. 2) were observed are presented in Table V. Group A nematodes of the first generation were recovered through the 31st day. Group B nematodes first appeared eight days after inoculation and persisted through the 39th day. Nematodes in groups C, M and D were observed as early as the 13th, 19th and 18th day, respectively. Appearance of group E nematodes was first noted on the 23rd day and infection by second generation larvae was first observed on the 39th day.

TABLE V

PERIODS OF RECOVERY OF NEMATODE DEVELOPMENTAL GROUPS

Nematode Group	Days After Inoculation
A	1 to 31
B	8 to 39
C	13 to 40
M	19 to 40
D	18 to 40
E	23 to 40
A[1]	39 to 40

[1]Second generation.

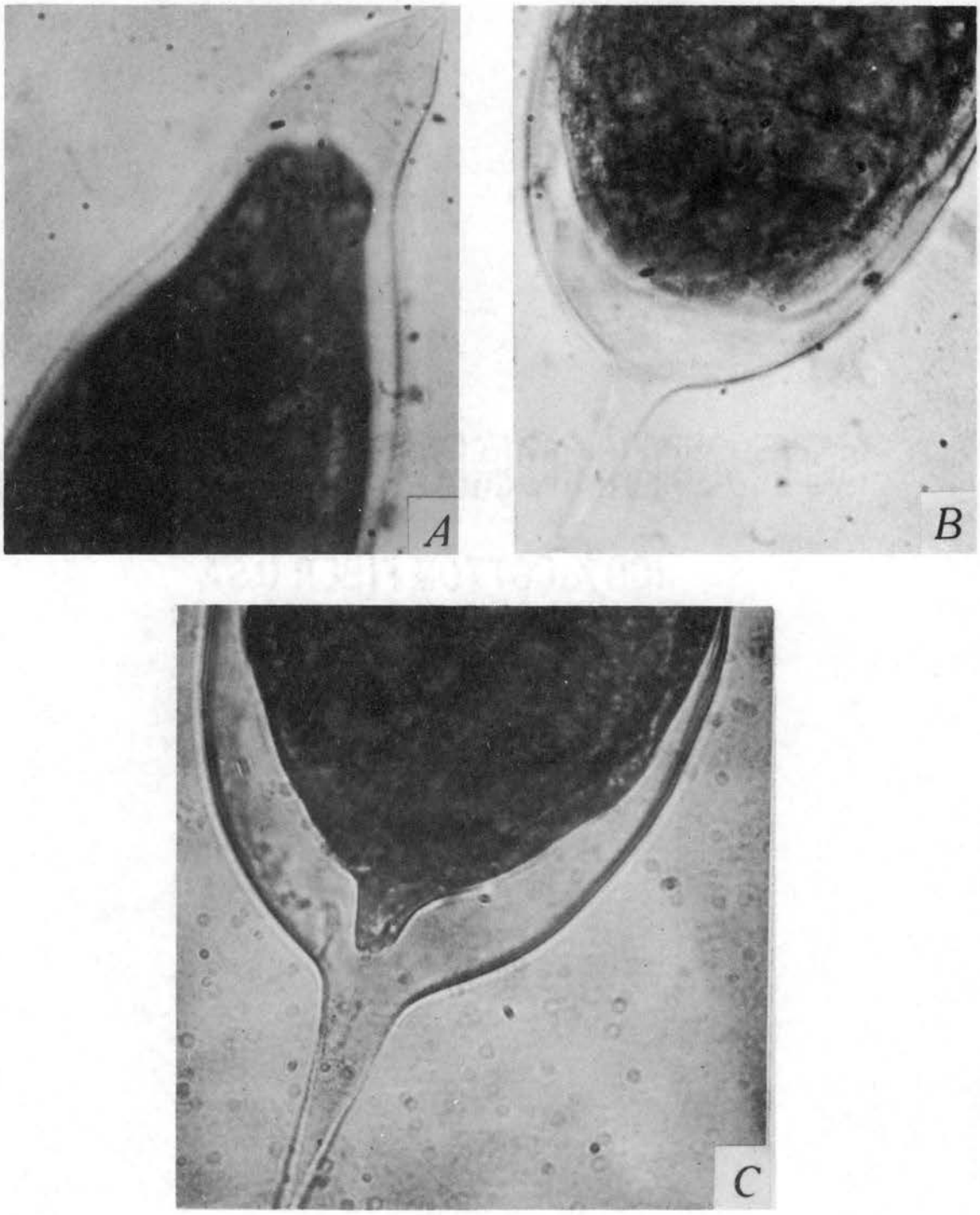

Figure 10. Third Stage Larvae. Note the One Molted Cuticle. A, Head Portion; B, Tail Portion with Cuticle of the Third Stage Without a Spike; C, Tail Portion with Cuticle of the Third Stage with a Spike.

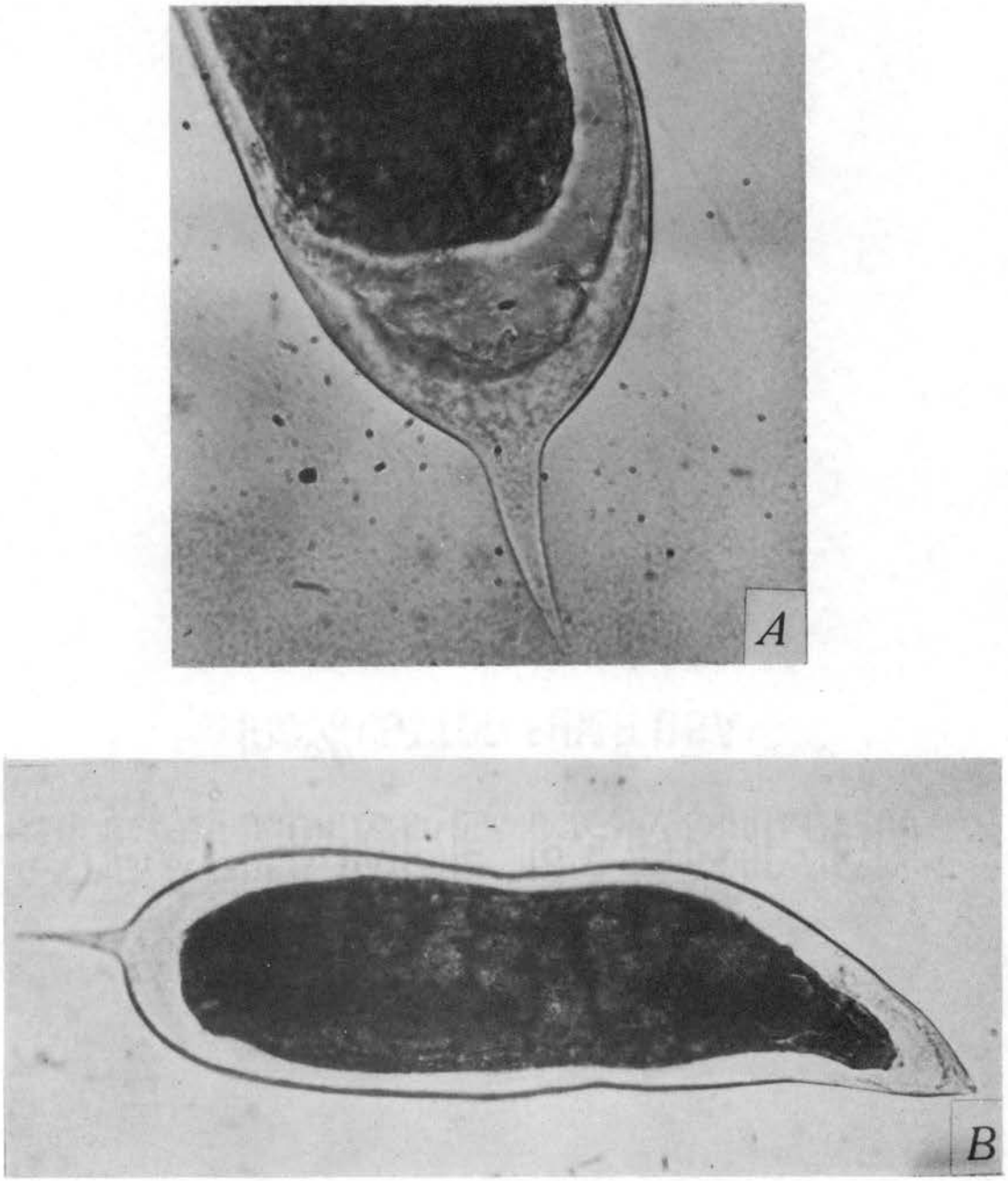

Figure 11. Fourth Stage Larvae. Note the Two Molted Cuticles. A, Tail Portion; B, Whole Larva.

Table VI shows that the number of group A nematodes was highest in the 6 to 10-day period, decreased continuously through the 31 to 35 and then increased in the 36 to 40 with the appearance of the second generation larvae. Group B nematodes were most abundant in the 11 to 15-day period after which their number decreased continuously through the termination of the experiment. Both groups C and D nematodes increased through the 21 to 25-day period and then decreased. Group M nematodes increased through the 31 to 35-day period and decreased in the 36 to 40. Group E nematodes were still increasing when the experiment was terminated. Nematode recoveries ranged from 4.0% to 10.7% of the original inoculum.

TABLE VI

POST-INFECTION NEMATODE DEVELOPMENT AT SELECTED PERIODS

Days After Inoculation	Nematode Groups[1]						
	A	B	C	M	D	E	Total
1 to 5	40.3	0	0	0	0	0	40.3
6 to 10	72.0	9.2	0	0	0	0	81.2
11 to 15	9.2	96.0	2.5	0	0	0	107.7
16 to 20	2.8	44.9	16.8	0.1	4.4	0	69.0
21 to 25	1.2	32.8	22.8	6.9	36.7	1.4	101.8
26 to 30	0.5	9.5	10.5	8.6	31.0	27.8	87.9
31 to 35	0.4	2.1	2.8	9.2	26.1	42.5	83.1
36 to 40	7.3	0.1	1.9	6.3	13.6	46.4	75.6

[1]Values are mean recoveries from 20 plants examined during the five-day period indicated.

Determination of Tolerance

Table VII shows that some peanut lines are more tolerant to nematode attack than others. When differences in yield between severely to very severely galled and uninfected plants were obtained and per cent reductions were determined, P.I. 288161 appeared to be the most tolerant among the peanut lines tested. Reduction in the yield of this line was 17.1%, compared to 48.6% in the control, Spantex. In decreasing order, the other lines which showed promise of tolerance were Early runner, P.I. 288138, P.I. 268684, Florigiant, P.I. 295244, P.I. 288167, and P.I. 295185.

TABLE VII

EFFECT OF M. HAPLA ON YIELD OF TOLERANT PEANUTS

Peanut Line	Gall Rating	Yield (g) Inoculated (I)	Yield (g) Uninoculated (U)	Difference in Yield (U-I)	Per Cent Yield Reduction $\left(\frac{U-I}{U} \times 100\right)$
Spantex (Control)	4.9	5.4	10.5	5.1	48.6
P.I. 295185	4.5	11.4	20.8	9.4	45.2
P.I. 288167	4.2	8.8	13.6	4.8	35.3
P.I. 295244	4.3	7.5	11.4	3.9	34.2
Florigiant	4.6	14.3	21.4	7.4	34.1
P.I. 268684	4.2	9.4	13.3	3.9	29.3
P.I. 288138	4.4	8.5	10.8	2.3	21.3
Early runner	4.2	11.4	14.1	2.7	19.1
P.I. 288161	4.3	10.2	12.3	2.1	17.1

Determination of Resistance

Based on galling, eight of the cultivated lines of A. hypogaea exhibited reduced susceptibility and four of the wild Arachis spp. showed varying degrees of resistance. No correlation between the degree of galling and root necrosis was obtained.

Table VIII shows the average nematode recovery in eight less susceptible cultivated peanut lines. F416 had the lowest gall rating of 3.0, compared to 4.2 in the susceptible control (Fig. 12). In decreasing order, NC4X, P.I. 295197, P.I. 295974, P.I. 288151, P.I. 288169, Dixie runner, and P.I. 295268 had higher gall ratings than F416, but lower than that of Spantex. The total number of nematodes recovered and the amount of development generally decreased with decrease in galling. For instance, only a total of 54.8 nematodes per 200-mg root sample, with no egg laying adults, were recovered from F416, compared to 152.6 with 11.1 egg laying adults in Spantex. In the remainder of the lines, a direct correlation between galling and nematode development and total recovery was also generally observed. No apparent correlations were obtained between galling and the numbers of larvae and non-egg laying adults recovered, except that higher numbers were recovered from Spantex than from any of the lines tested.

The average nematode recovery in resistant wild Arachis spp. is shown in Table IX. Arachis sp. P-246 had the lowest gall rating of 1.6, compared to 4.3 in the susceptible control. In increasing order, Arachis spp. P-237, P-258, and P-250 had higher gall ratings than Arachis sp. P-246, but much lower than that of A. hypogaea 'Spantex' (Fig. 13). The total number of nematodes recovered and the amount of development decreased with decrease in galling. For instance, only a

total of 15.2 nematodes per 200-mg. root sample, with no egg laying adults, were recovered from Arachis sp. P-246, whereas a total of 129.2, with 10.6 egg laying adults, were recovered from A. hypogaea 'Spantex'. Data collected from the other species also showed direct correlations between galling and nematode development and total recovery. Recovery of larvae and non-egg laying adults generally decreased with decrease in galling.

TABLE VIII

AVERAGE NEMATODE RECOVERY IN LESS SUSCEPTIBLE PEANUTS

Peanut Line	Gall Rating	No. of Nematodes Recovered			
			Adults		
		Larvae	Non-Egg Laying	Egg Laying	Total
Spantex (Control)	4.2	83.1	58.4	11.1	152.6
P.I. 295268	3.9	68.6	37.6	0.4	106.6
Dixie runner	3.7	67.9	46.6	0.1	114.6
P.I. 288169	3.5	41.6	48.6	0.6	90.8
P.I. 288151	3.4	43.9	37.7	0.6	82.2
P.I. 295974	3.4	42.3	27.5	0.1	69.9
P.I. 295197	3.4	41.9	42.2	0.6	84.7
NC4X	3.2	24.6	32.9	1.5	59.0
F416	3.0	30.3	24.5	0	54.8

Effects of Increased Inoculum Levels on Resistance

Host reaction to varying levels of nematode inoculum are shown in

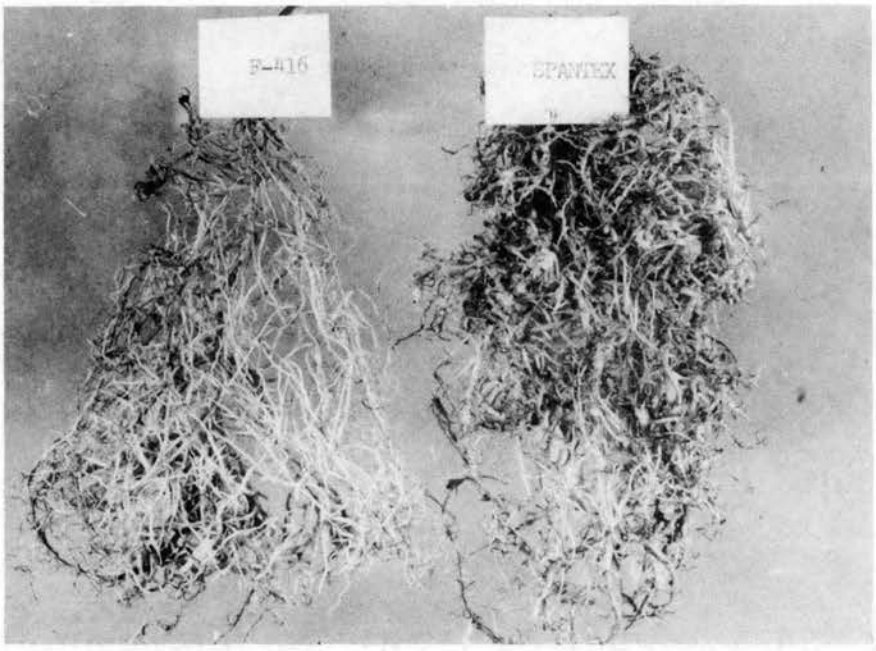

Figure 12. Galling of the Least Susceptible Cultivated Peanut Line (F-416) and the Susceptible Spantex.

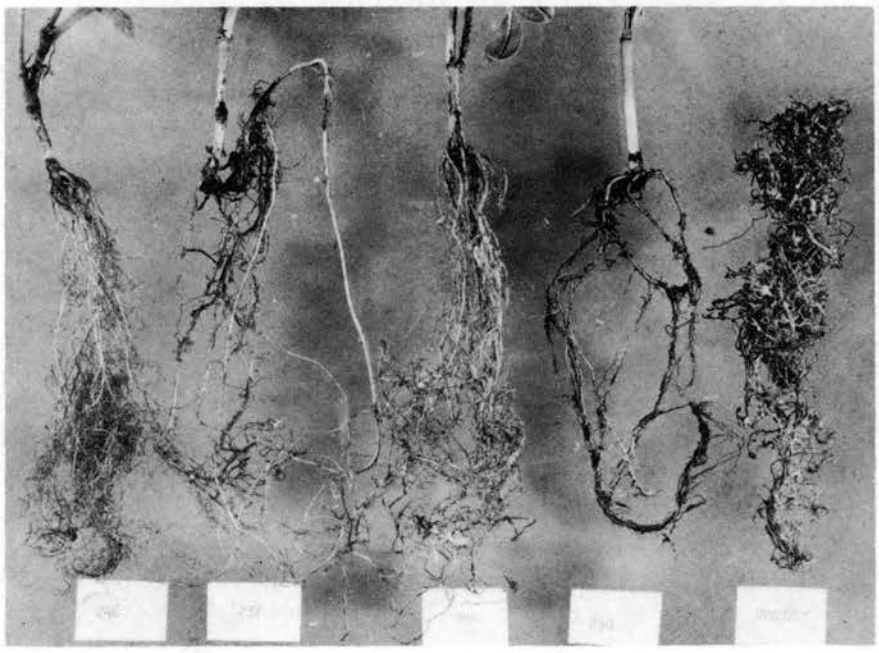

Figure 13. Galling of the Resistant Wild Arachis spp. and the Susceptible Spantex.

TABLE IX

AVERAGE NEMATODE RECOVERY IN RESISTANT WILD ARACHIS SPP.

Arachis spp.	Gall Rating	No. of Nematodes Recovered			
			Adults		
		Larvae	Non-Egg Laying	Egg Laying	Total
A. hypogaea 'Spantex' (Control)	4.3	75.9	42.7	10.6	129.2
Arachis sp. P-250	2.7	40.6	10.3	0.4	51.3
Arachis sp. P-258	2.3	22.2	14.4	0.4	37.0
Arachis sp. P-237	2.1	16.8	6.4	0.1	23.3
Arachis sp. P-246	1.6	10.6	4.6	0	15.2

Table X. Compared to A. hypogaea 'Spantex', gall ratings of Arachis spp. P-258, P-237 and P-246 were significantly lower at all levels, except in the zero egg mass level. There was a general increase in gall ratings as inoculum level was increased, but increases were not all statistically significant. For instance, the 40 egg mass level caused significantly less galling than the 80 egg mass level in A. hypogaea 'Spantex' and Arachis sp. P-258, but not in Arachis spp. P-237 and P-246. Gall rating at the 80 egg mass level was significantly lower than gall rating at the 160 egg mass level only in Arachis sp. P-237. At the 5 g chopped infected tomato root level, gall rating of Arachis sp. P-237 was not significantly different from gall rating of Arachis spp. P-246 and P-258. The gall rating of Arachis sp. P-258, however, was significantly greater than that of Arachis sp. P-246.

No statistically significant differences in root necrosis were obtained among levels and among species. Among levels, no significant

TABLE X

EFFECTS OF INCREASED INOCULUM LEVELS ON RESISTANT WILD *ARACHIS* SPP.

A. No. of Egg Masses Per Plant	Gall Rating[1]				Necrosis Rating[1]				Root Weight (g)[2]			
	Spantex (Control)	P-258	P-237	P-246	Spantex	P-258	P-237	P-246	Spantex	P-258	P-237	P-246
0	1.0a	1.0a	1.0a	1.0a	2.1a	2.4a	2.1a	2.1a	5.6a	4.3a	3.4a	4.8a
40	4.1b	1.8b	2.0b	1.8b	2.1a	1.9a	2.1a	1.8a	6.8a	4.3a	3.6a	4.2a
80	4.8c	2.4c	2.1b	2.1bc	2.0a	2.0a	2.0a	2.0a	5.8a	4.9a	3.8a	4.4a
160	5.0c	2.5c	2.4c	2.3c	2.4a	1.9a	2.0a	2.1a	5.3a	4.5a	3.8a	3.8a

B. Grams of Chopped Infected Tomato Root Per Plant	Gall Rating[1]			
	P-983 (Control)	P-258	P-237	P-246
5	5.0	2.8	2.5	2.4

[1]Among levels, similar letters indicate no significant differences at P = 0.05 and among species, lines indicate no significant differences at P = 0.05 with Duncan's multiple range test.

[2]Differences between means among species not determined.

differences in root weight were observed.

Effect of Nematode Isolates on Galling of Resistant Peanut

No resistance-breaking M. hapla isolate was indicated by the results shown in Table XI. Based on galling, Arachis sp. P-246 maintained resistance to the different nematode populations tested. Galling caused by the different populations did not differ significantly. The roots of the susceptible control were generally severely galled.

Effect of Plant Age on Resistance

Plant age affected the resistance of Arachis spp. P-246 and P-258 as shown in Table XII. Gall ratings of these species were significantly higher when inoculated at the age of one and one-half months than when inoculated at the age of three months. No significant differences in gall ratings of young and old plants of both the susceptible control and Arachis sp. P-237 were obtained. Except in Arachis sp. P-237, significantly more nematodes were recovered from the young than from the old plants. Significantly higher percentages of nematodes reached adulthood in the young than in the old plants of the resistant Arachis spp. P-237, P-246 and P-258, but not of the susceptible control.

Nematode Penetration and Development in Young Resistant and Susceptible Peanuts

Table XIII shows the differences in nematode penetration and development and time of galling in young resistant and susceptible peanuts. Galling had occurred in two to four days in the susceptible A. hypogaea 'Spantex', but it took 5 to 10 days for galling to occur in the resistant Arachis sp. P-246. The highest recovery, after 30 days, of

TABLE XI

GALLING OF RESISTANT AND SUSCEPTIBLE PEANUTS AS INFLUENCED BY *M. HAPLA* ISOLATES

Nematode Isolate	Gall Rating	
	Arachis sp. P-246	*A. hypogaea* 'Spantex'
Wells	2.1[1]	4.5[2]
Scott	2.2	4.0
Cain	2.1	4.0
Black	2.0	4.0
Ross	2.0	3.5
Davis	2.2	4.0
von Dirickson	2.0	3.5
Repp	2.4	4.0
Majors	2.1	4.0
O.S.U.	2.6	5.0

[1]Means of four replicates.

[2]Reading from one replicate.

first generation nematodes from the resistant peanut was 61% of the original inoculum, compared to 97% recovery from the susceptible peanut. None of the nematodes recovered from the resistant peanut were in group D after 20 days or in group E after 30 days, but nematodes in groups D and E were recovered from the susceptible peanut after 20 and 30 days, respectively. Examination of nematodes in the soil and nematode counts in the roots did not indicate completion of nematode life cycle in the resistant peanut after 50 days. Second generation larvae were found

TABLE XII

EFFECT OF PLANT AGE ON RESISTANCE[1]

Arachis spp.	Gall Rating		No. of Nematodes Recovered		Nematodes Reaching Adult Stage (%)	
	Young	Old	Young	Old	Young	Old
A. hypogaea 'Spantex' (Control)	4.3	4.2	150.2	69.7	35.4	49.9
Arachis sp. P-237	2.5	2.5	67.2	69.6	62.6	35.4
Arachis sp. P-246	2.9	2.2	52.0	25.7	48.4	33.9
Arachis sp. P-258	3.2	2.3	66.2	28.9	54.2	32.3

[1]Lines indicate no significant difference at P = 0.05.

TABLE XIII

NEMATODE PENETRATION AND DEVELOPMENT IN YOUNG RESISTANT AND SUSCEPTIBLE PEANUTS

Days After Inoculation	*Arachis* sp. P-246 (Resistant)							*A. hypogaea* 'Spantex' (Susceptible)						
	A	B	C	M	D	E	Total	A	B	C	M	D	E	Total
1	4.0						4.0	6.0						6.0
2[1]	5.7						5.7	11.2						11.2
3	9.0						9.0	25.0						25.0
4[2]	11.2						11.2	62.7						62.7
5	21.0						21.0	120.2						120.2
10[3]	67.5	2.7					70.2	177.5	9.7					187.2
20	309.0	262.0	22.7	.5			594.2	509.0	357.5	52.5	3.7	23.7		946.4
30	58.5	399.7	90.7	2.5	62.5		613.9	39.5	534.2	262.2	3.2	119.7	17.7	976.5
40	22.5	120.5	81.0	51.0	127.0	.7	402.5	103.2	165.2	199.0	64.0	314.2	150.2	995.8
50	11.7	52.5	49.7	33.2	133.2	5.0	285.3	187.7	54.5	61.7	109.7	313.5	387.7	1114.8

[1]Root tips of two plants of *A. hypogaea* 'Spantex' galled.

[2]Roots of all four plants of *A. hypogaea* 'Spantex' galled.

[3]Roots of all four plants of *Arachis* sp. P-246 galled.

both in the soil and in the roots of the susceptible peanut after 40 days. Appearance of second generation larvae was clearly indicated by the increased number of group A nematodes after 40 days in the susceptible peanut. The number of nematodes recovered from the resistant peanut started to decrease after 30 days, but the nematode recoveries from the susceptible peanut continued to increase until the termination of the experiment.

Nematode Reproduction in Resistant and Susceptible Peanuts

Little reproduction was observed in the resistant Arachis sp. P-246 up to 60 days after inoculation (Table XIV). Only an average of two and three-tenths egg masses per plant were found in the resistant peanut, but several hundred egg masses were found in the susceptible. Most of the egg masses that were found in the resistant peanut were empty and the average number of eggs per egg mass was only seven-tenths, compared to 222.9 in the susceptible. No second generation larvae were found in the roots of the resistant peanut, but they were found in the roots of the susceptible.

Histopathology of Nematode Infection in Resistant, Intermediate and Susceptible Peanuts

Nematode infections were accompanied by giant cell formation not only in the roots of the susceptible A. hypogaea 'Spantex' and of the intermediate A. hypogaea (F416), but also in the roots of the resistant Arachis sp. P-246 (Fig. 14). However, the galls were relatively smaller in the resistant peanut than in either the intermediate or the susceptible. Fewer infections were observed and less nematode development

TABLE XIV

NEMATODE REPRODUCTION IN RESISTANT AND SUSCEPTIBLE PEANUTS

	Arachis sp. P-246 (Resistant)	*A. hypogaea* 'Spantex' (Susceptible)
No. of Plants Examined	10.0	10.0
No. of Egg Masses Per Plant	2.3	Several Hundred
No. of Eggs Per Egg Mass[1]	0.7	222.9
Second Generation Larvae	Not Observed	Observed

[1]Means of counts made on one to four egg masses per plant of *Arachis* sp. P-246 and 10 egg masses per plant of *A. hypogaea* 'Spantex'.

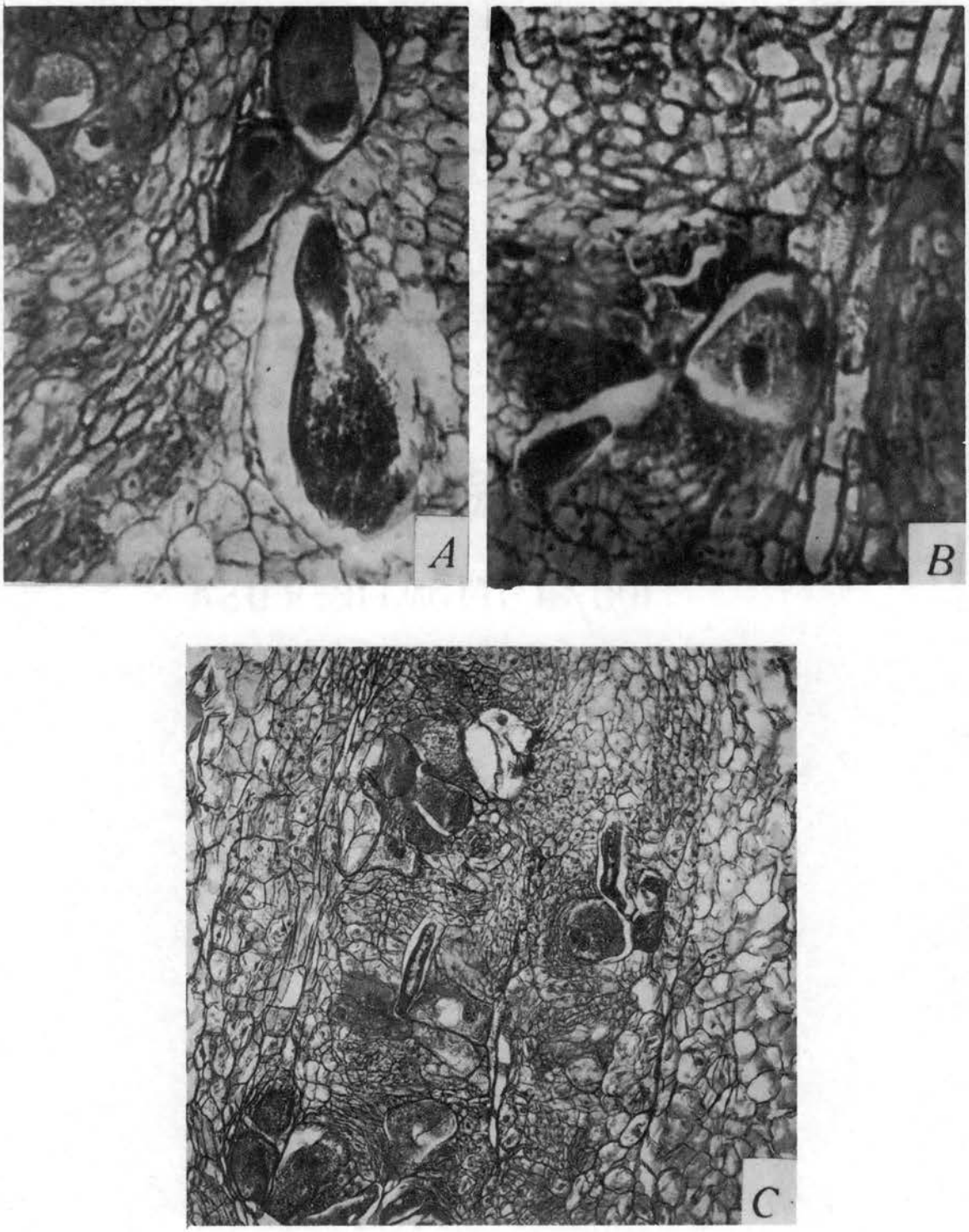

Figure 14. Longitudinal Sections of Galled Roots of Susceptible, Intermediate and Resistant Peanuts. A, Susceptible; B, Intermediate; C, Resistant.

occurred in the resistant peanut than in either the intermediate or the susceptible. Between the intermediate and the susceptible peanuts, no difference in the relative size of galls or number of infections was detected. On a per infection basis, no basic differences were observed in the anatomy of infected roots of the resistant, intermediate and susceptible peanuts. The size and number of giant cells formed by one nematode were nearly identical (Fig. 15) regardless of the degree of resistance or susceptibility of the plant. When present, giant cells in the resistant roots usually caused as much blocking, disruption and disorganization of vascular tissues as did the same number of giant cells in either the intermediate or the susceptible root. The other abnormalities, such as the formation of lateral roots from infection sites (Fig. 16), were also observed, regardless of the degree of resistance or susceptibility.

Attractiveness of Resistant and Susceptible Peanut Roots to M. hapla

Table XV shows the nematode recovery from each side of split pots seven days after inoculation. When no plants were grown on either side of the pot (NP/NP), 53.3 nematodes were found on one side and 61.7 on the other. In the R/NP treatment, there were 2.61 times more nematodes in the soil of the R side than in the soil of the NP side. The total numbers (nematodes in the soil plus nematodes in the roots) of nematodes found on the R and NP sides were 47.7 and 13.3, respectively. Of the total number of nematodes on the R side, 27.3% was in the roots. In the S/NP treatment, 1.64 times more nematodes were found in the soil of the NP side than in the soil of the S side. The total number of nematodes found on the S and NP sides were 101.7 and 31.3, respectively. Of the

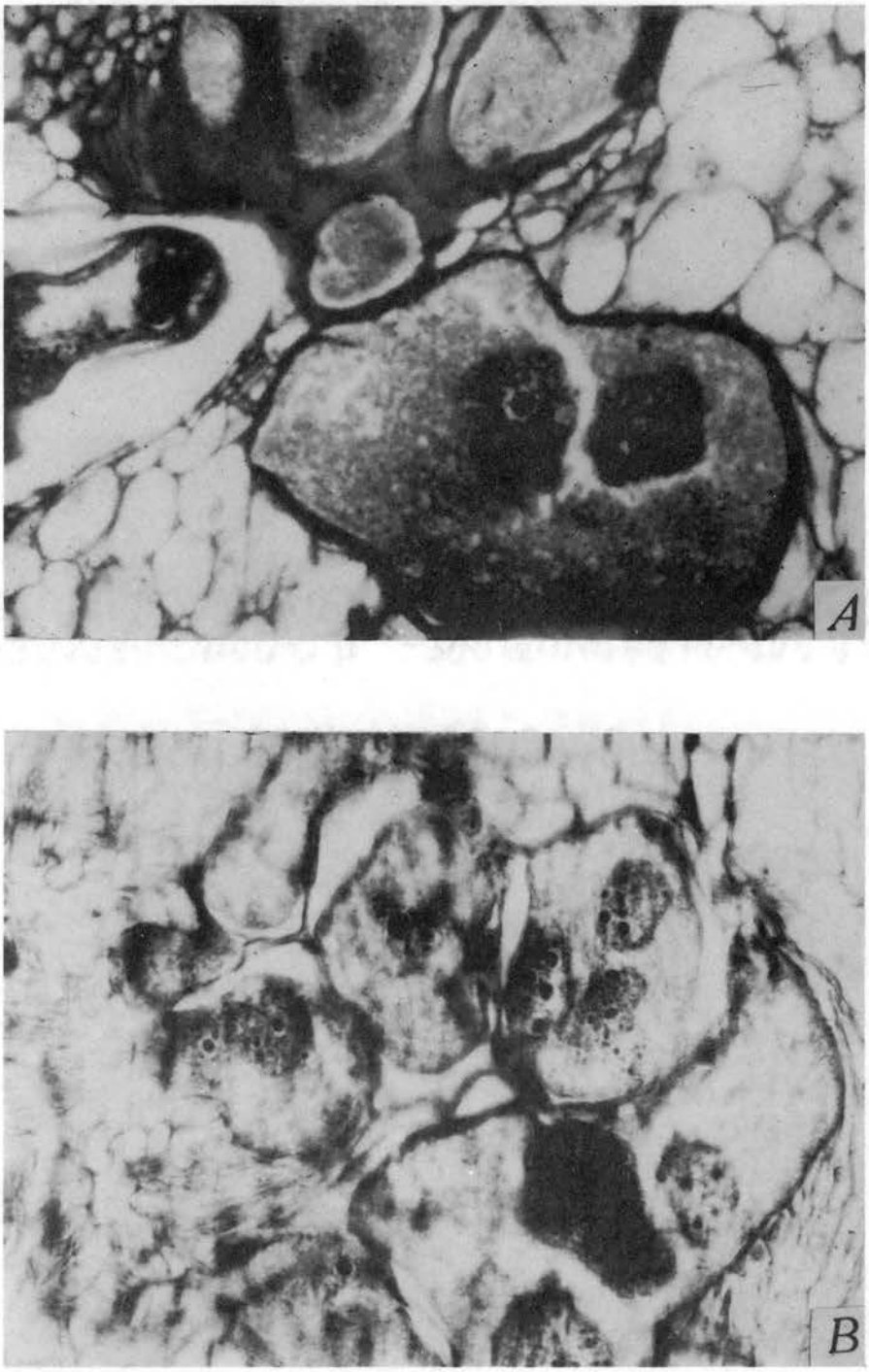

Figure 15. Enlarged View of Giant Cells. A, Giant Cells in the Root of Susceptible Peanut; B, Giant Cells in the Root of Resistant Peanut.

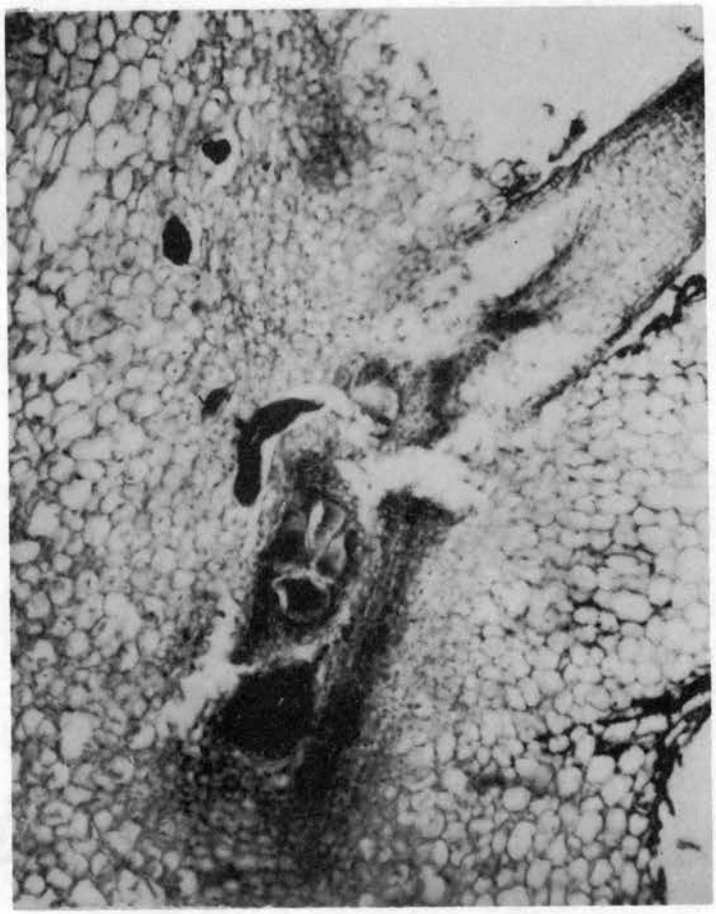

Figure 16. Longitudinal Section of a Galled Root of Resistant Peanut Showing the Formation of a Lateral Root from a Nematode Infection Site.

TABLE XV

NEMATODE RECOVERY FROM EACH SIDE OF SPLIT POTS SEVEN DAYS AFTER INOCULATION

Treatment[1]	Side of Pot Examined	No. of Nematodes Recovered[2]		
		Soil	Roots	Total
NP/NP	NP	53.3	-----	53.3
	NP	61.7	-----	61.7
R/NP	R	34.7 (72.7)	13.0 (27.3)	47.7
	NP	13.3	-----	13.3
S/NP	S	19.0 (18.7)	82.7 (81.3)	101.7
	NP	31.3	-----	31.3
R/S	R	27.7 (71.0)	11.3 (29.0)	39.0
	S	18.0 (11.9)	133.3 (88.1)	151.3

[1]Abbreviations: NP, no plant; R, resistant (*Arachis sp.* P-246); S, susceptible (*A. hypogaea* 'Spantex').

[2]Numbers inclosed in parentheses are percentages based on total counts per side.

total number of nematodes on the S side, 81.3% was in the roots. In the R/S treatment, 154 times more nematodes were found in the soil of the R side than in the soil of the S side. The total number of nematodes found on the R side was 39.0 and on the S side was 151.3. The percentages of nematodes in the roots of the R and S sides were 29.0 and 88.1, respectively.

DISCUSSION

The lack of root hairs in peanut plants probably restricted absorption of water and nutrients in either sand or perlite. Thus, poorer plant growth was obtained in these media than in soil. Soil has a finer texture and, therefore, more surface area was in contact with the roots. This could have accounted for the more vigorous plant growth in soil than in either sand or perlite. The poor growth of intact peanut seedlings and the failure of excised root tips to grow in White's (80) medium with varying concentrations of agar may have been due to a nutritional deficiency in the medium or to other unknown factors. Since excised tomato root tips were successfully grown and infected by larvae in the same medium, it was suggested that there is a difference in cultural requirements between excised peanut and tomato root tips and that larvae invade only actively growing roots.

The differences in galling, top weight and root weight of infected plants and in percentage recovery of egg laying females from these plants indicated a variation in pathogenecity among the isolates of *M. hapla* tested on a susceptible peanut variety. Variations in the ability of *M. hapla* isolates to parasitize alfalfa (35) and strawberry (64) have already been reported. Minton (49) observed a more pathogenic population of *M. arenaria* maturing rapidly and causing severe galling on peanut, in contrast with the less pathogenic population that did not mature and caused very little galling. In the present study, the Butler isolate of *M. hapla* caused significantly less galling than either the

Barger or the Wells isolate, but no correlation between galling of the susceptible peanut varieties used and nematode development was obtained.

The recovery of significantly higher percentage of egg laying females from plants grown at 24 C than from plants at 28 C agreed with previous observations (64) that reproduction of M. hapla was favored by low temperatures.

The reduction in the emergence of peanut seedlings in nematode-infested soil suggested that invasion by the nematode larvae of newly developing roots resulted in pre-emergent seedling mortality. Hence, the importance of pre-plant nematode control in heavily infested soils was indicated.

Galling of susceptible peanut plants, inoculated either at planting or 10 days after planting, correlated positively with inoculum levels up to 40 egg masses or 6000 larvae per plant. At the very high inoculum level of 2 g chopped infected tomato root, however, relatively fewer galls and more extensive root necrosis were observed than at 1 g level. This suggested that invasion by large number of larvae upset the host-parasite interaction and that instead of the usual stimulation of gall formation, death of root tissues resulted. This effect could have been due either to the large amount of nematode esophageal secretions or to the excessive mechanical injury caused by mass invasion in a limited area of the root. However, it is believed that much of the damage was caused by secondary invaders, the entry of which was perhaps facilitated by the large number of wounds created on the roots by nematode feeding. Later experiments indicated that nematode-infected peanut plant is very susceptible to root necrosis in the early stages of development when the tissues are succulent, but either a resistance to mechanical injury

or the ability to repair injured tissues seems to develop at maturity. This was evidenced by the lack of significant differences in root necrosis of both the susceptible and resistant peanuts between uninoculated plants and plants inoculated with up to 5 g chopped infected tomato roots at the age of three months or older. There was a stimulation of root growth at 5 egg mass and 4000 larval inoculum levels in the susceptible peanut variety Spantex. At 40 egg mass and 2 g chopped infected tomato root levels, a depression of root growth was observed suggesting that the effect of the nematode on root growth is not additive, but rather shifts from a stimulatory to a harmful effect at high inoculum levels. This finding agreed with the observation of Chitwood et al. (17) that stimulated root growth of peach variety S-37 resulted at moderate infestation levels of M. javanica, but depression of root growth resulted at high levels of infestation. Similarly, Madamba et al. (44) reported root growth stimulation of two unsuitable hosts each of M. javanica and M. incognita at low or moderate inoculum levels. The general reduction in top growth of peanut receiving 1 or 2 g chopped infected tomato roots or high levels of egg mass inoculum was probably a consequence of reduced root systems at these levels.

The development of M. hapla on the susceptible Spantex peanut variety was investigated under a 28 C 16-hr day and 20 C night regime. The intervals between molts of individual larvae were not determined, but it was apparent that the three parasitic molts of the most advanced larvae were completed within three days. This is in agreement with previously reported observations on tomato (9). However, the times required for initiation of parasitic molts, oviposition and infection by second generation larvae were less on peanut than those reported on

snapdragon and tomato (69). These variations may be attributable to the difference in the experimental conditions and in the suitability of the host plants.

When differences in yield between severely galled and uninfected plants were obtained and per cent reduction in yield was determined, a few cultivated peanut lines showed tolerance to M. hapla. The degree of tolerance varied among the different lines with the reduction in yield ranging from only 17.1% in the most tolerant line P.I. 288161 to 48.6% in the susceptible Spantex variety. Since it is difficult to accurately determine peanut yield under greenhouse conditions, confirmations from field tests are necessary before any conclusion can be made.

The present investigation has shown the resistance of the wild Arachis spp. P-237, P-246, P-250, and P-258 and the reduced susceptibility of the cultivated peanut lines F416, NC4X, Dixie runner, P.I. 288151, P.I. 288169, P.I. 295197, P.I. 295268, and P.I. 295974 to M. hapla. When inoculated at the age of three and one-half months, galling of the first three named wild Arachis spp. increased slightly with increasing level of nematode inoculum, but galling caused by inoculation with 5 g chopped infected tomato roots per plant was only moderate. Resistance or reduced susceptibility was characterized by low gall ratings and recovery of fewer and less developed nematodes. In the most resistant Arachis sp. P-246, resistance was further characterized by delayed gall formation, reduced larval penetration of roots, decline of nematode population after 40 days, little nematode reproduction, and failure of nematodes to complete their life cycle within 60 days after inoculation. There was a direct correlation between galling and nematode recovery and development. On this basis, galling alone may serve as an indicator of

resistance or susceptibility to M. hapla in peanut. No correlation, however, between degree of galling, root necrosis and root weight was obtained.

Since resistance in plants to root-knot nematodes is usually expressed after nematode penetration of roots (37), Christie's (19) report that some plants were resistant to nematode invasion has been questioned (25). In Christie's tests, plants were exposed to nematodes for 24 hr and examined for nematodes three to eight weeks after inoculation. Dean (25) argued that it was possible that larvae invaded resistant plants as readily as they invaded susceptible plants, but some died after entering resistant plants and could account for the reduced number of nematodes found. His argument was supported by the observation that there were as many M. incognita larvae in the susceptible as in the resistant tomato 24 hr after inoculation, but there were fewer larvae in the resistant than in the susceptible tomato one day later. Likewise, Riggs and Winstead (56) found similar invasion of resistant and susceptible tomatoes by M. incognita, but the larvae in the resistant tomato were dead 96 hr after inoculation. These reports suggested that the time factor is important in determining whether resistance is manifested before or after invasion of plant roots. In the present study on nematode penetration and development on peanut, the plants were exposed continuously to M. hapla larvae and examinations of roots were made at 1-, 5-, and 10-day intervals after inoculation. At all periods, fewer nematodes were found in the resistant than in the susceptible peanut. Unlike the observations of Dean (25) and Riggs and Winstead (56), increase in the number of nematodes was noted as the time of exposure to nematodes was increased up to 30 days after inoculation. A decrease in the number of

nematodes was only observed 40 and 50 days after inoculation. These observations suggested that the recovery of fewer nematodes in the resistant than in the susceptible peanut 1 to 30 days after inoculation cannot be attributed to death of some nematodes after entering the roots but rather to their inability to enter. Thus, a pre-infection phase of resistance was indicated. It should be noted that Dean (25) and Riggs and Winstead (56) worked on a host in which resistance was associated with hypersensitivity of infected tissue. Riggs and Winstead (56) found that larvae remained active in water for three or more weeks. Therefore, the death of nematodes in the hypersensitive tomato could not be attributed to starvation. Resistant and susceptible peanut roots examined for nematodes at different periods did not show root necrosis. Also, no dead cells around the head of invading nematodes, that Riggs and Winstead (56) observed in sections of resistant tomato roots, were detected in resistant peanut roots. This indicated that accelerated mortality of nematodes, similar to that observed on tomato, is not likely on peanut.

A random movement of nematodes in the absence of plant roots was demonstrated when no obvious difference in nematode counts was obtained between two unplanted sides of a pot seven days after inoculum was placed at the middle. However, when either a resistant or susceptible plant was grown on one side and none on the other, consistently more nematodes were found on the side with a plant than on the side without a plant. The attractiveness of peanut roots to M. hapla was, therefore, indicated. A susceptible plant appeared to be more attractive than a resistant plant, since there were 3.88 times more nematodes on the side of the pot where a susceptible plant was grown than on the side where a resistant plant was grown. This concurred with the observation of

Griffin (36) that susceptible alfalfa seedlings were more attractive to M. hapla than resistant alfalfa seedlings. Resistance to M. hapla at the root surface of peanut was suggested when only 27% to 29% of the nematodes that were found on the side of the pot with resistant plant was inside the roots, compared to 81% to 88% in the susceptible.

The recovery of significantly more nematodes in the young than in the old Arachis spp. P-246, P-258 and A. hypogaea 'Spantex' variety suggested that a mechanical barrier to nematode penetration seemed to have developed in peanut roots as the plants matured. Yarbrough (84) described the characteristic features of the root system of peanut. He emphasized that the young root of peanut is characterized by a total absence of a true epidermis and consisted anatomically of a cortex, and a central cylinder separated by an endodermis. The cortex was made up of several layers of parenchymatous cells and the central cylinder was differentiated into pericycle, primary phloem, and a tetrarch primary xylem. Such a relatively fragile anatomical framework of the young root would be quite incapable of providing a strong mechanical barrier to the nematode penetration. On the other hand, the mature root has two meristematic zones: (a) the phellogen which differentiates in the pericycle and, as it develops, causes a breakdown and death of the tissues external to it, and (b) the vascular cambium which forms the secondary phloem and a considerable amount of wood. The change in the internal construction of the mature root is probably accompanied by increased quantities of lignin, cellulose, and other polysaccharides deposited on the cell walls of the secondary tissues (1). Such an anatomical construction will definitely provide an effective mechanical barrier which would restrict nematode penetration of roots and could have

accounted for the recovery of fewer nematodes in the old than in the young plants. Loos (41) observed that tea was very susceptible to M. brevicauda Loos in the seedling stage, but developed a form of resistance with increasing age, until complete resistance was attained. In peanut, significantly higher gall ratings were observed in the young than in the old plants of Arachis spp. P-246 and P-258, but not of A. hypogaea 'Spantex'. This indicated a difference in response to nematode stimulus between resistant and susceptible peanut plants. Since plant age did not affect the galling and the number of nematodes recovered from Arachis sp. P-237, it is believed that the nature of resistance in this species is different from that in Arachis spp. P-246 and P-258.

The post-infection phase of resistance in peanut was characterized by the failure of nematodes to develop normally and reproduce and the decline in the number of nematodes after prolonged infection periods. This type of resistance has been attributed either to the failure of the host to respond favorably to the stimulus of nematode secretions with the resultant lack of gall and giant cell formation (18), to the synthesis by the host of a substance which neutralizes the giant cell-inducing agent of these secretions (8) or to the hypersensitivity of the host resulting in death of nematodes, probably from starvation (25) or toxicity (56). Sections of peanut roots showed that nematode infections were accompanied by gall and giant cell formation not only in the susceptible but also in the resistant plants. This suggested that the root tissues of resistant plants reacted favorably to the stimulus of nematode secretions as did those of susceptible plants, ruling out lack of favorable morphological response on the part of the host as an explanation to the nature of resistance in peanut. Barrons (8) suggested that, if a large

number of larvae entered a resistant root at about the same place, the plant might not be able to synthesize enough neutralizing substance in that area, so occasionally small galls and giant cells might form. Sections of resistant roots indicated that the formation of a group of giant cells may be incited by single nematodes and the relative number of giant cells incited by each nematode in the susceptible was apparently the same as in the resistant plants. The relatively larger galls and their earlier formation in the susceptible than in the resistant plants were probably due to the infections by more nematodes which developed faster in the susceptible plants. The absence of a detectable hypersensitive reaction of resistant peanuts to M. hapla was discussed earlier.

Since available hypotheses concerning resistance in plants, after root-knot nematode penetration of roots, do not explain the present findings, it is believed that resistance of a different nature exists in peanut. The formation of giant cells in resistant roots indicated that the inhibition of normal nematode development and reproduction in these roots could not be attributed to lack of giant cells. Any other physiological incompatibility between the resistant host and the parasite could have caused similar inhibition. The results have further indicated that the inhibitory effect seems to be cumulative and related to plant age, as evidenced by the significantly lower percentage of mature nematodes found in old than in young resistant plants. A change from an inhibitory to a lethal effect after prolonged host-parasite co-existence probably accounted for the decline in the number of nematodes as infection progressed.

In conclusion, the present findings have determined the host-para-

site relationships between peanut and M. hapla, under the conditions of this study. Resistance has been shown to be a combination of pre- and post-infection phases. The pre-infection phase was found to be due, at least partly, to less attractiveness of resistant roots. Resistance at the root surface is believed to be contributory to the restricted nematode invasion. A mechanical barrier to nematode penetration of roots probably accounted for the greater resistance of old plants than young plants. The causes of the post-infection phase of resistance have not been determined, but a probable divergence of the nature of this resistance in peanut from that found in other plants was indicated. The finding that the yield of some tolerant cultivated lines was less affected by nematodes than the yield of others may serve as a basis for the identification of this type of reaction in existing cultivated peanut lines. Although the genetic aspect has not been determined, it was demonstrated that some cultivated peanut lines are less susceptible and four wild Arachis spp. are resistant to M. hapla. With the use of efficient breeding techniques, it is hoped that this resistance or reduced susceptibility can be incorporated into commercially acceptable peanut varieties.

SUMMARY

Experiments conducted to study the host-parasite relationships with definition of peanut resistance to M. hapla indicated the following:

1. Peanut plants grew more vigorously in soil than in either sand or perlite.
2. Based on nematode development, 24 C was more optimum for M. hapla than 28 C, but the higher temperature was more favorable for peanut growth.
3. Nematodes in infested soil reduced emergence of peanut seedlings.
4. Galling of young susceptible plants correlated positively with inoculum levels up to 40 egg masses and 6000 larvae per plant, but galling at 2 g chopped infected tomato root level was reduced.
5. Generally, there was a stimulation of root growth of young susceptible plants at low infestation levels and depression of top growth and root growth, accompanied by increased root necrosis, at high infestation levels.
6. Nematode-infected susceptible plants were very susceptible to root necrosis in the early stages of development, but became resistant as they matured.
7. Meloidogyne hapla isolates Wells, Barger and Butler differed in their pathogenecity on the susceptible Spantex variety, based on plant and nematode interactions.
8. At 28 C, the second and third molts of the most advanced larvae occurred on the 11th and 12th day, respectively, after inoculation

to the susceptible Spantex variety. The fourth molt and shedding of the molted cuticles occurred between the 12th and the 13th day. Oviposition and infection by second generation larvae had occurred on the 23rd and 39th day, respectively.

9. In decreasing order, the cultivated peanut lines P.I. 288-161, Early runner, P.I. 288138, P.I. 268684, Florigiant, P.I. 295244, P.I. 288167, and P.I. 295185 were more tolerant to M. hapla than the other cultivated peanut lines tested, based on yield test in the greenhouse.

10. In decreasing order, the cultivated peanut lines F416, NC4X, P.I. 295197, P.I. 295974, P.I. 288151, P.I. 288169, Dixie runner, and P.I. 295268 were less susceptible and the wild Arachis spp. P-246, P-237, P-258, and P-250 were resistant to M. hapla, based on plant and nematode interactions.

11. Resistance was generally characterized by less galling and recovery of fewer and less developed nematodes.

12. In the most resistant Arachis sp. P-246, resistance was further characterized by delayed gall formation, reduced larval penetration of roots, decline of nematode population after 40 days, little nematode reproduction, and failure of nematodes to complete their life cycle within 60 days after inoculation.

13. The movement of M. hapla in the soil was random in the absence of roots. Either resistant or susceptible roots attracted the nematodes, but susceptible roots were apparently more attractive than resistant roots. Most of the nematodes that were attracted to the resistant roots failed to penetrate, but most of the nematodes that were attracted to the susceptible roots were able to penetrate.

14. No resistance-breaking isolate existed among the 10 M. hapla isolates from different localities in Oklahoma tested on Arachis sp. P-246.

15. Galling of plants correlated positively with nematode development and recovery. On this basis, galling alone may serve as an indicator of nematode resistance or susceptibility in peanut.

16. Galling of the susceptible Spantex variety and the resistant Arachis spp. generally increased with increasing inoculum levels, but old resistant peanuts maintained resistance to galling up to the 5 g chopped infected tomato root level.

17. Old plants of Arachis spp. P-246 and P-258 were more resistant to nematodes than young plants, based on galling and nematode development and recovery.

18. Nematodes developed more rapidly in young than in old resistant plants, but the speed of their development in young and old susceptible roots was apparently the same.

19. Plant age did not affect the gall rating of, and the number of nematodes recovered from, the resistant Arachis sp. P-237, nor the gall rating of, and the percentage of nematodes reaching adult stage in, the susceptible Spantex variety.

20. Meloidogyne hapla incited the formation of giant cells and excessive numbers of lateral roots in the susceptible, intermediate and resistant plants.

21. On a per infection basis, the anatomy of infected roots were relatively similar, regardless of the degree of resistance or susceptibility of the plant.

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VITA

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