HOST-PARASITE RELATIONSHIPS OF THE ROOT-KNOT NEMATODES, *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA*, ON BEANS (*PHASEOLUS VULGARIS*)

by

BENJAMIN WAMBUA NGUNDO

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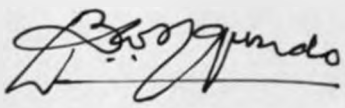
DECLARATION

(a) I, BENJAMIN WAMBUA NGUNDO, hereby declare that this thesis is my original work and has not been presented for a degree in any other University.



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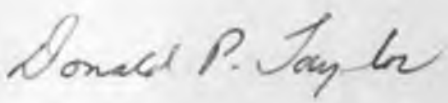

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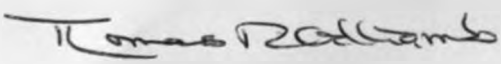

THOMAS R. ODHIAMBO

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SUMMARY

Field, laboratory, and greenhouse experiments were carried out to study effect of nematicide application on bean (Phaseolus vulgaris) yields in Kenya; penetration of larvae of Meloidogyne incognita and M. javanica in 6 bean varieties, and the subsequent development of the nematode parasites within the hosts; and histopathology of the roots of the hosts infected with the two parasites.

The nematicides were applied to a dark-red friable clay soil heavily infested with the two root-knot nematode species, at Kenya Canners Plantation Ltd., and Kuraiha Estate, Thika. It was found that, in the first field experiment at Kenya Canners Plantation Ltd., application of D-D as a broadcast (overall) and a row treatment, and EDB applied as a broadcast treatment, significantly reduced the number of M. incognita and M. javanica larvae in the soil. EDB row treatment did not reduce larval populations. Significant yield increases (45-60%) were obtained with Kikara and Mexico 142 with broadcast and row application of D-D, and broadcast application of EDB. This is consistent with the larval population decreases in these treated plots. Nematicides did not increase the yield of Marathon green bean. Yields of this variety were uniformly low, presumably because the

soil type was not conducive to good growth. In the second field experiment at Kuraiha Estate, D-D in the planting row did not significantly increase the yield of Kikara but a significant yield increase (32%) was obtained with Mexico 142. These results indicate that root-knot nematodes, in large numbers can reduce bean yields in Kenya by as much as 60%.

In the preliminary laboratory and greenhouse experiments, non-freshly-hatched larvae of M. incognita and M. javanica, at inoculum levels of 100 larvae, failed to penetrate root systems of Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon, and Premier bean varieties after exposure periods of 12, 24, or 48 hours. When the exposure period was increased to 48 hours, penetration of 5 of the 6 bean varieties by freshly-hatched larvae of the nematode parasites occurred at inoculum levels of 100, 150, 200 and 300. In Mexico 142, even at the inoculum level of 300 larvae, there was no penetration. Although larvae of the nematode parasites penetrated root systems in all 6 bean varieties, results showed that, when the experiment was conducted under low and variable room or greenhouse temperature conditions, no significant differences in penetration percentage existed between the varieties and the species. Results of a similar experiment conducted under controlled

growth chamber conditions at higher temperatures indicated that highly significant differences at 0.1% level occurred between the two species. At temperatures of 23° and 29°C for night and day respectively, M. incognita larvae penetrated root systems in all the 6 bean varieties in significantly greater numbers than M. javanica larvae. When the temperatures were raised to 25° and 30°C for night and day respectively, significantly higher numbers of M. incognita larvae penetrated root systems of Kikara, Canadian Wonder, Masterpiece, Marathon, and Premier than M. javanica larvae. There were no significant differences between M. incognita and M. javanica larvae in penetration of root systems of Mexico 142.

Within the hosts, it was found that rates of development from larvae to sexually differentiated nematodes occurred more rapidly in Mexico 142 for M. incognita and M. javanica, and in Masterpiece for M. javanica only, than in other bean varieties inoculated at the same time. However, the period taken for larvae of both nematode species to develop to the adult stage was identical in all 6 bean varieties. At growth chamber ambient temperatures of 25°C and 30°C night and day respectively, a high percentage of both species developed into females which laid eggs within 20 days after inoculation; these females completed their life cycle and a second

generation was developing within 30 days in all varieties. A low percentage of the nematode species developed to males. It is concluded from this study that M. incognita and M. javanica can develop and reproduce rapidly on Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon, and Premier bean varieties; hence, the varieties are highly suitable hosts of both nematode species. Under optimum temperature conditions for rapid development of both species, at least 3 generations are possible on these bean varieties in any of the growing seasons in East Africa.

- Results of the histopathological studies revealed extensive damage to the cortical and vascular tissue of roots of the 6 bean varieties infected with M. incognita and M. javanica. These studies showed that all 6 bean varieties were highly susceptible to both nematode species; the nematodes fed in the vascular area and caused the formation of 3-20 multinucleate giant cells around a single female. It was also shown that disruption of the stele by the giant cells, proliferated parenchyma cells, and the parasites themselves caused the conducting tissues to be scattered, so that, in cross section, the phloem and xylem occurred in irregular patches rather than in one continuous column. While most of the giant cells were found in the vascular cylinder in all 6 bean

varieties, giant cells were also observed in the cortex or between the pericycle and endodermis in Mexico 142, Canadian Wonder, and Premier bean roots.

A type of tissue designated "abnormal xylem", which is characterized by secondary wall thickenings of annular, reticulate, or pitted types, was observed associated with giant cells.

No nuclear division was observed in giant cells in this study in any roots of the bean varieties; it was assumed that nuclear division in giant cells was either not occurring at the time the tissues were killed and fixed, or did not exist in the root cells of the bean varieties.

C H A P T E R I

GENERAL INTRODUCTION

NEMATODES GENERAL DESCRIPTION

Nematodes have been described by Jenkins and Taylor (1967) as being multicellular, non-segmented animals having tissues assembled to form organ systems, and exhibiting a basic bilateral symmetry. They are most commonly vermiform (worm-shaped) and are further characterized by the possession of a pseudocoel, a body cavity which is not lined with an epithelial mesentery. In addition, according to these authors, nematodes have the following characteristics: their body wall consists of a cuticle beneath which is a hypodermis (epidermis) having 2 to 8 longitudinal chords which separate bands of longitudinal somatic muscles; the lumen of the esophagus (pharynx) is triradiate in cross section; the excretory system does not contain flame cells (cilia), but is composed of canals opening to the outside through the body wall by means of a ventrally located excretory pore; the anus and male gonopore are united to form a posteriorly located ventral cloaca; female gonopore is separate and ventrally located. No respiratory or circulatory systems are present. These authors stated that the majority of plant-parasitic

nematodes range in length from 0.5 mm to 2.0 mm. Although the typical, or basic, body form of nematodes is long, cylindrical, with the body tapering at both ends of the head and tail, in a number of plant-parasitic nematodes, the adult female becomes a swollen, pouch-like organism, with a form of a lemon, pear, kidney, or spherical configuration, while the male usually remains slender and worm-like. The best known examples of this type are the root-knot nematodes (species of Meloidogyne Goeldi, 1887), and the cyst nematodes (species of Heterodera Schmidt, 1871).

Nematodes are found in almost every kind of environment but, although often capable of withstanding drought for long periods, they cannot be active unless there is sufficient free water in their immediate environment. Thorne (1961) reported that nematodes constitute one of the most important groups of organisms which inhabits the soil about the roots of plants and which frequently plays a vital part in their growth and production. Rarely is any crop free from their attacks, whether in the field, the orchard, the home garden, or the greenhouse; yet we usually are unaware of their presence because of their microscopic size and inconspicuous position within the soil. These slender, active, wormlike creatures are found not only in the soil but also in fresh and salt water wherever organic matter exists, from the

arctic regions to the tropics, and from the ocean depths to the tops of high mountains.

Taylor (1971) pointed out that, in the past, nematode damage to crops was often ignored or attributed to other causes such as lack of soil fertility, deficient soil moisture, or soil exhaustion. This was partly because nematodes were too small to be seen without a microscope, and partly because definite information on their occurrence and pathogenicity was not available. When inexpensive soil nematicides became commercially available about 20 years ago, it was possible for the first time to control nematodes on a large scale in the field and thus demonstrate their adverse effect on crops. The author also stated that, in many developing countries, nematodes are a more serious and complex problem than in the developed countries. The reasons for this are many and varied. Most of the developing countries, lie in tropical or subtropical regions where the climate is suitable for activity and reproduction of nematodes throughout the year. Sandy and warm soils such as are found in many of these countries in the arid zone are very favourable for nematode infections, especially in irrigated areas which are used almost continuously for crop production. Perennial crops, and crops grown in the same fields year after year, are often so heavily attacked by nematodes that they barely survive;

yet these countries must depend on perennial crops and on crops grown without rotation for much of their food supply. According to Taylor, the nematode problem in developing countries is increased because of large areas, poor communications, and lack of trained personnel to study the problem and to advise the farmers. Often also, these countries lack quarantine regulations or ways of enforcing them which would prevent the introduction and internal spread of nematode-infected planting material. Nevertheless, developing countries must increase the availability of their agricultural products as rapidly as possible if the present decline in the *per capita* agricultural production is to be reversed. Taylor, therefore, suggested that prevention or reduction of crop losses due to plant-parasitic nematodes is one way of increasing crop yields.

NOMENCLATURE OF ROOT-KNOT NEMATODES

Because symptoms of root-knot disease are characteristic and easy to see, the nematodes in root galls were among the first plant-parasitic nematodes to be recognized. The first report of root-knot nematodes was by Berkeley (1855) when he studied galls on the roots of cucumber (*Cucumis sativus* L.) grown in a greenhouse in England; he referred to them as "Vibrios".

From Berkeley's discovery until 1949, the root-knot nematodes were regarded as one species. The name of this aggregate species was changed on several occasions and the history of nomenclature may be briefly summarised as follows. Schmidt (1871) proposed the generic name Heterodera for the sugar beet nematode, H. schachtii. Greeff (1872) gave the name Anguillula radicicola to a nematode that he found in galls on the roots of grasses (Poa annua L.). This was not a root-knot nematode but instead was the species known today as Ditylenchus radicicola (Greeff, 1872) Filipjev, 1936. Cornu (1879) found a root-knot nematode causing galls on the roots of sainfoin (Onobrychis viciaefolia Scop.) and named it Anguillula marioni. Müller (1884) found a root-knot nematode causing galls on the roots of Dodartia orientalis L. Under the erroneous impression that he had the same parasite as Greeff, he used the same specific name but placed the species in the genus Heterodera, thus making the combination H. radicicola. This was the accepted name for "the root-knot nematode" until 1932. Goeldi (1887) found a root-knot nematode causing galls on the roots of coffee (Coffea sp.) plants in Brazil and named it Meloidogyne exigua. Goodey (1932) pointed out that, as used by Müller, H. radicicola was misapplied and, under the Rules of Zoological Nomenclature, it was not a valid name for "the root-knot nematode". In these circumstances,

the oldest available name for the genus and species was that proposed by Schmidt and Cornu, respectively, and from then until 1949, Heterodera marioni (Cornu, 1879) Goodey, 1932 was the accepted name for "the root-knot nematode". Chitwood (1949) published his revision of this group, differentiated and designated 5 species and 1 subspecies of root-knot nematodes, removed them from Heterodera and placed them in a separate genus, Meloidogyne, first proposed by Goeldi. M. incognita (Kofoid and White, 1919) Chitwood, 1949 and M. javanica (Treub, 1885) Chitwood, 1949 were among the first 5 species designated by Chitwood. The former species was described from specimens collected from eggs encountered in fecal samples of soldiers at Camp Travis, Texas, U.S.A.; the latter species was described from specimens collected from sugar cane (Saccharum officinarum L.) grown in Cheribon and Buitenzorg, Java, Indonesia. Other workers have since described other species and the genus now comprises about 38 known species.

DISTRIBUTION AND HOST RANGE OF M. INCOGNITA AND M. JAVANICA

A pilot survey of East African cultivated soils for species of Meloidogyne showed that of 372 populations studied, 74 were of M. incognita and more than half (219) were of M. javanica; the former species has been

reported to be often the dominant root-knot nematode at lower altitudes, whereas the latter species is dominant at higher altitudes (Whitehead, 1965). Geographical distribution of these species includes Africa, Australia, Brazil, Ceylon, Colombia, Cyprus, India, Israel, Malaysia, Pakistan, Spain, Trinidad, U.S.A. and greenhouses in northern Europe. Goodey, et al. (1965) list over 340 and 770 plant species or varieties as hosts of M. incognita and M. javanica respectively. Many of these hosts are of economic importance, such as tea (Camellia sinensia L.), tobacco (Nicotiana tabacum L.), potato (Solanum tuberosum L.), pineapple (Ananas sativus Schult.), grapevine (Vitis sp.) tomato (Lycopersicum esculentum Mill.), and pepper (Capsicum frutescens L.).

ECONOMIC IMPORTANCE OF ROOT-KNOT NEMATODES AND NEED FOR RESEARCH ON BEANS

Species of Meloidogyne are responsible for considerable damage to crop plants in tropical and temperate zones, and in many regions their control is economically desirable. For instance, M. javanica is known to be the most serious pest of crop plants in Central Africa (Daulton and Curtis, 1963) and the predominant species in tobacco growing areas of Rhodesia and Malawi where losses have been estimated at 18-25 million pounds of cured leaf per annum, worth

£2,750,000 to £3,750,000 (Daulton 1963, 1964). LeClerg (1964) reported that nematodes (mainly root-knot nematodes) account for an estimated annual reduction in bean yields to over $4 million in the United States. In 1971, the Committee on Crop Losses of the Society of Nematologists estimated that the average annual loss of various crops caused by plant-parasitic nematodes (mainly root-knot nematodes) in the United States is about $1,590,696,000 (Special Publication No.1 of the Society of Nematologists).

With few exceptions, previous nematology research at the East African Agriculture and Forestry Research Organization (E.A.A.F.R.O.), Muguga, has been concerned with the distribution and taxonomy of nematode genera and species, particularly root-knot nematodes, Meloidogyne spp. (Whitehead, 1965). Recently, research priorities at E.A.A.F.R.O. have been reassessed, and major emphasis has been placed on increasing production of East Africa's cereals and legumes. In view of this, an investigation of the interaction of the root-knot nematode species and beans (Phaseolus vulgaris L.) was initiated. Beans are a popular and common food crop in East Africa. In Kenya they cover an acreage second only to maize (Zea mays L.), while in northern Tanzania the production of seed is of major value to the economy of that area. Bean seed has a high protein content

and is a useful supplement to the maize diet. Research work on beans, however, had lagged far behind that of maize in most respects. Relatively little was known of the effect of nematodes on bean yield.

In East Africa, Whitehead (1957) reported that beans were attacked by species of Meloidogyne. Hainsworth (1962) claimed that 10% (£7-8 million) of the agricultural produce of Kenya was lost each year through nematode attacks, principally from species of Meloidogyne. Hollis (1962) estimated that in Kenya smallholdings nematodes cause a 50-100% yield loss of food crops, including beans. During an E.A.A.F.R.O. survey in 1971, roots of severely stunted French beans were found to be heavily infected with two root-knot nematode species, M. incognita and M. javanica. Analysis of soil collected from the rhizospheres of both diseased and healthy plants indicated also a probable relationship between the root-knot nematodes and the stunted condition of the French beans.

Lordello and Santos (1960) indicated that dwarf beans (Phaseolus vulgaris L.) was found infected with M. javanica and M. incognita in different regions of Brazil.

In the United States, Miller (1936) reported that

root-knot nematodes, then considered to be the single species, H. marioni, were found generally on bean plants growing in sandy soils, and in some cases damage was sufficiently severe to cause yellow, stunted plants unable to set pods. Baker (1943) stated that beans were among the field crops whose "cropping power" might be considerably reduced by H. marioni. Harter and Zaumeyer (1944) reported that beans of all kinds were highly susceptible to root-knot nematodes, and that in some fields losses amounted to as much as 50%. Townsend and Ruehle (1947) pointed out that root-knot nematodes were occasionally a problem in some of the 80,000 acres devoted to snap beans (Phaseolus vulgaris L.) in Florida and that no resistant varieties were available at that time. The annual average loss in beans caused by plant-parasitic nematodes (mainly root-knot nematodes) has been estimated at approximately $26 million (Committee on Crop Losses of the Society of Nematologists; Special Publication No.1 of the Society of Nematologists, 1971).

Blazey et al. (1964) showed that 55 varieties of Phaseolus vulgaris were found to vary in their response to M. incognita from being heavily galled with many females and egg masses to having occasional small galls and a few scattered females and egg masses. Seven of the most resistant varieties were heavily parasitized

by other root-knot nematodes including M. hapla Chitwood, 1949, M. arenaria (Neal, 1889) Chitwood, 1949, and M. arenaria thamesi Chitwood, Specht and Havis, 1952. Fassuliotis et al. (1967) reported that selections in F4 and F5 from a hybrid (B-3864) of snap bean were highly resistant to M. incognita acrita Chitwood, 1949 in both greenhouse and field evaluations.

On the basis of reported damage to beans by root-knot nematodes in other parts of the world and the importance of beans in Kenya agriculture, there was thus an urgent need to appraise critically, under field and greenhouse conditions, the extent of losses in bean yields caused by root-knot nematodes under Kenya conditions. Although the objectives of this study were mainly to determine the host-parasite relationships of M. incognita and M. javanica on beans, the effect of the nematodes on bean yields had to be known first in order to justify these studies. For this reason, therefore, a chapter on the "Effect of nematicide application on bean yields" is included (Chapter 3), which precedes the chapters on the host-parasite relationships. Aspects of the relationships studied included penetration of larvae of M. incognita and M. javanica in 6 bean varieties as affected by temperature; comparative development of the two species on the 6 bean varieties; and

comparative histopathology of roots of the 6 bean varieties infected with the two parasites.

C H A P T E R 2

MATERIALS AND METHODS

EFFECT OF NEMATICIDE APPLICATION ON BEAN YIELDS

Location and characteristics of experimental plots

The first field experiment was initiated in 1971, at the beginning of the April-June rainy season (known locally as the long rains). Nematicide treatments were applied to soil heavily infested with a mixed population of two root-knot nematodes, Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 and M. javanica (Treub, 1885) Chitwood, 1949, on land operated by Kenya Canners Plantation, Ltd., Thika (1,493 m altitude, 37°6'E, 1°1'S). The nematicide plots were established on a site where sisal (Agave sp.) had been grown for several years until 1969. After the land was cleared of sisal, two successive crops of green beans (Phaseolus vulgaris L. var. Marathon) were grown. This was followed by one crop of okra (Hibiscus esculentus L.), which was heavily attacked by the root-knot nematodes, after which the bean nematicide experiment was initiated.

The second field experiment was carried out in

1971, at the beginning of the November/December rains (the short rains) at Kuraiha Estate, Thika (1,493 m altitude, 37°3'E, 1°2'S). Nematicide treatments were applied to soil heavily infested with the root-knot nematode, M. incognita; the experiment was sited on plots where beans (var. Premier) had been grown continuously under overhead sprinkler irrigation for several years and had been heavily attacked by the nematode. This method of irrigation was also used during the experiment.

The mean annual rainfall of both sites is 986 mm (10 years of records). The soil type is a Kikuyu dark-red friable clay. In general, this soil is mantled primarily by red volcanic loam of high clay content exhibiting a high degree of aggregation due to iron and aluminium oxides. It has a friable consistency and moderate permeability.

Soil and root sampling methods

Ten subsamples of soil were taken to a depth of 20 cm from every experiment plot; the total amount of soil was mixed thoroughly, and a composite sample (approximately 930 g) for nematode analysis was placed in a 15 x 25 cm labelled polythene bag.

In the first experiment, both pre-and post-

fumigation soil samples were taken for nematode analysis; the post-fumigation soil samples were taken 14 days later prior to planting. No soil samples for nematode analysis were taken in the second experiment; treatments were compared on the basis of yield of bean seed only.

Root sampling for identification of species of root-knot nematodes was carried out once in each experiment. Ten heavily galled root systems from each of the 3 bean varieties used (Kikara, Mexico 142 and Marathon) in each of the experiments were taken at random from guard rows and placed in separate labelled polythene bags.

Nematode extraction, enumeration and identification

In the first experiment, soil samples from every plot were thoroughly mixed and analysed as follows. A 100 ml subsample of each composite sample was processed through an Oostenbrink (1954) modified flotation funnel and the resulting nematode suspension brought up to 100 ml. Of this suspension, two 10-ml samples were analysed under a dissecting microscope, giving numerical data on the population densities of the nematodes present. Specimens of plant-parasitic nematodes found were identified to genus.

In each experiment, 10 egg-laying Meloidogyne females from each of the bean varieties were dissected from infested root systems. Their perineal patterns, the characteristic cuticular markings on the posterior end of females of species of Meloidogyne, were studied and identified using the methods described in detail under studies on penetration, development and histopathology.

Preparation, treatment and maintenance of plots

In each experiment, the fields were ploughed and harrowed before fumigation. Fumigation plots were tilled again by hand and brought as near as possible to seed bed condition before treatment; the ground was marked in 30 cm squares for the broadcast (overall) treatment plots, and in rows 45 cm apart for the row treatment plots. Two guard rows separated plots and surrounded replicates.

In the first experiment, 5 treatments and 3 bean varieties were compared using a 3 x 5 factorial design replicated 5 times; each experimental plot (4.1 x 4.6 m) contained 200 plants in 10 rows. The 5 treatments were: soil fumigation with D-D (1,3-dichloropropene and 1,2-dichloropropane) broadcast and row; EDB (ethylene dibromide) broadcast and row; and non-fumigated controls. The 3 bean varieties used included both dry and green

beans: dry bean varieties were Kikara (a local selection) and Mexico 142 (a canning variety); the green bean variety was Marathon (a canning variety).

In the second experiment, 2 treatments and 2 bean varieties were compared using a 2 x 2 factorial design replicated 6 times; each experimental plot (3.2 x 4.3 m) contained 126 plants in 6 rows. The 2 treatments were non-fumigated control and D-D applied as a row treatment. The 2 bean varieties used were Kikara and Mexico 142. Sources of the bean seeds used are described in detail later in this chapter under penetration, development and histopathology.

Fumigants were applied 14 days prior to planting with a hand injector at 30 cm intervals to a depth of 15 cm at the following rates: D-D at 336.8 l/ha broadcast and 224.5 l/ha in the row at 3.2 ml per injection; EDB at 67.3 l/ha broadcast and 44.9 l/ha in the row at 0.6 ml per injection. After application the chemicals were immediately sealed in by closing the injection holes with soil. Bean seeds were dressed with Agrosan-D (40% Aldrin w.p. and mercuric chloride) at the rate of 70.9 g/45.4 kg before planting for the control of bean fly (Melanagromyza phaseoli Tryon, 1895).

Furrows about 15 cm in depth were opened and,

for the first experiment, double superphosphate (40-42% P_2O_5) at the rate of 167.9 kg/ha and potassium sulphate (K_2SO_4) at the rate of 112.1 kg/ha were applied by hand at planting. In the second experiment, a compound fertilizer ($N:P_2O_5:K_2O:M_gO$ = 15:15:6:4) at the rate of 167.9 kg/ha was applied between the bean seeds also by hand at planting. Two bean seeds were planted at 20 cm spacing in rows 45 cm apart. Thinning took place one week after emergence leaving only one seedling per original planting site. The experiments were weeded by hand three times during the experiment.

Two and a half weeks after emergence, it was found that germination of the beans in the first experiment, particularly Marathon, was in the range of 45-80%, and growth was not uniform. Where plants were missing, seeds were resown in order to achieve a uniform stand of all varieties. In the second experiment, stands of all varieties were uniform and there was no need for replanting.

When the plants were one month old, calcium ammonium nitrate was applied in each experiment at the rate of 167.9 kg/ha. It was also found necessary to apply 80% Dithane M-45 w.p. (80% zinc and manganese ethylene bis-dithiocarbamate) to control anthracnose, caused by the fungus, Colletotrichum lindemuthianum

(Sacc. and Magn.) Briosi and Cov., and bacterial halo blight, caused by Pseudomonas phaseolicola (Burk.) Dowson.

PENETRATION OF THE ROOT-KNOT NEMATODES, M. INCOGNITA AND M. JAVANICA INTO SIX BEAN VARIETIES, DEVELOPMENT OF THE NEMATODES IN THE BEAN VARIETIES, AND HISTOPATHOLOGY OF THE BEAN ROOTS INFECTED WITH THE NEMATODE SPECIES

Bean varieties and their sources

The 6 bean varieties used in these experiments included both dry and green beans. The dry bean varieties were Kikara (a local selection), Mexico 142 (a canning variety), Canadian Wonder and Masterpiece (both for local marketing and export). Green bean varieties were Marathon (a canning variety) and Premier (for local marketing and export). Bean seeds were originally obtained from different sources: Kikara was purchased locally at Muguga; Mexico 142 was obtained from Katumani Experimental Station, Machakos; Canadian Wonder and Masterpiece were purchased from Kirchoff's East African Ltd., Nairobi; Marathon was obtained from Kenya Canners Plantation Ltd., Thika; and Premier was obtained from Kuraiha Estate, Thika. The bean varieties were increased in field trials at

Embu, Thika and Muguga and are now maintained at E.A.A.F.R.O., Muguga.

Nematodes and their sources

The two species of Meloidogyne used were M. incognita and M. javanica.

M. incognita was originally obtained from egg masses produced on heavily infested 'Premier' bean roots collected in the field at Kuraiha Estate, Thika; it was maintained on the 6 bean varieties in the greenhouse at E.A.A.F.R.O., Muguga.

M. javanica was originally obtained from a single egg mass isolated from a tomato plant found in a sugar cane plot at E.A.A.F.R.O., Muguga. The culture was maintained on tomatoes and beans in the greenhouse.

Species identification

Ten egg-laying Meloidogyne females from each source were collected from the greenhouse cultures and dissected from their respective infested root systems with dissecting needles and placed in two separate watch glasses containing water. Each female was removed individually from the watch glasses, placed on a piece of perspex and the posterior end cut off

with a sharp point of a small scalpal. The piece of cuticle, bearing anus, tail tip and vulva was cleaned of adhering body tissues, trimmed down to the area containing the perineal pattern and mounted in glycerin (Goodey, 1963) on a 2.5 x 7.5 cm glass slide. A dissecting needle was also used to steady the female during this operation. Ten perineal patterns were mounted on one slide. A coverslip, 19 mm in diameter was gently lowered over the specimens on each slide and sealed with glyceel (Thorne, 1935). The specimens were identified under compound microscope by the configuration of their perineal patterns (Taylor, et al., 1955).

Nematode hatching and enumeration

Egg masses of previously identified species of Meloidogyne were removed from tomato and bean roots with forceps and placed in watch glasses containing distilled water and incubated at 25°C (Godfrey, 1931) for 24 hours.

Inoculum consisting of larvae of M. incognita and M. javanica obtained as described above was prepared as follows. Larvae of each of the species of Meloidogyne were collected in two separate beakers and the resulting suspension in each of the beakers brought up to 100 ml. A 3-ml aliquot of each

suspension was pipetted into a counting dish and the number of larvae counted under a dissecting microscope using a hand tally counter. For each experiment in the penetration studies, each time the count was made, the inoculum was accurately adjusted to the required number of larvae by the addition or removal of individual larvae. In the development and histopathology experiment, only the initial nematode populations were counted; the subsequent inocula levels were estimated by dilution based on the initial counts.

Pregermination of seeds

Thirty petri dishes, each containing a moistened filter paper 9 cm in diameter, were sterilized in a pressure-cooker at 1.05 kg per sq cm for 15 minutes. Three seeds from each of the 6 bean varieties were surface sterilized by soaking them in 0.1% mercuric chloride for 5 minutes. The petri dishes (Fig. 1) were incubated at 25°C for 60 hours.

Medium for transplanting seedlings

Sandy soil obtained from E.A.A.F.R.O., Muguga, was steam sterilized at 93°C for 15 minutes. The sandy soil consisted of the following particle sizes: gravel (> 2 mm) = 10.3%, coarse sand (0.2-2 mm) = 84.2%

and fine sand, silt, and clay (<0.2 mm) = 5.5%.

Inoculation and preparation of seedlings for examination

(a) Penetration studies

Experiment 1 was designed to determine the exposure time necessary for larvae of M. incognita and M. javanica to penetrate bean roots at a constant inoculum level. Exposure times of 12, 24 and 48 hours and an inoculum level of 100 larvae for each of the two species of Meloidogyne used for this study were chosen arbitrarily.

Sterilized sandy soil was put in paper cups, each 9.5 cm in height and 7.5 cm in diameter. Seedlings, with roots between 2 and 4 cm long of each of the 6 bean varieties, 36 in total, pregerminated as previously described, were placed on the sterilized sandy soil in the paper cups. A suspension of 100 M. incognita larvae, which had been kept in a water suspension in a watch glass for more than 24 hours, was placed in each of 18 (5-ml) pyrex beakers. Similarly, 100 M. javanica larvae were placed in another set of 18 beakers. The suspension of each species was inoculated by pouring it directly on the roots of each of the 6 bean varieties and the

roots were immediately covered lightly with additional sterilized sandy soil. All the seedlings were watered with a gentle stream of water, after which the cups were kept in the greenhouse for 12, 24 and 48 hours; no replicates were used. The inoculated seedlings were maintained in the greenhouse at the following temperatures: 21°C at 0830 hours; 24°C at 1430 hours; and 24.5°C at 1830 hours.

After 12, 24 and 48 hours, 12 cups, one for each of the two species inoculated on the 6 varieties, were removed from the greenhouse. The root systems were gently washed free of soil and stained for 1 minute in hot 0.01% cotton blue lactophenol (Franklin and Goodey, 1949). The root systems were removed from the solution and cleared in cold lactophenol in petri dishes. The destained root systems were examined for larval penetration by carefully dissecting the tissues under a dissecting microscope.

Experiment 2 was designed to determine the number of larvae penetrating the bean root systems in a constant period of time. Materials and methods used for this study and for the subsequent experiments are described in detail in Experiment 1. An arbitrarily chosen inoculum level (300 freshly-hatched larvae) and exposure time (48 hours) were used for this

study: the experiment was replicated twice.

Experiment 3 was designed to verify results obtained in Experiment 2. While the number of replicates and the exposure period were the same as in Experiment 2, the number of Meloidogyne larvae used was reduced to 250 and 200 per seedling for each species.

Experiment 4 was designed to verify further the results obtained in Experiment 3. Here, the number of replicates and time between inoculation and the assay were again the same as in Experiment 2, but the number of Meloidogyne larvae was reduced to 150 and 100 per seedling for each species.

Experiment 5 was designed to determine whether 100 freshly-hatched Meloidogyne larvae, as opposed to larvae kept for 24 hours, would penetrate the root systems within 24 hours. Two replicates were used in this experiment. Larval penetration percentages were not determined for Experiments 1-5, as these were preliminary experiments designed to determine the required inoculum level and exposure time for subsequent experiments.

Experiment 6 compared penetration of the 6 bean varieties by freshly-hatched larvae of the two species at a constant inoculum level of 100 and a constant

exposure period of 48 hours at room temperature. The experiment was replicated 5 times.

Experiment 7 was designed to verify results obtained in Experiment 6; the experiment was studied under greenhouse temperature conditions rather than at room temperature as in Experiment 6.

In Experiment 8 the inoculum level, number of replicates and exposure period were the same as in Experiment 6, but larvae of the two species were not of the same age. Larvae of M. javanica were obtained from tomato soil in a pot maintained in the greenhouse whereas larvae of M. incognita were freshly-hatched from egg masses as described previously. The experiment was studied in a growth chamber at ambient temperatures of 23° and 29°C night and day respectively. The temperatures were recorded with a thermograph (Fig. 2).

The inoculum level, number of replicates and exposure time used in Experiment 9 were the same as in Experiment 6. However, larvae of each of the species were of the same age, freshly-hatched from egg masses. In addition, the ambient temperatures in the growth chamber were raised to 25° and 30°C night and day. Root systems were examined and larval penetration percentages were determined in Experiments 6-9.

(b) Developmental and histopathological studies

Seedlings, with roots between 2 and 4 cm long of each of the 6 bean varieties, 72 in total, pregerminated as described earlier, were placed on sterilized sandy soil in the paper cups. A suspension of 200 freshly-hatched larvae of M. incognita was inoculated by pipetting it directly on the roots of each of the 6 bean varieties. Similarly, 200 freshly-hatched larvae of M. javanica, were pipetted directly on the roots of each of the 6 bean varieties. In each case, the roots were immediately covered lightly with additional sterilized sandy soil. All the seedlings were watered with a gentle stream of water, after which the cups were kept in a growth chamber with ambient temperatures of 25° and 30°C night and day respectively, for 10, 20, 30 and 40 days; no replicates were used.

After 10, 20, 30 and 40 days, 18 cups, one of each of the two species inoculated on the 6 varieties and one for the control in each variety, were removed from the growth chamber. The root systems were gently washed free of soil, roots containing galls from infected and portions of healthy roots were cut from the root systems of each variety and placed in formalin-acetic acid-alcohol (FAA) fixative for a minimum time of 18 hours (Johansen, 1940).

In the developmental studies, three heavily galled roots, from each of the varieties infested with each of the observation intervals, were placed in watch glasses containing lactophenol. Nematodes were carefully dissected from the individual galls, placed on glass slides in a drop of lactophenol, and mounted under cover slips. The slides were observed under a microscope to determine the sex and development stage of each nematode.

In the histopathological studies, for every observation interval, one infected and one healthy root from each of the varieties, for each species, were dehydrated in the ethyl alcohol and tertiary butyl alcohol series, infiltrated with and embedded in paraffin, and sectioned transversely and longitudinally at 12 μ thick with a rotary microtome. The sections were fixed to glass slides with Haupt's adhesive and 4% formalin, stained with safranin and fast green, and mounted permanently under cover slips with Canada balsam (Johansen, 1940). Seventy-two pieces of root were sectioned; the sections were studied under a microscope to determine the histological changes, if any, within infected and healthy roots.

C H A P T E R 3

EFFECT OF NEMATICIDE APPLICATION ON BEAN YIELDS

INTRODUCTION

Root-knot nematodes, species of Meloidogyne Goeldi, 1887, have been shown to attack and cause severe stunting and yellowing of bean (Phaseolus vulgaris L.) plants (Miller, 1936), and to reduce the crop yield by as much as 50% (Harter and Zaumeyer, 1944; Hollis, 1962).

The effectiveness of soil treatments in reducing root-knot nematodes and in increasing vegetable yields have been demonstrated in the United States on various crops such as tomatoes (Lycopersicum esculentum Mill.) by Lear and Thomason (1956); Wade beans by Winstead et al. (1958); sweetpotatoes (Ipomoea batatas Lam.) by Nielsen and Sasser (1959); and head lettuce (Lactuca sativa L.) by Radewald et al. (1969).

Although studies in the United States have repeatedly demonstrated the effectiveness of soil fumigants, it was not known whether similar results would be obtainable under Kenya conditions. This field experimentation was conducted to determine the effect of nematicide application on bean yields in

fields known to be heavily infested with root-knot nematodes under Kenya conditions. If these tests demonstrated that root-knot nematodes were capable of causing large reductions in bean yield under field conditions, the host-parasite relationships of root-knot nematodes and beans would be investigated under greenhouse, growth chamber, and laboratory conditions (reported in Chapters 4, 5, and 6).

EFFECTS OF D-D AND EDB APPLICATION ON MELOIDOGYNE LARVAE

Plant-parasitic nematodes found associated with the bean varieties in the 75 plots of the first experiment in the pre-fumigation sampling dates included species of Meloidogyne; Helicotylenchus Steiner, 1945; Scutellonema Andrassy, 1958; Rotylenchus Filipjev, 1936; Criconemoides Taylor, 1936; Pratylenchus Filipjev, 1936; and Longidorus (Micoletzky, 1922) Thorne and Swanger, 1936. Of these, specimens of Meloidogyne were the only nematodes which occurred consistently enough and in high numbers throughout all samplings to permit statistical analysis. Numbers of Meloidogyne spp. in the first experiment, and yields for each experiment, were statistically analysed.

Means of Meloidogyne larvae recorded in the first

experiment on pre-fumigation and post-fumigation sampling dates are given in Table 1. Analyses of variance carried out on numbers of larvae per 100 ml soil (data transformed to logarithms) showed the following effects. Prior to fumigation there were no significant differences in numbers of Meloidogyne larvae in the plots. Post-fumigation data (Table 2 and Appendix 1) indicated that there were highly significant differences ($P < 0.001$) between D-D broadcast, D-D row, EDB broadcast and the control treatment means. Application of D-D broadcast, D-D row and EDB broadcast significantly reduced the numbers of Meloidogyne larvae, but there was no significant difference between control and EDB row treatment means.

EFFECTS OF D-D AND EDB APPLICATION ON BEAN YIELD

Data on the yields for each experiment are given in Tables 3 and 4. Analysis of variance carried out on the yields of Kikara and Mexico 142 are also given in Appendices 2 and 3.

In the first experiment, there were significant differences ($P=0.01$) between D-D broadcast, D-D row, EDB broadcast and the control treatment means. Significant yield increases were obtained with Kikara and Mexico 142 with D-D broadcast, D-D row and EDB

TABLE 1

Effects of D-D and EDB application on populations of Meloidogyne larvae from the bean nematicide experiment at Kenya Canners, Thika, 1971.

Treatment	Dosage (l/ha)	Type of application	Means[a] of Meloidogyne larvae per 100 ml soil	
			Pre-fumigation	Post-fumigation
Control	-	-	341	11
D-D	336.8	broadcast	125	2
D-D	224.5	row	165	0
EDB	67.3	broadcast	388	5
EDB	44.9	row	335	22

[a] Mean of 5 replicates of the raw nematode population counts before being transformed to logarithms

TABLE 2

Effects of D-D and EDB application on populations of Meloidogyne larvae from the bean nematicide experiment at Kenya Canners, Thika, 1971.

Treatment	Dosage (l/ha)	Type of application	Means[a] of Meloidogyne larvae (transformed to logarithms) per 100 ml soil	
			Pre-fumigation	Post-fumigation
Control	-	-	2.01	0.63
D-D	336.8	broadcast	1.74	0.15
D-D	224.5	row	1.74	0.05
EDB	67.3	broadcast	2.11	0.24
EDB	44.9	row	2.10	0.72

L.S.D[b] (P = 0.05) between two pre-fumigation treatments = 1.44

L.S.D (P = 0.05) between two post-fumigation treatments = 0.23

[a] Mean of 5 replicates

[b] Least Significant Difference (the smallest difference between two means which can be considered significant at some specified probability (P) level).

TABLE 3

Effects of D-D and EDB application on mean yields of Kikara and Mexico 142 bean seeds from the nematicide experiment at Kenya Canners Plantation, Thika, 1971.

Treatment	Dosage (l/ha)	Type of application	Mean bean seed yields (kg/plot)
Control	-	-	2.090
D-D	336.8	broadcast	2.714
D-D	224.5	row	3.141
EDB	67.3	broadcast	3.051
EDB	44.9	row	2.652

L.S.D (P=0.05) between two treatments = 0.621

TABLE 4

Effects of D-D application on mean yield of Mexico 142 bean seeds from the nematicide experiment at Kuraiha Estate, Thika, 1971-72.

Treatment	Dosage (l/ha)	Type of application	Mean bean seed yield (kg/plot)
Control	-	-	1.84
D-D	224.5	row	2.62

L.S.D. (P=0.05) between two treatments = 0.341

TABLE 5

Effects of D-D and EDB on yield increases of Kikara and Mexico 142 bean seeds, expressed in kilogram per hectare and in percentage, from the nematicide experiment at Kenya Canners Plantation, Thika, 1971.

Treatment	Dosage (l/ha)	Type of application	Yields					
			(kg/ha)		Increase (kg/ha)		Increase (%)	
			Bean variety		Bean variety		Bean variety	
			Kikara	Mexico 142	Kikara	Mexico 142	Kikara	Mexico 142
Control	-	-	1,143	1,107	-	-	-	-
D-D	336.8	broadcast	1,652	1,269	509	162	45	15
D-D	224.5	row	1,612	1,769	469	662	41	60
EDB	67.3	broadcast	1,783	1,502	640	395	56	36
EDB	44.9	row	1,685	1,170	542	63	47	6

TABLE 6

Effects of D-D on yield increases of Kikara and Mexico 142 bean seeds, expressed in kilogram per hectare and in percentage, from the nematicide experiment at Kuraiha Estate, Thika, 1971-72.

Treatment	Dosage (l/ha)	Type of application	Yields					
			(kg/ha)		Increase (kg/ha)		Increase (%)	
			Bean variety		Bean variety		Bean variety	
			Kikara	Mexico 142	Kikara	Mexico 142	Kikara	Mexico 142
Control	-	-	1,596	1,347	-	-	-	-
D-D	224.5	row	1,801	1,919	205	572	13	42

broadcast, but there was no significant difference between EDB row and control treatment means (Table 3 and Appendix 2). Nematicide application did not increase the yield of Marathon green bean.

In the second experiment, there were significant differences (P=0.01) between D-D and control treatment means. D-D in the row did not significantly increase the yield of Kikara, but a significant yield increase was obtained with Mexico 142 (Table 4 and Appendix 3). Yields of Kikara and Mexico 142 in the first experiment and of Mexico 142 in the second experiment, expressed in kg/ha, are given in Tables 5 and 6 respectively.

DISCUSSION

Results of the first experiment indicated that application of D-D as a broadcast and a row treatment, and EDB applied as a broadcast treatment, significantly reduced the numbers of *M. incognita* and *M. javanica* larvae in the dark-red friable clay soil. EDB row treatment did not reduce larval populations. Significant yield increases (45-60%) were obtained with Kikara and Mexico 142 in the first experiment, with three treatments: broadcast and row application of D-D, and broadcast application of EDB. This correlates well with the larval population decreases

in these treated plots. Similar root-knot nematode control and yield increases by these chemicals have been reported on various crops by Lear and Thomason (1956); Good and Steele (1958); Lear and Raski (1958); Nielsen and Sasser (1959); and Colbran (1962). Nematicides did not increase the yield of Marathon green bean; yields were uniformly low, presumably because the soil type was unfavourable for this variety.

In the second experiment in which overhead sprinkler irrigation was used, results indicated that D-D in the planting row did not significantly increase the yield of Kikara, but increased the yield of Mexico 142 significantly (42%). Reasons why D-D applied in the row, on a similar soil type to that of the first experiment, did not significantly increase the yield of Kikara in this experiment are not known. Winstead *et al.* (1958) reported that beans planted in rows treated with D-D were severely stunted and yields were less than those of the non-treated plots. Ellis *et al.* (1949) reported that yields of beans on plots treated with D-D were significantly lower than on those treated with EDB and confirmed previous work by Clayton and Ellis (1949) showing that both date of treatment and inherent response of certain crops are important factors to be considered in soil fumigation.

Field observations at each experiment indicated that vegetative growth of the beans in plots treated with D-D or EDB was more vigorous than in the untreated controls (Fig. 3). Roots of the bean varieties dug from the untreated control plots were found to be heavily infested with the root-knot nematodes, M. incognita and M. javanica, whereas roots from the treated plots were apparently healthy (Fig. 4). These field observations were confirmed by greenhouse pot tests (Figures 5 and 6); stunting and severe root galling were produced by growing plants in the greenhouse in soil heavily contaminated with a mixed population of M. incognita and M. javanica.

These field and greenhouse results indicate that high population of the root-knot nematodes M. incognita and M. javanica can reduce bean yields in Kenya by as much as 60%: this supports claims made by Hollis (1962) that nematodes reduce yields of beans in Kenya smallholdings by 50 to 100%. Although increases in yields of beans were obtained with soil fumigation, field control is not considered economical in Kenya because of the relatively low cost per kilogram of beans and the relatively high cost of the fumigants. Alternatively, it is suggested that control of root-knot nematode on beans in this country, may have to depend on non-chemical control practices, that is, crop

rotation and/or breeding or selecting for tolerant or resistant varieties. Discussion on these control measures will be found in Chapter 7.

CHAPTER 4

PENETRATION OF LARVAE OF *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA* INTO SIX BEAN VARIETIES AS AFFECTED BY TEMPERATURE

INTRODUCTION

Byars (1914) reported that larvae of a species of *Meloidogyne* Goeldi, 1887 (referred to then as *Heterodera radicicola* (Greef, 1872) Müller, 1884) penetrated epidermal cells near the embryonic region of a cowpea (*Vigna* sp.) root tip the first day after their inoculation into nutrient agar. Godfrey and Oliveira (1932) observed that the first larvae of *H. radicicola* (=*Meloidogyne* sp.) penetrated the roots of pineapple (*Ananas sativus* Schult.) some time prior to 6 hours after inoculation. They found that a few nematodes had already penetrated into the interior of the root tips at the 6-hour observation and that penetration took place in the meristematic region. Barrons (1939) demonstrated that, when both plants were given an equal opportunity to become infected, just as many larvae of a species of *Meloidogyne* (reported as *H. marioni* (Cornu, 1879) Goodey, 1932) entered the roots of a highly resistant bean (*Phaseolus*

vulgaris L. var. Alabama No.1) as entered the roots of a susceptible bean (var. Kentucky Wonder). Christie (1946) found that larvae of H. marioni (=Meloidogyne sp.) did not enter the roots of some plants as readily or in as large numbers as they did others. As all this work was done before Chitwood's (1949) reclassification of the root-knot nematode, the actual species used in these investigations are unknown.

More recently, in experiments with the entry of larvae into roots, Christie (1949) found that when alfalfa (Medicago sativa L.) and Lantana sp. were equally exposed to infection, the roots of alfalfa were heavily invaded by larvae of a species of Meloidogyne but only very rarely were the roots of Lantana entered. These results suggest the existence of plants whose roots may not be invaded freely by larvae of all species. Riggs and Winstead (1959) reported that larvae of M. incognita (Kofoid and White, 1919) Chitwood, 1949 penetrated roots of resistant and susceptible tomatoes (Lycopersicum esculentum Mill.) in similar numbers but necrotic areas appeared within 24 hours around the larvae in resistant plants. Minton (1963) showed that equal numbers of two morphologically similar populations of M. arenaria (Neal, 1889) Chitwood, 1949 penetrated

peanut (Arachis hypogaea L.) roots, but differed widely in pathogenicity. Brodie and Cooper (1964) found that larvae of 5 single egg-mass isolates of Meloidogyne, representing 4 species and subspecies, M. arenaria arenaria (Neal, 1889) Chitwood, 1949; M. javanica javanica (Treub, 1885) Chitwood, 1949; M. hapla Chitwood, 1949; and 2 isolates of M. incognita, penetrated roots of upland cotton (Gossypium hirsutum L.) seedlings in equal numbers and significantly reduced growth of cotton seedlings. Bird and Wallace (1965) found that in a preliminary pot experiment four times as many M. javanica entered tomato roots as did M. hapla but their growth rates were not significantly different. Milne et al. (1965) reported that larvae of M. javanica were found to penetrate the roots of tobacco (Nicotiana tabacum L.) and N. repanda L. to the same degree and in the same region. Reynolds et al. (1970) showed that larvae of M. incognita acrita entered both resistant and susceptible alfalfa varieties in approximately the same numbers. Wallace (1966) observed that several larvae of M. javanica entered a tomato root through the same opening.

No references were found in the literature comparing penetration of bean varieties by M. incognita and M. javanica. The present study was

undertaken to compare percentage penetration of 6 selected bean varieties by larvae of M. incognita and M. javanica.

EFFECT OF EXPOSURE TIME ON LARVAL PENETRATION OF M. INCOGNITA AND M. JAVANICA AT A CONSTANT INOCULUM LEVEL

When inocula of 100 larvae of each of the two species of Meloidogyne, which had been kept in water for more than 24 hours, were tested for penetration at exposure periods of 12, 24 and 48 hours, no larvae of either species were found in the roots of any of the bean varieties. This suggested that larvae which had been kept in a water suspension at room temperature for prolonged periods were unable to penetrate roots even during a 48 hour exposure.

Similarly, 100 freshly hatched larvae of the two species did not penetrate roots after a 24 hour exposure.

EFFECT OF INOCULUM LEVEL OF M. INCOGNITA AND M. JAVANICA ON PENETRATION AT A CONSTANT EXPOSURE TIME

When a constant exposure time of 48 hours was

maintained and inocula levels of 100, 150, 200 and 300 freshly-hatched larvae were used, both M. incognita and M. javanica entered the root tips in all bean varieties except Mexico 142 at the inoculum level of 300 larvae only. Although germination of varieties was simultaneous, at inoculation Mexico 142 root systems were longer (Fig.7) than were the systems of any of the other varieties incubated for the same period of time (Fig.8): rate of growth of the roots of Mexico 142 was faster.

PENETRATION PERCENTAGE OF M. INCOGNITA AND M. JAVANICA AT A CONSTANT INOCULUM LEVEL AND A CONSTANT EXPOSURE TIME

Analysis of variance made on the penetration percentage of M. incognita and M. javanica at a constant inoculum level of 100 freshly-hatched larvae of each of the species of Meloidogyne, after 48 hours exposure, revealed no significant differences between the bean varieties or the species of Meloidogyne, whether the experiment was conducted at room or greenhouse temperatures. When the experiment was studied under growth chamber conditions, data on the penetration percentage (Tables 7 and 8; Appendices 4 and 5) indicated that while there were no significant differences between varieties, there were highly

TABLE 7

Percentage penetration means of Meloidogyne incognita and M. javanica laryae in roots of Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon and Premier bean varieties. (M. incognita inoculum consisted of larvae derived from egg masses; M. javanica inoculum consisted of larvae derived from soil).

Bean variety	Percentage penetration[a] of species of Meloidogyne[b]	
	M. incognita	M. javanica
Kikara	46.4	11.2
Mexico 142	43.4	9.6
Canadian Wonder	36.2	4.2
Masterpiece	43.6	7.6
Marathon	43.2	2.8
Premier	41.6	3.6

L.S.D. ($P = 0.001$) between two species means = 14.16

[a] Mean of 5 replicates

[b] Inoculum = 100 larvae of each of the two species per root system

TABLE 8

Percentage penetration means of *Meloidogyne incognita* and *M. javanica* larvae in roots of Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon and Premier bean varieties. (Inoculum of both species was derived from egg masses).

Bean variety	Percentage penetration[a] of species of *Meloidogyne*[b]	
	M. incognita	*M. javanica*
Kikara	42.6	21.6
Mexico 142	28.4	28.4
Canadian Wonder	41.8	14.2
Masterpiece	50.0	22.8
Marathon	54.2	31.4
Premier	56.0	28.0

L.S.D. ($P = 0.001$) between two species means = 14.94

[a] Mean of 5 replicates

[b] Inoculum = 100 larvae of each of the two species per root system

significant differences ($P < 0.001$) between species. At growth chamber ambient temperatures of 23° and 29°C for night and day respectively in Experiment 8, in which larvae of the inoculum were of varying age, a striking difference was noted between *M. incognita* and *M. javanica*: penetration percentage of *M. incognita* was significantly higher in all 6 bean varieties (Table 7). When ambient temperatures of the growth chamber were raised to 25°and 30°C for night and day in Experiment 9, in which larvae were of the same age, differences between species were again highly significant. In addition, differences in penetration of different bean varieties were observed: *M. incognita* did not differ significantly from *M. javanica* in penetrating root systems of Mexico 142 (Table 8). *M. incognita*, however, penetrated roots of Kikara, Canadian Wonder, Masterpiece, Marathon and Premier in significantly greater numbers than *M. javanica*.

DISCUSSION

In the preliminary experiments, *M. incognita* and *M. javanica* larvae, at inoculum levels of 100 larvae, failed to penetrate root systems of any of 6 bean varieties after exposure periods of 12, 24 or 48 hours. Failure of the larvae to penetrate may have been due to the weak and inactive condition of the

larvae used for inoculation: these had been kept in a water suspension at room temperature for more than 24 hours. Dropkin (1957) reported that larval infectivity of H. rostochiensis Woll. declined rapidly when larvae were stored in tap water at room temperature for extended periods. Similar observations were reported by Thomason et al. (1964) who found that infectivity of M. javanica was reduced more rapidly than motility after 4 days storage in tap water at 3° or 27°C. They suggested that larvae of M. javanica were injured by low temperature while at high temperatures the decline in infectivity was due to high respiratory rate and depletion of energy needed for penetration of the host root. Van Gundy et al. (1967) reported that another nematode, Tylenchulus semipenetrans Cobb, 1913 was motile and infective in vitro for a longer time than M. javanica.

Reasons for M. incognita and M. javanica larvae not penetrating the bean root systems after an exposure period of 24 hours are not known. Godfrey and Oliveira (1932) reported that at an inoculum level of approximately 500 larvae of H. radicicola (=Meloidogyne sp.), penetration in pineapple roots occurred some time prior to 6 hours after inoculation. Siddiqui and Taylor (1970) found that at an inoculum level of approximately 500 larvae, large numbers of

larvae of M. naasi Franklin, 1965 entered oat roots within 24 hours after inoculation at a growth chamber temperature of 26°C. In the experiment reported here, it seems possible that numbers were below the numerical threshold for penetration: if the inoculum levels had been increased, penetration within 24 hours might have occurred.

When the exposure period was increased to 48 hours, penetration of 5 of the 6 bean varieties by freshly-hatched, active larvae of M. incognita and M. javanica occurred at inoculum levels of 100, 150, 200 and 300. In Mexico 142, even at the inoculum level of 300 larvae, there was no penetration. Mexico 142 root systems were of greater length at the time of inoculation even though all varieties had been incubated for the same period of time. It was assumed that because of this, larvae did not locate the root tips, the focal point of entry by most nematode larvae (Byars, 1914; Godfrey and Oliveira, 1932; Christie, 1936; Widdowson et al., 1958; Peacock, 1959).

Although larvae of M. incognita and M. javanica penetrated root systems in all 6 bean varieties, results show that no significant differences in penetration percentage existed between the varieties and the species whether the experiment was conducted under room or greenhouse temperature conditions.

Failure to achieve significant differences between the species was probably due to low and variable room and greenhouse temperatures. Results of a similar experiment conducted under controlled growth chamber conditions at higher temperatures indicated that highly significant differences at 0.1% level occurred between the two species. At temperatures of 23° and 29°C for night and day respectively, M. incognita larvae penetrated root systems in all the 6 bean varieties in significantly greater numbers than M. javanica larvae. When the temperatures were raised to 25° and 30°C for night and day, significantly higher numbers of M. incognita larvae penetrated root systems of Kikara, Canadian Wonder, Masterpiece, Marathon and Premier than M. javanica larvae. There were no significant differences between M. incognita and M. javanica larvae in penetration of root systems of Mexico 142. It is probable that in this experiment again rapid root growth of Mexico 142 in some way precluded penetration, probably a result of failure of the larvae to locate the root tips.

It appears from these results that penetration is influenced by age of larvae and temperature: high percentage of both M. incognita and M. javanica were recovered when young freshly-hatched larvae were used, and the temperature was raised to 25° and 30°C for night and day respectively. Bird and Wallace (1965)

reported that at 15-20°C, M. hapla invaded tomato roots in significantly greater numbers than M. javanica, but numbers of M. hapla invading at 20-25°C were not significantly different. Similarly, at 25-30°C, M. javanica had a significantly high invasion rate than M. hapla, but there were no significant differences detected between the numbers of M. javanica invading tomato roots at 15-20, 25-30 and 30-35°C temperature ranges. Hu (1966) reported that optimum temperature range for M. incognita and M. javanica for penetration of sugar cane was 20-30°C, and Wallace (1966) reported a similar temperature range for M. javanica for penetration of tomato roots.

Although larvae of both species were recovered from all parts of the root systems of each of the bean varities, the largest concentrations were in areas behind the root tips. This agrees with observation made by O'Brien and Prentice (1931); Godfrey and Oliveira (1932); Linford (1939); Wieser (1955); Widdowson et al. (1958); Peacock (1959); Bird (1962b); Lavallee and Rohde (1962). The area of root elongation behind the apical meristem is possibly more easily penetrated than other portions of the root system. Regions of a root within the same root-system are known to be differentially attractive or repellent to nematodes. Wieser (1955) working on the effect of tomato seedlings and excised roots on

M. hapla found that the region of cell elongation of the tomato root exuded an attractive substance to the larvae of M. hapla, while the meristematic region and the root cap gave off a repellent substance. The distribution of the larvae in the roots indicated that some roots had a greater chance of being invaded than others. In some cases as many as 50 larvae were recovered from a single root whereas in other roots of the same bean variety none were observed. Mass action appears to be a factor in heavy penetration of root tips; large numbers of larvae often enter the root at or near the same point.

CHAPTER 5

COMPARATIVE DEVELOPMENT OF MELOIDOGYNE INCOGNITA AND M. JAVANICA ON SIX BEAN VARIETIES

INTRODUCTION

The root-knot nematode is an obligate parasite. Though it can remain alive for long periods in the soil in either the larval or the egg stages, it cannot complete its life cycle outside the roots of the host plant. Once inside a plant, the nematode may feed on existing cells or it may elicit changes in host tissues that are essential for the parasite's growth.

Many plants are regarded as resistant to root-knot nematode, not because larvae fail to enter but because they fail to develop after entering. Sasser and Taylor (1952) showed that, in addition to normal entry and development of the root-knot larvae, there are also other reactions depending on plant species involved. Larvae may either fail to enter the roots, enter in reduced numbers with little or no development, or enter in large numbers with varying degrees of development ranging from none at all to a few of the individuals reaching maturity. Development is generally slower in resistant varieties than in

susceptible and few, if any, females reach maturity (Rohde, 1965). In general, factors for resistance operate after entry and are most often indicated by failure of the host plant to respond to nematode secretions in a manner favourable to nematode development. Fassuliotis (1970) found that although as many larvae of Meloidogyne incognita acrita Chitwood, 1949 penetrated the roots of resistant Cucumis ficifolius Bouche, and C. metuliferus Mey as those of the susceptible melon (C. melo L.), few developed to adult females. Resistance in C. ficifolius and C. matuliferus was associated with hindrance of larval development beyond the second stage, delayed development of larvae to adults and stimulation toward maleness. A high proportion of males is often a characteristic of root-knot nematode populations of resistant plant varieties. This has been interpreted as an indication of stress since other adverse environmental factors induce larvae to develop as males instead of females (Triantaphyllou, 1960). Siddiqui and Taylor (1970) demonstrated considerable variation in susceptibility among oat (Avena sativa L.) varieties screened against the Illinois isolate of the barley root-knot nematode, M. naasi Franklin, 1965. All varieties rated as susceptible contained typical females and egg masses in galled tissue. Differences in the developmental rates of nematode isolates have been shown by Michell and Taylor (1972)

with five geographical isolates of M. naasi, on barley (Hordeum vulgare L.); oat; sorghum (Sorghum bicolor Moench); and quack grass (Agropyron repens Beauv.). Sorghum was the most suitable host for the Kansas isolate but did not support development beyond the infective larval stage in the other isolates.

Development of the root-knot nematode is influenced by environmental factors other than the host, of which temperature seems to be the most important. Demonstrating the effect of this factor, Tyler (1933b) found that in tomato (Lycopersicum esculentum Mill.) grown in soil at 27°C, 16 to 19 days elapsed from the time larvae entered the roots until females began to lay eggs. When plants were cultured at 24°C, this interval was 25 to 27 days, and at 15.5°C it was 67 days. Davide and Triantaphyllou (1967) found that the majority of M. incognita (Kofoid and White, 1919) Chitwood, 1949 larvae reached the adult stage on tomato within 10 and 50 days after inoculation at, respectively, 35° and 15°C. Males of M. incognita consituted less than 1% of the nematodes developed at 20°, 30° or 35°C and about 6.7% at 15°C.

These differences in parasite development resulted from interactions between the same race of nematode and different host plants, and from interactions between

different races of the parasite and the same host plant. The existence of races with host range differences within the root-knot nematodes could create many problems relating to crop rotation recommendations and the screening of parental lines for resistance to the nematodes.

Although in the previous studies described in Chapter 4 on the reaction of the 6 bean varieties to invasion by M. incognita and M. javanica (Treub, 1885) Chitwood, 1949 large numbers of larvae were recovered in the roots 48 hours after inoculation, it was not known whether the varieties were suitable or unsuitable hosts to the root-knot nematodes. It is well known that beans (Phaseolus vulgaris L. var. Pinto) are suitable hosts of M. hapla Chitwood, 1949 (Wieser, 1956). To determine the effects of the hosts on the parasite, studies on comparative rate of development of M. incognita and M. javanica in roots of the 6 bean varieties were conducted at different time intervals after inoculation, under conditions of controlled environment.

TEN DAYS AFTER INOCULATION

Samples taken 10 days after inoculation showed that sexual differentiation, which normally takes place

during the second larval stage, had occurred in 92% of M. incognita and 100% of M. javanica in Masterpiece; 98% of M. javanica in Marathon; and 100% each of M. incognita and M. javanica in Mexico 142 (Table 9). In most of the bean varieties the predominant developmental form of both species was the third-stage female larva; no adult females or males of either species were observed in any of the varieties 10 days after inoculation.

TWENTY DAYS AFTER INOCULATION

At 20 days after inoculation, sexual differentiation occurred in 100% of each of M. incognita and M. javanica in all bean varieties (Table 10). The majority of the nematodes observed were adult females with egg masses extruded outside the root; percentages of oviparous females ranged from 23 to 96. A low proportion of males, 1% or less, was observed in Mexico 142, Masterpiece, Marathon and Premier.

THIRTY DAYS AFTER INOCULATION

In the 30-day samples the predominant nematodes observed were adults, 55% or more being egg-laying females in all bean varieties (Table 11). Numbers of differentiated M. incognita and M. javanica males in the varieties Mexico 142, Masterpiece,

TABLE 9

Development of *Meloidogyne incognita* and *M. javanica* on six bean varieties 10 days after inoculation

Host	*Meloidogyne* spp.	Total no. of nematodes examined in three heavily galled roots	Sexually undifferentiated 2nd-stage larvae, %	Sexually differentiated nematodes, %			
				Female larvae			Total
				2nd	3rd	4th	
Kikara	*M. incognita*	54	72	4	20	4	28
	M. javanica	57	35	2	61	2	65
Mexico 142	*M. incognita*	11	0	0	73	27	100
	M. javanica	63	0	2	81	17	100
Canadian Wonder	*M. incognita*	118	47	2	50	1	53
	M. javanica	127	27	3	68	2	73
Master-piece	*M. incognita*	156	8	4	83	5	92
	M. javanica	96	0	2	69	29	100
Marathon	*M. incognita*	85	54	0	40	6	46
	M. javanica	92	2	0	92	6	98
Premier	*M. incognita*	172	33	2	56	9	67
	M. javanica	358	56	2	39	3	44

TABLE 10

Development of Meloidogyne incognita and M. javanica on six bean varieties 20 days after inoculation

Host	Meloidogyne spp.	Total no. of nematodes examined in three heavily galled roots	Sexually undifferentiated 2nd-stage larvae, %	Sexually differentiated nematodes, %									
				Females					Males				Total
				Larvae			Adults		Larvae			Adults	
				2nd	3rd	4th	w/o eggs	with eggs	2nd	3rd	4th		
Kikara	M. incognita	52	0	0	0	25	52	23	0	0	0	0	100
	M. javanica	45	0	0	0	0	4	96	0	0	0	0	100
Mexico 142	M. incognita	77	0	0	3	14	9	73	0	0	0	1	100
	M. javanica	18	0	0	0	11	22	67	0	0	0	0	100
Canadian Wonder	M. incognita	161	0	0	0	21	29	50	0	0	0	0	100
	M. javanica	138	0	0	0	9	38	53	0	0	0	0	100
Master-piece	M. incognita	165	0	0	4	13	29	53	0	0	0	1	100
	M. javanica	157	0	1	2	26	16	53	1	0	0	1	100
Marathon	M. incognita	121	0	0	1	17	28	52	0	0	0	2	100
	M. javanica	49	0	0	0	10	31	59	0	0	0	0	100
Premier	M. incognita	55	0	0	0	5	33	62	0	0	0	0	100
	M. javanica	118	0	0	0	8	17	74	0	0	0	1	100

TABLE 11

Development of Meloidogyne incognita and M. javanica on six bean varieties 30 days after inoculation

Host	Meloidogyne spp.	Total no. of nematodes examined in three heavily galled roots	Sexually undifferentiated 2nd-stage larvae, %	Sexually differentiated nematodes, %									
				Females					Males				Total
				Larvae			Adults		Larvae			Adults	
				2nd	3rd	4th	w/o eggs	with eggs	2nd	3rd	4th		
Kikara	M. incognita	148	0	0	0	4	15	81	0	0	0	0	100
	M. javanica	39	0	0	0	0	3	97	0	0	0	0	100
Mexico 142	M. incognita	206	0	0	1	12	16	70	0	0	0	1	100
	M. javanica	156	0	0	2	19	13	65	0	0	0	1	100
Canadian Wonder	M. incognita	173	0	0	0	4	32	64	0	0	0	0	100
	M. javanica	33	0	0	0	3	9	88	0	0	0	0	100
Master-piece	M. incognita	262	0	1	2	24	16	55	0	0	1	1	100
	M. javanica	187	0	0	1	9	26	62	0	2	0	0	100
Marathon	M. incognita	180	0	0	0	11	23	64	0	0	1	1	100
	M. javanica	97	0	0	0	15	19	65	0	0	1	0	100
Premier	M. incognita	229	0	0	1	7	20	72	0	0	0	0	100
	M. javanica	167	0	0	2	12	23	62	0	0	1	0	100

TABLE 12

Development of *Meloidogyne incognita* and *M. javanica* on six bean varieties 40 days after inoculation

Host	*Meloidogyne* spp.	Total no. of nematodes examined in three heavily galled roots	Sexually undifferentiated 2nd-stage larvae, %	Sexually differentiated nematodes, %									
				Females					Males				Total
				Larvae			Adults		Larvae			Adults	
				2nd	3rd	4th	w/o eggs	with eggs	2nd	3rd	4th		
Kikara	*M. incognita*	315	0	0	0	7	15	78	0	0	0	0	100
	M. javanica	177	0	0	0	14	10	76	0	0	0	0	100
Mexico 142	*M. incognita*	78	0	0	0	5	8	87	0	0	0	0	100
	M. javanica	164	0	0	0	5	21	74	0	0	0	0	100
Canadian Wonder	*M. incognita*	60	0	0	0	0	7	93	0	0	0	0	100
	M. javanica	90	0	0	0	0	12	88	0	0	0	0	100
Master-piece	*M. incognita*	75	0	0	0	0	0	100	0	0	0	0	100
	M. javanica	105	0	0	0	3	15	82	0	0	0	0	100
Marathon	*M. incognita*	68	0	0	0	0	25	74	0	0	0	1	100
	M. javanica	87	0	0	0	11	22	67	0	0	0	0	100
Premier	*M. incognita*	135	0	0	0	14	28	57	0	0	1	0	100
	M. javanica	229	0	0	0	2	26	72	0	0	0	0	100

Marathon and Premier were again low, with little or no increase in frequency. At this sampling date, roots of all varieties contained second generation, sexually undifferentiated second-stage larvae of both species.

FORTY DAYS AFTER INOCULATION

Results of observations made after 40 days were similar to those at 30 days, except that no sexually differentiated second- and third-stage larvae of the initial population were present in any of the bean varieties. The percentage of egg-laying females had increased, and now ranged from 57 to 100 (Table 12). Only 1% or less of M. incognita males was observed in Marathon and Premier. Roots of all varieties contained a second generation of sexually differentiated second- and third-stage larvae of both species.

DISCUSSION AND CONCLUSION

Rates of development from larvae to sexually differentiated nematodes occurred more rapidly in Mexico 142 for M. incognita and M. javanica, and in Masterpiece for M. javanica only, than in other bean varieties inoculated at the same time. However, the period taken for larvae of both nematode species to develop to the adult stage was identical in all 6 bean

varieties. At growth chamber ambient temperatures of 25° and 30°C night and day respectively, a high percentage of both species developed into females which laid eggs within 20 days after inoculation; these females completed their life cycle and a second generation was developing within 30 days in all varieties. A low percentage of the nematode species developed to males. Michell and Taylor (1972) reported a similar length of life cycle and a low percentage of males for a Kansas isolate of M. naasi on sorghum. They concluded that, based on the amount of galling and rate of nematode development, sorghum appeared to be an excellent host for the Kansas isolate. In contrast, Christie (1949) observed that in a highly suitable host for a given species of Meloidogyne, growing at the temperature optimum for rapid development of the parasites, the first females to mature began to lay eggs in from 25 to 30 days after they entered the roots as larvae. Suitability of host plant is thus suggested where a high percentage of a nematode population developes to females compared with a low percentage developing to males. After making numerous cultures of Meloidogyne sp. on tomato roots grown asceptically on agar, Tyler (1933a) found more males in old or heavily parasitized roots than in lightly infested ones, which she attributed to inadequate nutrition when the parasites were crowded. Linford (1941) came to a similar conclusion after

observing the development of root-knot nematodes at the edges of cowpea leaves where the small veins provided poor nutrition for the parasites. Observing M. incognita in numerous experiments, Triantaphyllou (1960) found that many more larvae became males in crowded galls formed in root tips than in less crowded parts. He concluded that, when conditions are favourable, most second-stage larvae in a root develop into females; when conditions are unfavourable, most develop into males.

The formation of egg masses outside the roots of beans in both species, the position in which eggs often begin to hatch immediately, thus releasing larvae directly into the surrounding soil, is a factor of considerable significance in the rapid build-up of nematode populations in the field. Similar observations have also been made in root-knot nematodes on cowpea by Godfrey and Oliveira (1932) and Peacock (1957); the latter author suggested that this may be important in root-knot control since external egg masses are likely to be much less resistant than egg masses protected by a gall tissue.

It is concluded from the present studies that M. incognita and M. javanica can develop and reproduce rapidly on Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon and Premier bean varieties;

hence, the varieties are highly suitable hosts of both nematode species. Under optimum temperature conditions for rapid development of both species, at least 3 generations are possible on these bean varieties in any of the growing seasons in East Africa.

C H A P T E R 6

COMPARATIVE HISTOPATHOLOGY OF SIX BEAN VARIETIES INFECTED WITH MELOIDOGYNE INCOGNITA AND M. JAVANICA

INTRODUCTION

In the previous studies (Chapter 5), it was shown that all 6 bean varieties supported development beyond the infective larval stage, of M. incognita (Kofoid and White, 1919) Chitwood, 1949, and M. javanica (Treub, 1885) Chitwood, 1949. Invasion of a susceptible root by larvae of Meloidogyne spp. Goeldi, 1887, usually causes major histological changes in plant cells in the vacinity of the nematode. These histological changes in root tissues of susceptible plants brought about by the presence of developing root-knot nematodes have been described by several investigators. In general, histological changes involve hypertrophy of the cortex, phloem and xylem parenchyma; hyperplasia of the pericycle and xylem parenchyma; formation of giant cells with breakdown of cell walls; and the formation of galls (Christie, 1936; Crittenden, 1958; Krusberg and Nielsen, 1958; Bird, 1962a; Hodges and Taylor, 1966; Heald, 1969; Baldwin and Barker, 1970; Siddiqui and Taylor, 1970). Dropkin and Nelson (1960) studied the

histological responses of 19 soybean (Glycine max Merr.) varieties infected with M. incognita incognita (Kofoid and White, 1919) Chitwood, 1949, and M. incognita acrita Chitwood, 1949. Giant cell development in roots of susceptible varieties began with intense cellular multiplication, followed by hypertrophy of cells immediately surrounding the head of the infective larva. Dissolution of the walls of hypertrophied cells resulted in the development of several large multinucleate cells, termed giant cells; nuclear division within giant cells was rare. These giant cells are believed to arise in response to substances emanating from the parasite's esophageal glands and to provide nutriment for the parasite. Bird (1962a) reported that giant cell development and maintenance in roots of dwarf bean (Phaseolus vulgaris L. var. Brown Beauty) infected with M. javanica, appear to depend on a continuous stimulus from the nematode; physical removal of the nematode resulted in breakdown of the giant cells. Although the histological changes in root tissues caused by root-knot nematodes have been described on many hosts, such as tomato (Lycopersicum esculentum Mill.), soybean, sweetpotato (Ipomoea batatas Lam.), maize (Zea mays L.), creeping bentgrass (Agrostis palustris Huds.), and wheat (Triticum aestivum L.), there are no reports on the histopathology of beans in addition to Bird's work.

The purpose of this study was to observe and compare, the histopathology of 6 bean varieties infected with M. incognita and M. javanica, at different time intervals after inoculation.

NON-INFECTED BEAN ROOT

Figures 9 and 10 show, respectively, a longitudinal section of a non-infected root of a Canadian Wonder bean plant 20 days after germination, and a transverse section of a non-infected root of a Mexico 142 bean plant 30 days after germination. Typical dicotyledonous root anatomy, including epidermis, cortex, and stele, is clearly seen.

POSITION OF THE NEMATODE IN RELATION TO ROOT TISSUES

Root sections of all 6 bean varieties observed 10, 20, 30 and 40 days after inoculation showed that after penetration, larvae of M. incognita and M. javanica migrated both intercellularly through the cortex, and intracellularly to the stele of the root (Fig. 11). Only the head of the larva was inserted into the vascular system, the remaining sausage-shaped portion of the nematode was located in the cortical cells adjacent to the stele of the root (Fig. 12, 13, 14).

EPIDERMAL DAMAGE

At 10 days after inoculation, the larvae caused slight injury in penetrating the epidermis. However, observations made 20, 30 and 40 days after inoculation showed that the passageway formed by the larvae had increased in size; the epidermal cells were collapsed forming a cavity (Fig. 13, 14, 15).

CORTICAL DAMAGE

The 10-day samples showed that the larvae caused slight injury in penetrating the cortex and endodermis, but induced marked cortical changes after permanently establishing their feeding positions in the vascular system. Hypertrophy of the cortical parenchyma cells was observed in the area immediately surrounding the larva (Fig. 12). It would appear, in such cases, that the presence of the parasite suppressed mitotic activity in the apical meristem and hence retarded or terminated growth. Much of the swelling and galling of the infected roots, 20, 30 and 40 days after inoculation, appeared to be caused by the increase in size of the mature female nematodes and egg masses within the cortex; the body of the nematodes and the egg masses caused considerable disruption of the cortical cells (Fig. 13, 14, 15).

STELAR DAMAGE

In root sections taken 10 days after inoculation, it was observed that the infective larva made its way through the cortex, endodermis and pericycle to the vicinity of the xylem cells (Fig. 12). Soon after the larva permanently established its feeding position, it induced marked stelar changes. Cells of the pericycle, lying near the path of the larva, showed slight hypertrophy. Rapid proliferation of pericycle cells in the infected root caused swellings or galls. A conspicuous feature of these swollen tissues was the frequency with which lateral roots developed in the region of these galls (Fig. 16, 17). In this instance, the presence of the nematode seemed to stimulate mitotic activity in the pericycle; once cell division was initiated it proceeded in a more or less normal manner and lateral roots were formed. Hyperplasia of the phloem and xylem parenchyma cells was observed in the area immediately surrounding the larva (Fig. 12). Enlargement of a few phloem and xylem cells was observed about the larval head indicating the initiation of giant cell formation; the hypertrophied cells were in direct contact with the nematode's head and probably represented cells into which secretions had been injected (Fig. 12). Root sections taken 20, 30 and 40 days after inoculation, showed that affected phloem and xylem cells appeared

to be coalesced, with the stele more or less completely transformed into giant cells (Fig. 13, 18). Frequently, 2-3 nematodes were observed feeding in the vascular tissues in close proximity, which resulted in the formation of extensive giant cells (Fig. 19, 20).

A type of tissue designated "abnormal xylem", which is characterized by secondary wall thickenings of annular, reticulate, or pitted types, was observed associated with giant cells at 10, 20, 30 and 40 days after inoculation (Fig. 21, 22, 23). The cells of this tissue seemed to form directly from xylem parenchyma and assumed the shape of parenchyma cells but with no apparent intercellular spaces (Fig. 23). This differentiation response around giant cells occurred in the vascular tissue and was most abundant around giant cells located in xylem parenchyma (Fig. 22, 23). Abnormal xylem cells were not absorbed by giant cells and restricted their expansion.

GIANT CELLS

Ten days after inoculation, giant cells, 3-10 in number, were present around the head of the larva in the phloem and xylem parenchyma cells in all 6 bean varieties (Fig. 12). However, giant cells were occasionally observed in the cortex in Mexico 142, Canadian Wonder, and Premier roots (Fig. 24, 25, 26).

Giant cells formed in the cortex were usually smaller than those in cambial or vascular tissues. The cytoplasm of the giant cells was more granular and dense than that of the surrounding cells. Giant cells measured 22-175 x 29-184 μ, whereas the normal cells in that area were 12-16 x 16-23 μ in cross section. A range of 1-19 nuclei measuring 7-23 μ in diameter were present in each giant cell. Each nucleus contained 1-2 nucleoli measuring 2-7 μ in diameter. After 20 days, secondary thickening of giant cell walls, irregular in pattern, was frequently observed; the cytoplasm of the giant cells became highly granular and dense (Fig. 27). Giant cells around the head of the nematodes increased in number from 3-10 to 6-20, as well as in size, and now measured 55-189 x 97-262 μ in cross section. Their enlargement appeared to have resulted from the dissolution of adjacent parenchyma cell walls, and incorporation of the protoplasm into the giant cell. The giant cells became highly multinucleate, the nuclei were hypertrophied and although scattered throughout the cytoplasm, tended to be aggregated somewhat toward the centre of the giant cells (Fig. 28). The nuclei, 1-28 in number in each giant cell, measured 14-28 μ in diameter; each of their deep-staining nucleoli were 5-9 μ in diameter. After 30 days, no increase in the number of giant cells or the size of nuclei and nucleoli was observed, but there was increase in size

of the giant cells that now measured 83-271 x 299-784 μ in cross section. The content of the giant cells deteriorated; the cytoplasm became highly vacuolate, and many giant cell nuclei degenerated and their contents were diffused into the cytoplasm (Fig. 29). Many giant cells were partially or completely degenerated after 40 days; the giant cell cytoplasm, the nuclei and nucleoli disappeared (Fig. 30). Nuclear division was not observed in giant cells in any sections at the various time intervals after inoculation.

DISCUSSION

The histological changes in the roots of Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon, and Premier bean varieties infected with M. incognita and M. javanica were similar. In these comparative histopathological studies, it has been shown that the ability of the 6 bean hosts to support growth of the root-knot nematodes depends on the host-cell response to the presence of the parasite. The studies have greatly emphasized the sequence of histological changes that occur in roots of highly susceptible hosts following infection by these nematodes. Results of the histopathological studies revealed extensive damage to the cortical and vascular tissue of roots. These studies showed that both nematode species fed in the vascular area and caused the

formation of 3-20 multinucleate giant cells, around a single female, surrounded by a thickened secondary wall. While most of the giant cells were found in the vascular cylinder in all 6 bean varieties, giant cells were also observed in the cortex or between the pericycle and endodermis in Mexico 142, Canadian Wonder and Premier bean roots; similar observations were reported by Christie (1936) on tomato.

The general sequence of giant cell formation under conditions most favourable for root-knot nematode development, was similar to the sequence outlined for other susceptible hosts such as tomato (Christie, 1936); sweet potato (Krusberg and Nielsen, 1958); and soybean (Dropkin and Nelson, 1960). During the first 10 to 20 days after inoculation, the giant cells continued to invade adjacent tissues, coalescing with cell contents after the dissolution of cell walls. This did not take place equally in all directions; tissues along the central cylinder were evidently more susceptible than the lateral parenchyma, as was also observed in tomato by Christie (1936). This author reported that at the beginning of giant cell formation, it is usually several adjacent members of a row of undifferentiated cells in the central cylinder that first coalesce through the dissolution of the separating cross walls. This same tendency persisted throughout the early stages of development with the

result that giant cells tend to extend longitudinally along the central cylinder rather than laterally into the parenchyma. As the giant cells became older this invasion of tissues gradually lessened until, after about 40 days, it appeared to have largely stopped, and the giant cell cytoplasm, nuclei and nucleoli disappeared. It appears that the disappearance of the giant cell cytoplasm was related to the natural ageing and death of the female nematodes. Bird (1962a) reported, based on observations made on tomato and bean infected with M. javanica, that giant cell development and maintenance depends on a continuous stimulus from the nematode and that once this stimulus is removed the cytoplasm of the giant cell becomes vacuolated, breaks down and is eventually encroached upon by the surrounding cells of the host. Christie (1936) working on tomato similarly observed that gall and giant cell development in the root are brought about through the stimulating action of some substances secreted through the mouth of the parasite. The disruption of the stele by giant cells, proliferated parenchyma cells, and the parasites themselves caused the conducting tissues to be scattered, so that, in cross section, the phloem and xylem occurred in irregular patches rather than in one continuous column.

It appears that abnormal xylem was associated

with giant cells; these cells and/or the nematodes seemed to influence abnormal xylem formation, development of abnormal xylem was massive around giant cells, and none was present in giant cell free tissues. This suggested that the formation of abnormal xylem was apparently stimulated by nematode feeding, as it was not formed around other portions of a nematode body or egg mass. Similar observations have also been made by Krusberg and Nielsen (1958) on sweet potato infected with M. incognita acrita. They suggested that these cells are also formed as a response to injured xylem parenchyma rather than being peculiar to nematode infections, as was also reported by Siddiqui and Taylor (1970) on wheat roots infected with M. naasi. The latter authors noted that the cells serve to contain the giant cell development in a susceptible host.

Nuclear divisions in giant cells have been observed in various root-knot nematode-infested plants (Smith and Mai, 1965; Littrell, 1966; Siddiqui and Taylor, 1970). However, no nuclear division was observed in giant cells in this study in any roots of the bean varieties. It is suggested that since the giant cells and the cell walls were diffused and the cytoplasm appeared to be continuous, at least until the 20th day, it is possible that the initial multinucleate condition in the young giant cells

resulted from the incorporation of nuclei of neighbouring cells, as was suggested by several previous investigators (Christie, 1936; Dropkin and Nelson, 1960). Occurrence of mitotic division was observed in root cells of okra infected with M. incognita acrita 72 hours following inoculation and continued to be frequently observed until after 144 hours (Littrell, 1966), in root cells of onion infected with M. hapla 4 days after inoculation until after 14 days (Smith and Mai, 1965) and, in root cells of wheat infected with M. naasi 9 days after inoculation until after 12 days (Siddiqui and Taylor, 1970). However, results of the ultrastructural studies reported by Huang and Maggenti (1969), working on the host-parasite relationships between broad bean and M. javanica, indicated that the multinucleate condition was derived from repeated mitoses of the original diploid cells without subsequent cytokinesis. It is probable, therefore, that in this study nuclear division in giant cells was either not occurring at the time the tissues were killed and fixed, or did not exist in the root cells of the bean varieties. Further investigation is required to establish the precise nature of the mitotic division, if any, in beans.

CHAPTER 7

GENERAL DISCUSSION

The research reported in this thesis was designed to study critically three important aspects of nematode infestation of beans (Phaseolus vulgaris L.) in Kenya. These were: the course of infestation and the subsequent development of the parasite within the host; the reaction of the host to infection; and crop loss likely to be encountered in Highland areas. These objectives have been achieved in the laboratory, greenhouse, and field; the results represent the first detailed studies of the parasitism of root-knot nematodes to be carried out in East Africa.

Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon and Premier bean varieties were found to be highly susceptible to Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949, and M. javanica (Treub, 1885) Chitwood, 1949. The nematode larvae were able to penetrate the bean roots, develop and reproduce rapidly. The reaction of the hosts to the parasites was not a hypersensitive one as is sometimes true of plants resistant to species of Meloidogyne Goeldi, 1887 (see later discussion on nematode control);

instead, the reaction of the hosts was to develop giant cells.

It was also found that under conditions of heavy root-knot nematode infestation in Kenya, yields were depressed by 60%.

In order to appraise these results in relation to those found elsewhere, life history, penetration and feeding habits, symptoms, host reaction, disease complex, and control of the species of root-knot nematodes, will now be reviewed in general, with illustrations of some particular host-parasite reactions.

LIFE HISTORY OF THE SPECIES OF ROOT-KNOT NEMATODE, MELOIDOGYNE.

Although different species of root-knot nematodes differ from one another in their host-parasite relationships, and in various physiological and morphological characteristics, all have substantially the same life history.

In a favourable host several hundred eggs are produced by each female. The maximum counted by Tyler (1938) was 2,882. She also estimated that

between 34 and 78 eggs could be laid in a day, the number depending on the host plant. The figures are averages, the rate of egg production varying greatly at different times in the 6 weeks during which she made her observations. It is not known which species Tyler had under observation.

Mature females in roots lay eggs into a gelatinous egg sac, the secretion of 6 large rectal gland cells. If the female is deeply embedded in the plant tissue, the egg sac may also be embedded and the gelatinous matrix remains soft, but if the egg sac is exposed on the root surface, the outer matrix layers may dry and become tough, and sometimes coloured orange-brown. In either case the eggs are partially protected against drying and can survive in soil for a period that depends on temperature, moisture, and on the species of the root-knot nematode. Bergeson (1959) found that eggs and larvae of M. incognita acrita Chitwood, 1949 survived for over a year at 10°C but that at 10-16°C the survival time was reduced by half, while at 21°C some nematodes survived for 4 months. At 0-4°C, larvae died in 1-2 weeks. Daulton and Nusbaum (1961) reported that at a soil temperature of -2°C, eggs of M. hapla Chitwood, 1949 can survive for a longer period than

those of M. javanica. The authors stated that although a longer period is required to kill eggs of these nematodes in dry soil than in damp soil, however, at 36° and 40°C, eggs of M. hapla are killed more rapidly in dry soil than eggs of M. javanica; eggs of the former are less tolerant to these high temperatures than eggs of the latter nematode.

Larvae hatch from the eggs in water. In dry conditions and in solutions of high osmotic pressure, hatching is inhibited though embryos continue to develop. Dropkin and Martin (1957) found that solutions of various salts and of dextrose at a molar concentration equivalent to a potential osmotic pressure of 15 atmospheres prevented the emergence of larvae from eggs of M. arenaria (Neal, 1889) Chitwood, 1949, and M. javanica. Development of eggs of M. arenaria proceeded normally in 0.3M NaCl, but the larvae remained motionless until the eggs were transferred to water. Eggs of M. javanica hatched after inhibition by NaCl for up to 150 days. The authors stated that the value for complete inhibition of larval emergence coincides with the moisture stress that brings on permanent wilting of plants. This response of nematode eggs to moisture stress, according to these authors, is

probably an important factor in the distribution and infectivity of plant parasitic nematodes.

Although larvae hatch in large numbers in water, root diffusate (emanation or exudate from germinating seedling roots) from, for example, tomato (Lycopersicum esculentum Mill.) acts as a stimulus. In experiments with egg sacs of M. hapla, M. incognita acrita, and M. javanica, Viglierchio and Lownsbery (1960) reported a significant increase of 15-30% more larvae in tomato (var. Rutgers) root diffusate than in water, and the rate of hatching was also greater. Loewenberg et al. (1960) found that larvae of M. incognita incognita (Kofoid and White, 1919) Chitwood, 1949, though not requiring a hatching factor to emerge, depend greatly on the pH and mineral composition of the environment. According to these authors, the optimum pH for hatching of the larvae of this nematode in Heller's nutrient solution is 6.5.

The 1st-stage larva moults within the eggs (Christie and Cobb, 1941). According to Tyler (1933b) development from egg-laying to hatching takes 9 days at 27°C and 31 days at 16.5°C. On hatching, an infective 2nd-stage larva emerges from the egg sac, moves through the soil and enters a plant root usually at a point in the elongating

region close behind the root tip (Fig. 31). Van Gundy et al. (1967) discussing ageing and starvation in larvae of M. javanica found that the 2nd-stage larvae of this nematode were mobile and infective for up to 32 days in vitro by which time body contents were exhausted. 1% of larvae stored in soil in the absence of food survived and were infective after 64 days. Within a day of penetration of the host, there is a rapid loss of mobility and infectivity; this is even more pronounced after 2 days (Bird, 1967). If the plant is a favourable host, as is in the case of beans, after penetration, the larva moves through the cortex to the vascular system and lies parallel to the long axis of the root with its head in the pericycle layer (Fig. 32). Feeding begins and the nematode remains sedentary in this position until maturity.

The majority of nematodes grow in a series of steps as they feed, grow and moult. However, species of Meloidogyne are peculiar in that, after entering the plant and commencing to feed, they grow and increase considerably in size before the onset of moulting. After moulting the nematodes grow rapidly and increase in size. Bird (1959) found that growth of M. javanica larvae in tomato was slow at first. During the first 8 days of parasitism the cross-sectional area of the larva

only doubled, but in the next 5 days it trebled. About the 14th day, the time of the 2nd moult, the larvae had thickened and possessed the characteristic spiked tail (Fig. 33), differentiating this stage of species of Meloidogyne from species of Heterodera Schimdt, 1871. From the 14th through the 19th days there was no increase in size. During the 2nd moult the anterior part of the stylet (the hollow feeding structure of sclerotized cuticle located in the buccal cavity) was shed and the posterior part resorbed; the adult stylet was reformed after the final (4th) moult. The 3rd and 4th moults followed rapidly within the moulted cuticle of the 2nd-stage larva. Under Bird's temperature (11-40°C) conditions the parasitic moults (2nd, 3rd, and 4th) occurred within 3 days; by the 19th day all specimens were in the final moult or were adults. Growth was very rapid between the 20th-27th days when egg sac formation commenced, followed 2 days later by egg laying, by which time the females were almost at their maximum size (Fig. 34). From 30th-40th days the rate of growth of the nematodes was slight; however, egg sac growth and rate of egg laying increased rapidly, the egg sac usually becoming bigger than the female which produced it (Fig. 35).

While the female root-knot nematode is a

sedentary parasite during larval development and throughout its entire adult life, the male is a sedentary parasite only during its larval development. When the male matures, it uses its stylet to break through the larval cuticles and root tissue and so emerges into the soil. The subsequent history of the adult male is not very well known. Males are said to live free in the soil and frequently are found there, sometimes in quite large numbers. They are also found embedded in egg masses at the posterior ends of females where they go, presumably, to copulate (Christie, 1959). Christie further stated that males have been found occasionally within roots, including those of certain resistant plants like crotalaria. During their parasitic development, males produce the same effects as females on their hosts and will stimulate the development of galls in the same manner as females. The only difference is that the effect of the male extends over a much shorter period.

No one seems to have described root-knot nematodes in copula. Copulation may occur; however, it is not necessary since parthenogenesis occurs in these nematodes. Tyler (1933a) found that reproduction without males appears to be regular and normal. One family of the root-knot nematode,

referred to then as H. marioni (Cornu, 1879) Goodey, 1932, was carried through 12 generations in the absence of males by repeated isolations; the females laid eggs and reproduced normally. In any case when mature females are completely embedded in the host tissue, as happens in potato (Solanum tuberosum L.), carrot (Daucus carota L.), and plants with similarly swollen underground parts, it is very unlikely that the males can reach them. Triantaphyllou (1962) did not observe fusion of the sperm and egg pronuclei in M. javanica, and fertilization did not occur even when sperm was present in the oocytes. He concluded that the main and, perhaps, the only mode of reproduction was by parthenogenesis. Dropkin (1953) reared populations of M. incognita acrita, and M. arenaria from single larva infections, thus demonstrating parthenogenesis in these species.

Under stress conditions such as overcrowding, food shortage, high temperature, or unsuitable host plant, a high percentage of males and intersexes is produced. In experiments with M. incognita and M. javanica, Triantaphyllou (1960) proposed an hypothesis of sexual differentiation, which was supplemented by Davide and Triantaphyllou (1968). According to these authors, under favourable conditions, larval development proceeds

towards the production of a female. Adverse conditions produce a masculizing effect on larvae which develop into males with one testis, males with two testes, or intersexes, depending on the timing of the adverse stimulus. If it occurs before differentiation, small males with a single testis are produced. If conditions worsen after differentiation has started, sex reversal occurs and males with two testes or intersexes result. The authors produced female intersexes (females with some male characters such as incompletely developed spicules) from plants sprayed with high rates of maleic hydrazide; partial sex reversal had occurred due to adverse conditions in late development of the female larvae.

PENETRATION INTO ROOT CELLS, AND FEEDING HABITS OF ROOT-KNOT NEMATODES

Penetration and feeding of root-knot nematodes are closely linked, for both require the invasion of the outer cell layer of the host. Linford (1942) observed the feeding of the 2nd-stage larva of H. marioni (=Meloidogyne sp.) before and during penetration into roots of lettuce (Luctuca sativa L.). He found that a larva may begin thrusting its stylet intermittently soon after it makes contact with the epidermal root cells. After a usually

prolonged repeated series of stylet thrusts in one place, the stylet is finally inserted into the cell with a series of 3 or more very rapid jerks, the stylet not sliding back between thrusts, and each one carrying it farther forward until the maximum possible degree of protrusion is achieved. Once the stylet tip is fully inserted into a cell, all perceptive motion of the nematode stops. However, according to Linford, after a period of 15-30 seconds of rest, the esophageal bulb begins a rapid and rhythmic pulsation that may continue 10-40 seconds. This rhythmic pulsation of the esophageal bulb is the mechanism with which the nematode injests its food. As soon as action of the bulb stops, the larva rectracts its stylet and either immediately resumes thrusting in approximately the same place, turns its head to a different cell, or moves to a different position before the next period of feeding. After an epidermal cell has been fed in one or more times and battered by the innumerable stylet thrusts during a period of minutes, its wall may break, allowing the head of the nematode to slip in while the disorganized remains of the protoplast flow out into the water film on the root surface. The larva then commonly continues to penetrate into the root, feeding as it goes. Movement through the epidermis is halting as though there are periods when the larva feeds on different cells. The author observed

larvae advancing approximately one cell length or less, and then lying at rest for 2 minutes or more before moving farther in. He pointed out that such halting advance was observed with larvae penetrating almost directly into the meristem through the side of the root cap and with others entering the maturing zone. Other larvae also may be attracted to this point, where they attempt to invade the root together (Godfrey and Oliveira, 1932; Peacock, 1959). Linford further observed that, after the larva has forced its head into a cell, its feeding continues much as on the root surface, periods of rapid thrusting of the stylet alternating with periods of rest and of sucking. The larva also uses its stylet both to break through cell walls and to separate cells along the middle lamella. Cells are destroyed chiefly by entry of a nematode after the cell has been fed in and the cell wall has subsequently been weakened by numerous stylet thrusts. The larvae feed persistently during intercellular migration to their permanent sites.

Linford (1937) also observed the feeding of the adult female of this root-knot nematode both in nutrient solution and in sections of live galls from roots of pea (Pisum sativum L.). He found that the feeding process in the adult female was very similar to that already described for 2nd-stage larva. The

female obtains its food not by secreting saliva into intercellular spaces and then sucking up nutrient substances exosmosed from adjacent giant cells, but rather by penetrating cells with its slender stylet and feeding directly from their substance. Within the root, the anterior end of the mature female becomes tightly pressed against the adjacent cell walls; at this stage, however, the head still is moved freely and through a wide angle, pointing the stylet in all directions. Linford reported also that in solution, saliva coming apparently from the dorsal esophageal gland duct, was observed flowing outward from the extreme tip of the protruded stylet. This was seen only in nematodes found with the stylet fully protruded when removed from the root. Saliva flow into a giant cell was not seen but, according to Linford, the protoplasm of normal giant cells would obscure it except in a most favourable situation. The usual pause after penetration of a cell before actual feeding begins, as indicated by pulsation of the bulb, suggests that it occurs frequently. The author further observed that the longest period of continuous pulsation, slightly exceeding an hour, was insufficient to empty the cell fed upon. By feeding briefly and in irregular rotation upon all the giant cells within reach of its mobile head, this nematode avoids destruction of cells and thus

maintains, for a long period, an immediately accessible and abundant food supply. Linford concluded therefore, that, clearly this nematode feeds, in all stages, with its stylet inserted into cells.

SYMPTOMS OF ROOT-KNOT NEMATODE INJURY TO PLANTS

On susceptible host plants, root-knot nematodes cause severe growth retardation, characteristic root galls, and several disease complexes.

(a) Above-ground symptoms. Above-ground symptoms are essentially the same as those caused by any condition that deprives a plant of an adequate and properly functioning root system. These symptoms include:

(i) Stunting or reduced growth. Stunting and slow growth are common in root-knot nematode attacks and are often seen as patches in a field. Seinhorst and Sauer (1956) observed poor growth and stunting in vines (Vitis sp.) caused by M. javanica in Australia. The young shoots remain short, according to these authors, and, in the case of severe damage, may be so sparse that the vine shows bare arms.

(ii) Discolouration of foliage. Die-back in

plants accompanied sometimes by leaf chlorosis is indicative of a nutritional deficiency and, with root-knot nematode infestations, the cause often lies in root destruction. Chitwood and Berger (1960) observed lower stems, leaf chlorosis, and even death of the mature coffee (Coffea arabica L.) plants infested with M. exigua Goeldi, 1887 in Guatemala. The authors also observed that the chlorotic leaves of the coffee seedlings in nurseries often drop and the plants die. Discolouration may range from light yellow to deep red, purple and even black, and at best this symptom can only serve as a warning of possible root-knot nematode infestation. Zaumeyer and Thomas (1957) reported that root-knot disease of beans may be suspected when the plants are pale and yellowish, somewhat dwarfed, and have a tendency to wilt during the warmer part of the day.

(iii) Stem and leaf galls. Sometimes root-knot nematodes cause galls in above-ground parts of plants. Steiner et al. (1934) reported giant galls on stems of some ornamentals, Thunbergia laurifolia Lindl., and T. grandiflora Roxb. caused by the root-knot nematode, H. marioni (=Meloidogyne sp.). The authors observed that the gall-like masses of tissue, often reach a diameter of 45-60 cm, and may attain this degree of development within a

period of a few months to a year. Neither of the Thunbergias seemed to suffer much from these galls, except perhaps by a reduction in the number of flowers; older galls soon decay and crumble. In a fresh condition the gall tissue, according to these authors, is extremely tough, consisting mainly of lignified fibres. The gall surface is very rugose and of blackish colour, whereas the interior has a waxy, yellowish-white appearance. The authors further stated that the growth of these galls is due mainly to autoinfection, the progeny of the first and succeeding generations of the parasite remaining within the plant. The final result is an enormous accumulation of specimens of the parasite, which, after the final break-down of the host plant, will invade the surrounding soil. Another point of interest, as the authors pointed out, is the fact that these galls usually are fully exposed to the sun. Since the lethal temperature point of larval root-knot nematodes is around 46-47°C, it would appear that autoregulation by the host plant must keep down the temperature of at least the fully insulated portions of the galls, so that this lethal is not reached. They suggested that the rugose structure of the galls through increase of the evaporating surface may be favourable to such a function.

Linford (1941) observed galls on stems and leaves of various plants caused by root-knot nematodes. He found that in leaf laminae and petioles of Emilia sonchifolia DC., numerous galls developed within which nematodes came to maturity and laid eggs. In laminae, the parasites were distributed on both large and small veins where they produced chiefly small, simple galls. On petioles, however, long compound galls soon developed rugose surfaces. In the succulent leaves and stems of Portulaca oleracea L., the relatively few nematodes that established caused so little gall formation that when the first generation egg masses were well developed, infected parts were distinguished only with difficulty. In cowpea (Vigna sinensis Endl.) leaves infected in the bud while very young, galling was accompanied by distortion, crinkling, mild mottling, vein clearing, and occasional enations. Tomato leaves, and the stems of both tomato and cowpea, developed large galls under the influence of successive generations of parasites.

Miller and DiEdwardo (1962) observed leaf galls on Siderasis (=Tradescantia) fuscata Moore caused by a root-knot nematode, M. incognita incognita. They also observed that galls, usually occurring along the leaf veins or midrib, contained adult females, viable eggs, and larvae.

(b) Below-ground symptoms

(i) Root galls. Species of root-knot nematodes form characteristic galls on the roots of host plants. The knotted, swollen and distorted root system is usually diagnostic of Meloidogyne infestations and has earned them the name of root-knot nematodes. The size, number and shape of the galls vary, however, for different species and for different host plants. Southey (1965) reported that most graminaceous plants fail to form galls, except Pennisetum clandestinum Hochst. infested with M. kikuyensis de Grisse, 1960, and on legumes the galls are often very small. M. hapla causes small galls characterized by the development of lateral roots above and below the galls. At high temperatures, according to Southey, nematodes develop rapidly and, when mature, often protrude on the root surface (Fig. 34, 35), galls not being formed. The author stated that galls are not formed on Brassica spp. infested with M. artiellia Franklin, 1961, and the same is probably true for other root-knot nematodes that have so far remained undetected because they do not cause gall formation.

Townsend and Ruehle (1947) reported that the most noticeable symptom of root-knot disease in beans is the knots which are found on the roots of

affected plants. These differ from the bacterial nodules on bean roots in that the swelling involves the entire circumference of the root, whereas, the bean nodules occur as growths attached to the side of the smaller rootlets. The authors observed that nematode galls also occur on the larger roots and even on the underground portion of the bean stems. When the infestation of bean roots is heavy the entire root may become one large gall; the gall on small rootlets may enlarge the root to 5 or 10 times its normal diameter (Fig. 4,6). These authors further observed that root-knot galls appear to be composed of normally healthy but overgrown tissues in their early development and may reach considerable size before there is any evidence that the tissues are breaking down. Most galls become soft and begin to disintegrate before the bean plants die; old galls are brown and have the appearance of rotten cork. According to these authors, the bean plants may not show other symptoms if the infection occurred late in the growth of the crop, or if the development of the nematodes has been slow. Bean plants which are severely infected in their early growth become stunted and have few branches (Fig. 5). They may become chlorotic or may simply wilt and die. The death of the plants does not occur until the galled roots begin to break down. This may be caused by the activity of other organisms, such as fungi

and bacteria which will be discussed later, infecting the diseased roots as much as by nematodes.

(ii) Excessive root branching. Galls are not the only symptom of root-knot nematode injury. Christie (1959) reported that roots of most plants, when infected with M. hapla, tend to form branches near the region of invasion which may result in a dense, reticulate type of root system. Symptoms of injury by this species might be characterized as a combination of galls and stubby root. In other words, if the branch rootlets manage to attain a moderate length before their growth is stopped, the resulting root system may be composed of numerous short stubby branches often arranged in clusters. The galls tend to be small and located on the small roots.

(iii) Injured or devitalized root tips. Christie (1959) observed that for all the other root-knot nematodes, other than M. hapla, that occur in the United States, infected plants tend to have much fewer small rootlets than normal, and symptoms might be characterized as a combination of galls and coarse root. In other words, if the growth of lateral roots is stopped just as most of them are breaking through the cortex or while they are very short, an open system made up mostly of the main roots

largely devoid of small branch rootlets may result. The galls tend to be large and to involve the main roots. The author also observed that curly tip, injury at the side of a root close to the tip, caused by root-knot nematodes, may retard growth and elongation on that side and result in curling.

HOST REACTION CAUSED BY ROOT-KNOT NEMATODE FEEDING

Although not all plants attacked by species of Meloidogyne develop pronounced galls, Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon, and Premier bean varieties infected with M. incognita and M. javanica develop pronounced galls (Fig. 4,6); the internal structure of the bean roots is also modified where the nematodes feed (Fig. 36). In the host, larva of the root-knot nematodes feeds on the cells of the vascular system adjacent to the head. These cells undergo a series of modifications finally becoming the so called 'giant cells', which are many times the size of the original cell and bounded by irregularly thickened walls. Jenkins and Taylor (1967) defined a giant cell as a term applied to a host response in which 40-60 adjacent cells coalesce to form a multi-nucleate mass of protoplasm. The authors further stated that it is also termed a syncytium or lysigenoma.

Giant cells disrupt the vascular system which is probably the main cause of the general unthriftiness, stunting, tendency to wilt and even death of infected plants. Giant cell formation by M. javanica was also described by Bird (1961). He observed that when a larva of the nematode reaches a position where it can develop, it begins to feed in turn on 5 or 6 cells adjacent to its head. In about 4 days, these cells develop into giant cells. Some cell walls break down and the walls surrounding such a group thicken and become the giant cell wall. The nuclei thus enclosed divide several times and neighbouring cells coalesce, adding more nuclei until a syncytium results that encroaches on the surrounding vascular tissue and contains many nuclei and a highly granular protoplasm. The nuclei stain strongly and have conspicuous nucleoli. The walls of the giant cells become irregularly thickened, in places 5-10 times the normal thickness, but fine channels allow cytoplasmic connections between adjacent cells. Giant cells in the stele cause disruption of the xylem strands and irregular xylem elements often develop. If the attack is light, the plant can compensate and the roots, though galled, continue to grow, sometimes with increased branching. According to Bird, root-knot nematodes not only stimulate cortical hyperplasia but also induce break-down of cell walls, mitosis and protein synthesis.

The stimulus is thought to be a growth-promoting substance, which the author found in nematodes and galls but not in adjacent roots, but he did not consider his results to be conclusive.

Bird (1962a) also showed that giant cells require the repeated stimulus of the feeding nematode for their continued existence. When he killed nematodes in roots without destroying the roots, the surrounding giant cells collapsed.

Gall formation on the roots, a separate phenomenon from giant cell formation, according to Dropkin (1955), is brought about by hypertrophy of the cortical tissues around the nematode and its feeding site, and later the cortical cells may divide and enlarge the gall.

ROOT-KNOT NEMATODES IN DISEASE COMPLEXES

Root-knot nematodes have been shown to interact with other pathogens causing a disease complex in which symptoms are worse than those produced by either pathogen alone.

Schindler *et al.* (1961) reported an experiment where carnations (*Dianthus caryophyllus* L.) were inoculated with the root-knot nematodes, *M. hapla*,

M. incognita, M. incognita acrita, M. arenaria, M. arenaria thamesi Chitwood, Specht, and Havis, 1952, and M. javanica; and with the carnation Fusarium wilt fungus (F. oxysporum Schlect. f. dianthi (Prill. and Del.) Snyder and Hansen). When Fusarium inoculum was applied 1 week, 2 months, or 5 months after the root-knot nematode inoculations, there was a synergistic interaction between the two pathogens which greatly increased the severity of the Fusarium wilt disease; the carnations were more severely wilted by the fungus in the presence of the two organisms than the sum of the damage due to the fungus and nematode acting alone.

Johnson and Littrell (1969) conducted an experiment on the effect of M. incognita, M. hapla, and M. javanica on the severity of Fusarium wilt (F. oxysporum) of chrysanthemum, C. morifolium Ram., 'Yellow Delaware' (Fusarium-susceptible), and 'White Iceberg' (Fusarium-resistant). They found that nematodes did not break Fusarium wilt resistance of 'White Iceberg'; however, wilt symptoms appeared earlier and were more severe among 'Yellow Delaware' plants inoculated with M. javanica and F. oxysporum than similar combinations of the fungus and M. incognita or M. hapla or with the fungus alone.

Thomason et al. (1959) observed that when

plants of the wilt-tolerant cowpea variety Grant were inoculated by dipping roots of seedlings in a Fusarium, F. oxysporum f. tracheiphilum (E.F.Sm.) Snyder and Hansen spore suspension, only slight xylem necrosis of the lower stem (Fusarium wilt value) occurred compared to a severe expression of the same symptom for the susceptible variety Chino 3. However, when soil was infested with the Fusarium and the root-knot nematode, M. javanica, the Fusarium wilt value was higher on the tolerant Grant variety than on the susceptible Chino 3 variety.

McGuire et al. (1958) determined the effect of the root-knot nematode species, M. hapla, M. javanica, M. incognita, M. arenaria, and M. incognita acrita, on the development of Fusarium wilt, F. oxysporum f. vasinfectum (Atk.) Snyder and Hansen, in the Buffalo variety of alfalfa (Medicago sativa L.). They found that at the end of 4 months the percentage of plants infected with Fusarium wilt in each treatment was as follows: M. hapla and Fusarium, 95; M. javanica and Fusarium, 60; M. incognita and Fusarium, 50; M. arenaria and Fusarium, 50; M. incognita acrita and Fusarium, 10; Fusarium, 15; Meloidogyne spp. alone and control, 0. Percentage of dead plants in the above treatments was 40, 5, 10, 4, 0, 3, 0, respectively. The number of

plants infected with Fusarium and the severity of infection were in proportion to the severity of root-knot.

Effect of root-knot nematodes, M. incognita, and M. javanica on Fusarium wilt, F. oxysporum f. perniciosum (Hepting) Toole, of mimosa (Albizzia julibrissin Durazz.) seedlings was investigated by Gill (1958). He found that a greater reduction in the percentage of seedlings surviving occurred in the soil infested with the combination of Fusarium and either M. incognita or M. javanica than in the soil infested with Fusarium alone. Plants in root-knot-nematode-infested soil did not exhibit wilt symptoms, while plants wilting in soil infested either with Fusarium alone or with Fusarium plus root-knot nematodes exhibited typical wilt symptoms including vascular discolouration. He concluded that the presence of the root-knot nematodes, M. incognita and M. javanica, therefore, increased the incidence of Fusarium wilt of mimosa seedlings.

Porter and Powell (1967) investigated the influence of the root-knot nematodes, M. incognita, M. arenaria, and M. javanica on Fusarium wilt, F. oxysporum f. nicotianae (J. Johnson) Snyder and Hansen, development in flue-cured tobacco (Nicotiana tabacum L.). They found that interactions between

the root-knot nematodes and Fusarium were distinct in the tobacco, and that significant infection occurred only when the nematodes and fungus pathogens were both present. Interactions were evident in both the resistant (Dixie Bright 101), and the susceptible (North Carolina 402) tobacco varieties. The nematode species did not differ in their ability to enhance wilt development. Plants of the Fusarium wilt-susceptible tobacco variety, North Carolina 402, became infected earlier and to a greater extent than those of the resistant tobacco variety, Dixie Bright 101.

Fusarium wilt, F. oxysporum f. vasinfectum of cotton (Gossypium hirsutum L.) is more severe in the presence of M. incognita and M. incognita acrita than in their absence. Martin et al. (1956) found that even the wilt-resistant cotton, Coker 100 wilt, became severely wilted when inoculated with the wilt fungus together with either M. incognita or M. incognita acrita. The wilt-susceptible variety Deltapine 15 was 27% wilted in the presence of Fusarium alone compared with 100% when inoculated with root-knot in addition to Fusarium.

Taylor and Wyllie (1959) investigated the interrelationship of root-knot nematodes and Rhizoctonia solani Kühn on emergence of soybean

(Glycine max Merr.). They found that 3 weeks after planting, the average emergence for each treatment, expressed as a percentage of the control, was as follows: M. javanica alone, 98%; M. hapla alone, 83%; R. solani alone, 50%; M. javanica plus R. solani, 17%; and M. hapla plus R. solani, 2%.

Tu and Cheng (1971) conducted an experiment on the interaction of the root-knot nematode, M. javanica and the fungus (Macrophomina phaseoli (Maubl.) Ashby) in root-knot of kenaf (Hibiscus cannabinus L.). They found that the incidence and severity of root-knot caused by the fungus was increased in screenhouse-grown kenaf seedlings simultaneously infected by the root-knot nematode. In seedlings inoculated at 5, 10 and 15 days of age, root-rot lesions increased 70.3, 44.1, and 21.8%, and nematode penetration increased 49.0, 36.7, and 12.3% when both the fungus and nematode were present.

Alfieri and Stokes (1971) reported that the combined pathogenic effects of the charcoal rot fungus (M. phaseolina (Tassi) Goid.) and M. javanica were greater on plants of Ligustrum japonicum L. than the independent effects of either. Leaf chlorosis and abscission, twig die-back, stunting, reduction and necrosis of roots, and eventual plant loss were greater on plants infected with both pathogens.

Sasser et al. (1955), and Powell and Nusbaum (1960) investigated the black-shank-root-knot complex in flue-cured tobacco. Sasser et al. (1955) found that the tobacco plants inoculated with the black-shank fungus (caused by Phytophthora parasitica Dast. var. nicotianae (B. de Haan) Tucker) in the presence of the root-knot nematodes, M. incognita acrita, M. incognita and M. javanica, developed black-shank symptoms earlier and to a greater extent than did plants grown in soil infested with only the black-shank fungus. Similar observations were also reported by Powell and Nusbaum (1960) on the same fungus in the presence of M. incognita incognita, and M. incognita acrita. Histopathological observations made by the latter authors further indicated that root-knot nematode infection predisposes plants to black-shank invasion, to the extent that roots of black-shank-resistant and -susceptible plants become a suitable substrate for fungus development. They stated that the fungus thrived on hyperplastic and hypertrophied root tissue; hyphae appeared to grow directly into such regions, and colonization was rapid and extensive. The mycelium that occupied galled tissue appeared extremely vigorous; the hyphae were large and densely filled with protoplasm, and contained conspicuous nuclei.

Stewart and Schindler (1956) showed that the bacterial disease of carnations caused by Pseudomonas caryophylli Burk. was increased in the presence of the root-knot nematodes, M. hapla, M. javanica, M. incognita acrita, M. arenaria, and M. incognita. All 5 root-knot nematode species increased the rate of wilting in the presence of bacteria; only M. incognita acrita had a similar effect when no bacteria were present. They considered that mechanical wounding, also, increased the rate of wilting in the presence of bacteria, but showed no effect in their absence.

NEMATODE CONTROL:

DISCOVERY OF D-D AND EDB AS SOIL FUMIGANTS

Soil fumigation with various biocidal chemicals has been used for many years to control various soil inhabiting pests. For example, carbon disulfide (CS_2) was widely used in France in the 1860's to control root aphids (Phylloxera vitifoliae Fitch, 1856) on grapes. Its use was discontinued because of its high degree of inflammability. During the 1920's and 1930's, chloropicrin (CCl_3NO_2) was used widely in the Hawaiian Islands to control nematodes in pineapple (Ananas sativus Schlt.) fields. Although it was highly effective,

it was expensive, corrosive, irritating to the eyes and lungs, and it required a soil seal. Methyl bromide (commonly referred to as MBr) was used in the early 1940's as a soil fumigant, but its use is still limited by its high cost and its need for a soil seal.

Lower cost materials that could be applied on a large area basis were still needed. The nematicidal efficacy of a mixture of 1,3-dichloropropene and 1,2-dichloropropane was discovered in 1943, when Carter (1943) an entomologist of the Hawaiian Pineapple Research Institute, reported that this mixture, produced by the Shell Chemical Corporation, was a promising new soil fumigant when applied at the rate of approximately 224.5 litres per hectare. He stated that the fumigant not only controlled root-knot nematodes in pineapple fields, but also controlled destructive larvae of a beetle (Anomala orientalis Wth., 1920). The discovery of D-D, as it was named, marked the beginning of the present soil fumigation industry. Experimental plots were soon established throughout the United States by the Shell Chemical Corporation, cooperating with federal, state, and private agencies. These early demonstrations were so successful that within 5 years many thousands of acres had been treated and soil fumigation was accepted as a successful and

profitable practice for control of nematode on many crops. In 1945, Christie made preliminary experiments with ethylene dibromide, a Dow Chemical Company product, and found it effective as a preplant treatment in controlling root-knot nematodes on tomato. In the same year, the Dow Chemical Company introduced the chemical as a successful soil fumigant and made field-scale demonstration plots near Ventura, California. EDB, as it became known, not only proved to be effective against root-knot nematodes, but also gave remarkable control of wireworms (Lane and Stone, 1954). McBeth (1969) stated that during the past 20 years, the amount of soil fumigants used for controlling plant nematodes has increased from almost nothing to more than 60 million pounds each year. Unlike other agricultural pesticides, there have been very few new nematicides of commercial importance introduced since the advent of D-D type compounds and EDB; these compounds were the first products to be used on a large scale to control plant nematodes and still command a major part of the market. The two fumigants are similar in that they are halogenated hydrocarbons, possess a moderately high vapour pressure, are highly phytotoxic, and are used extensively and successfully as pre-planting treatments for annual crops.

FACTORS AFFECTING SOIL FUMIGATION WITH D-D AND EDB

D-D and EDB are volatile liquid soil fumigants; the chemicals are brought into contact with nematodes by gaseous diffusion. The liquid must volatilize and the gas diffuse through the soil pore spaces, become dissolved in soil moisture in concentrations high enough to reach levels toxic to nematodes, and then escape into the air. Soil diffusion is, however, affected by many factors including soil temperature, soil moisture, soil type, soil compaction, and the sorption relationships between the fumigant and the soil components (McBeth, 1954).

McBeth (1954) stressed the effect of temperature on diffusion when he estimated that D-D (boiling range about 50-115°C) in a sandy soil diffuses 15 cm in less than 24 hours at soil temperature of 24°C, whereas at 7°C it takes 96 hours. He also stated that for optimum results soil moisture in sandy loam or clay soils should not be more than 85% nor less than 50% of field capacity; where soils have a moisture equivalent of 25% or more, the dosage of fumigants must be increased. Heavy clay soils are difficult to fumigate owing to sorption of the fumigant on the very large active surface area of the soil particles and the blocking of pore spaces by soil moisture. Soil should be in seed bed

condition for most efficient nematicide treatment with compaction at a minimum. Fumigants are sorbed more in dry than in moist soils. Peat soils are not favourable for fumigation because of the high level of sorption on the organic matter. Siegel *et al.* (1951) studied the diffusion patterns of D-D and EDB using sorption isotherms and radioactive tracer technics in Brookston clay loam soil containing 7.86% organic matter, a well-decomposed muck, and Wyoming bentonite (montmorillonitic clay). They found that the extent of diffusion of the compounds was approximately equal in identical soils at the same moisture level. Diffusion was mostly lateral and downward, with very little penetration into the soil above the point of injection. Optimum diffusion was obtained with the soils at the moisture equivalent or field capacity (the percentage of water remaining in the soil 2 or 3 days after being saturated and after free drainage has practically stopped); wetting the soil to saturation decreased the extent of vertical diffusion, and air drying decreased diffusion in all directions. The two compounds were sorbed to about an equal extent; sorption was highest in the muck, less in Wyoming bentonite clay, and least in the Brookston clay loam. The bentonite clay was completely desorbed by passage of air, but the muck and the Brookston retained quantities of the compounds that could not be removed in this manner.

Wade (1954a) studied the sorption of EDB on 3 different soils: a sandy soil, soil with high organic matter, and a clay soil with 10% of added sand. He found that sorption of EDB by the soils was rapid; most of the amount sorbed was taken up within half an hour. Over the usual field range of moisture contents, the amount of EDB held by the soil remained constant although there was an increase with increase in moisture content, owing to solution of the fumigant in the soil water. The amount of EDB sorbed was proportional to the organic carbon content of the soil and decreased with increase in temperature. In determining the stability of EDB in soil, Wade (1954b) found that once EDB was introduced into soils, it remained stable at moisture contents in the field range of moisture for at least one week. Wade (1955) also found that in dry soils (an organic soil and clay) below the field range of moisture content, the sorptive capacities decreased with increasing humidity. Call (1957a) also determined the sorption of EDB but on 20 different soils at their field capacity. In all the soils, which ranged from coarse sands through silts and clays to heavy peats, the sorption coefficient was correlated with surface area, organic matter content, moisture content, and less closely with clay content. Call (1957b) carried

out another study on distribution of EDB vapour round an injection point under ideal conditions of uniform soil porosity, soil compaction, and constant soil temperature. He concluded that diffusion was the most important factor controlling the distribution of EDB vapour in soil. Low concentrations near the surface were caused by loss of fumigant into the air. The only way to prevent this was to seal the soil surface with water or with an impervious covering, or to decrease the porosity by rolling.

METHODS OF APPLYING D-D AND EDB

Soil fumigation with D-D and EDB is an exacting procedure that must be done properly if satisfactory results are to be obtained (Turner and Dieter, 1955). Both these fumigants are extremely phytotoxic; in order to avoid injury to crops, careful attention must be given to aerating the soil and allowing an adequate time interval between application and planting. Christie (1959) listed the following considerations relating to application of the compounds:

(a) Before applying D-D or EDB, the land should be prepared to the consistency of a good seedbed.

It should be smooth and reasonably free of lumps, clods, and undecomposed crop residues. Where cover crops or crop residues are plowed under, they should be allowed to decay. Heavy soils should be plowed, as disking alone will not loosen them to a sufficient depth.

(b) The soil temperature 15 cm below the surface should be between 10° and 30°C, preferably between 21° and 27°C.

(c) D-D or EDB is not fully effective in very dry or very wet soil; for best results in most soils the moisture content should approach but not exceed field capacity.

(d) After the fumigant is applied, holes, channels, or furrows left by the applicator should be filled promptly and firmly and the land left smooth and firm at the surface. Operations necessary to accomplish this should be performed immediately after the fumigant is applied. The land should remain undisturbed for one week, then it should be deeply and thoroughly disked.

(e) The time that must elapse between application and planting to avoid injury to the crops depends on the rate at which the fumigant was

applied and the kind, temperature, and condition of the soil. The fumigants leave the soil quickly when the weather is hot and dry and may linger tenaciously when it is cold and wet. Soils high in organic matter are especially difficult to aerate. When weather and soil conditions are ideal, two to three weeks are usually adequate.

Application of the fumigants is laborious and costly; to inject small measured liquid quantities of D-D or EDB into the soil at closely spaced intervals might seem, at first glance, to be a comparatively simple operation, but it has proved to be surprisingly difficult. Much time, energy, and money has been spent in devising, testing, perfecting, and adjusting application equipment. For small-scale fumigant application, a hand gun applicator is used either for a row or a broadcast (over-all) treatment. The applicator resembles an hypodermic syringe and a needle; it consists of a long, hollow "needle", and a plunger or trip device. The "needle" is inserted into the soil to the desired depth, usually 15 to 20 cm, and the plunger pressed to a calibrated stop to force the liquid into the soil. For a row treatment, spacing the injection points 30 cm apart in the row has become more or less standard practice. For a broadcast application, before treatment, the soil surface is usually marked

off into 30 cm squares. The fumigant is applied in a diamond pattern by marking injections at the junctions of the cross in the first row and halfway between the second row, at the junctions in the third row, halfway between in the fourth row and so on across the garden or plant bed. For fumigation of large areas, various types of tractor-drawn or implement-mounted injectors, designed to deliver a constant volume at the correct depth, are available, and their methods of application are described in detail by Good (1969).

Except for plant beds and in situations where the highest possible degree of control is desired, Christie (1959) reported that the usual procedure is to apply the fumigants at low to moderate rates, just enough to give one crop sufficient protection to prevent serious reductions in yields. For broadcast applications under most conditions, this means 224.5 to 280.8 litres per hectare of D-D, 168.5 to 224.5 litres per hectare of a 40% EDB fumigant or 50.6 to 67.4 litres per hectare of an 85% EDB fumigant. Row fumigation requires about half or less the normal broadcast rate, depending on row spacing.

HOW D-D AND EDB KILL NEMATODES

Penetration of nematodes by chemicals is a

necessary prerequisite of the physiological reactions associated with death. However, the precise manner in which soil fumigants such as D-D and EDB kill nematodes has not been determined. Nematodes are resistant to most soil fumigants; their resistance is due, in part at least, to the impermeability of the cuticle and the protective covering of the egg. Bird (1958) identified 15 amino acids in the cuticle of M. hapla and M. javanica; he suggested that the chemical reaction of the cuticle indicated the presence of collagen as well as phenolic compounds and polyphenol oxidase. Bird and Rogers (1965) defined three distinct cuticle layers of M. javanica: outer cortex, inner cortex and fibre layers. According to Chitwood (1952) the cuticle of a nematode is composed of a complex of several proteins, including collagens, fibroids, elastoids, keratoids, and, in some cases at least, lipid material. He pointed out that the egg shell proper is a chitinoid and is relatively permeable to water, gases and chemicals, although a mucoid covering opposes drying out and the penetration of chemicals such as fat solvents. The vitelline membrane, inside the egg shell, composed of a waxy material chemically related to beeswax provides a further barrier to the penetration of chemicals into the egg. The egg has no natural openings which may be the reason why this is the stage in the life cycle that tends to be the most

resistant to chemicals. It appears, therefore, best to assume that the soil fumigants enter the nematodes through the natural openings of the body such as mouth, anus, and vulva. Chitwood (1952) suggested that the halogenated hydrocarbons including D-D and EDB kill nematodes by precipitating proteins, blocking nerve endings and by destroying nerve sheaths and cellular membranes.

ECONOMIC LIMITATIONS OF SOIL FUMIGATION, AND SUGGESTED CONTROL MEASURES OF ROOT-KNOT NEMATODE ON BEANS IN KENYA

While soil fumigation with D-D or EDB can control root-knot nematodes with significant bean seed yield increases (Chapter 3), this control method cannot be recommended in Kenya. For effective and continuous control of root-knot nematode with soil fumigation, the procedure has to be repeated seasonally; the fumigants are very expensive and the cash value of the beans is relatively low. At present, costs of the soil fumigants to control root-knot nematodes restrict their use to crops having high cash value such as cotton, tobacco, tea (Camellia sinensis L.), coffee, pineapple, etc.; these crops and others may be almost wholly dependent upon soil fumigation. For example, it is estimated that the nematodes which

survive pre-plant and post-plant treatments contribute to the 5% loss in pineapples (Special Publication No.1 of the Society of Nematologists, 1971). The Society also reported that at present, annual estimates of total U.S. nematicide costs are $60 million for 0.6 million hectares (average = $86 per ha); they pointed out that this is likely to increase with improved methods for estimating losses in a wider range of crops. The future method(s) to be used in Kenya to control root-knot nematodes on beans will, therefore, have to depend upon non-chemical control practices, that is, crop rotation and/or breeding or selecting for tolerant or resistant crop varieties. The use of these non-chemical control measures too are not without limitations.

Although crop rotation is one of the oldest methods of controlling nematodes, its use in controlling root-knot nematodes is complicated by many factors among which are: the wide host range of the root-knot nematodes, and the existence of different species and of strains within species that exhibit different host preferences (Allen, 1952; Martin, 1954; Sasser and Nusbaum, 1955; Colbran, 1958; Dropkin, 1959; Riggs and Winstead, 1959; Goplen et al., 1959; Triantaphyllou and Sasser, 1960; Michell and Taylor, 1972). Jenkins

and Taylor (1967) suggested that the f
criteria should be met for rotations t
effective:

(a) The nematode population must
to damage the primary crop or be poten
damaging.

(b) Complete knowledge of the ne
range, including weeds, must be known,
requires extensive testing of the loca
against potential hosts.

(c) The nematode population mus
appreciably reduced by the rotation s
primary crop can be economically prod

(d) The crop or crops to be inc
rotation must be adapted to the pract
grower and easily marketable.

The authors also stated that if
conditions are met, crop rotation can
successful method of control, and if
used in the rotation produce an incon
to the susceptible plant, rotation is
than chemical control since there is
materials.

Rotations with non-host plants to control root-knot nematodes have been attempted by several workers. In Rhodesia, Daulton (1964) and Mitchell et al. (1971) reported that 4 years of a non-susceptible grass preceding tobacco and potato respectively, reduced populations of M. javanica to low levels and increased yield and quality; the grasses they recommended were Eragrostis curvula Nees. var. Ermelo, Chloris gayana Kunth. var. Katambora, and Panicum maximum Jacq. var. Sabi. Navarro and Barriga (1970) reported that, in Colombia, root-knot nematode infestations in tomato and bean fields were considerably reduced in rotation with either Crotalaria spectabilis Roth. or Tagetes minuta L. Numbers of nematodes were greatly reduced after Tagetes and, to a lesser extent, after Crotalaria; the latter is a more acceptable crop because of its use as green manure. Ayala (1971) reported that, in Puerto Rico, 12 months rotation with Pangola-grass (Digitaria decumbens Stent.) controlled M. incognita on pineapple. In India, Chandwani and Reddy (1971) conducted a pot experiment to select non-hosts of M. javanica for use in the crop rotation programme for controlling root-knot disease of tobacco. The following 6 plant species were found to be non-susceptible: Cuminum cyminum L., Gaillardia picta Gray, Gomphrena globosa L., Phlox drummondii Hook., Setaria italica Beauv.,

and Zinnia linearis L.

The main problem in Kenya as regards crop rotation system, is that farmers and peasants want a food or cash crop, and are only willing to include such a crop in a rotation. For instance, a farmer or a peasant who grows beans is willing to include potatoes, pineapples, maize (Zea mays L.), and tomatoes in his rotation. If such a crop is found and a mixed population of the root-knot nematodes, M. incognita and M. javanica occurred, the crop might not be a non-host to both nematode species. Any of the above crops, except maize, is a suitable host to both nematode parasites; the crops are therefore unsuitable in a rotation in this country because the parasites will infect the roots or tubers of these crops and reproduce freely. It is with great difficulty that farmers and peasants can be persuaded to include any of the non-host plant species in their rotations, since none of them is a food crop nor has a cash value. A crop rotation programme in Kenya, therefore, must be acceptable to the farmers or peasants and non-host crops must be worth growing. Van der Linde (1956) reported similar experiences in South Africa where farmers were reluctant to grow E. curvula or C. spectabilis in a rotation to control M. javanica, M. arenaria thamesi, M. hapla, and M. incognita acrita on tobacco,

even though the land was heavily infested with the root-knot nematodes.

Non-susceptible economic crops, some of which could be utilized in a rotation programme in this country have, however, been recommended elsewhere. Results of the greenhouse and field studies conducted in the United States by Allen *et al.* (1970) using crop rotation to control *M. naasi* Franklin, 1965, showed that growing non-host crops, oat (*Avena sativa* L.), onion (*Allium cepa* L.), and potato for one season prior to planting barley (*Hordium vulgare* L.), greatly increased yields. The best yields were obtained when potato was grown for two seasons preceding barley. This rotational cropping system, however, cannot be applied in Kenya because of the following reasons. Potatoes in this country are highly susceptible, as was reported earlier in the preceding paragraph, to *M. incognita* and *M. javanica*, the predominant root-knot nematode species found infesting the bean areas. Onion, also, has been shown to carry through at least two generations of these root-knot nematode species (Lewis *et al.*, 1958). Rotation with oat, barley and beans will not be practical either, since these crops are not grown successfully under the same climatic conditions. Oat and barley are grown at higher and cooler areas (2,000 m altitude, and above) with heavy rainfall

(1,270 mm mean annual, and over), whereas beans thrive better at lower and warmer areas (1,500 m altitude, and below) with moderate rainfall (980 mm mean annual, and lower). In Ghana, Peacock (1957) working on the comparative development on susceptible and resistant host species to root-knot nematodes, showed that groundnut (Arachis hypogaea L.) and cowpea (var. Machakos) were highly resistant to attack by the root-knot nematode, M. incognita acrita; no larva was observed to gain entry into the roots of these crops. Although these crops were found to be resistant to M. incognita acrita in Ghana, a complete knowledge of the host range, including weeds, of M. incognita and M. javanica in Kenya, will have to be known before definite recommendations for a specific cropping system utilizing these crops can be recommended. It has been shown that host specificity for species of Meloidogyne, is essentially a problem which is determined by the species and races of the nematodes present; it follows, therefore, that a crop rotation system also be based on local information. A typical example of populations within a species possessing host preferences is that of M. javanica, which Sasser (1954) observed producing galls on tomato (L. peruvianum Mill.) in Maryland, U.S.A.; yet Sauer and Giles (1957) in Australia found that this nematode failed to produce galls on the tomato

plant in the field, thereby differing from the M. javanica population studied by Sasser. The frequent occurrence of mixed populations of M. incognita and M. javanica in this country could also complicate development of satisfactory rotations. An example of this was observed by Thorne (1961) who reported that an area in California, U.S.A. occurred in which there was a mixed population of M. incognita acrita and M. arenaria. After three years, alfalfa had to be ploughed because of damage of M. arenaria. This was followed by cotton for two years, during which the population of M. incognita acrita increased, and the field was then sown again with alfalfa.

The use of crop varieties resistant to root-knot nematode attack offers another promising method of control of the species of Meloidogyne on beans in Kenya. Christie (1959) defined resistance as that quality in a plant that makes it an unsuitable host. Rohde (1965) defined resistance in plants to nematodes as a set of characteristics of the host plant which act more or less to the detriment of the parasite. He pointed out that this may range from mere failure of the host to be a suitable food supply through production of toxins that kill the entering parasite. The author noted that resistance is variable, ranging from slight to complete, is measured in terms of ability of the parasite to survive, and

is not always directly related to plant growth. A susceptible plant may support large numbers of nematodes without showing much injury, whereas a resistant plant may be severely injured reacting to an invading pathogen which does not survive. Some resistant plants are entered or fed upon by few nematodes, even when exposed to conditions favouring heavy infection, whereas other resistant plants are as heavily invaded as susceptible plants. Nematode development is generally slower in resistant varieties than in susceptible and few, if any, female nematodes reach maturity. In general, resistance shows up after infection and is most often based on failure of the host to respond to nematode secretions in a manner favourable to nematode development.

Host-parasite interactions in plants resistant to root-knot nematodes may include one or more of the following characteristics:

(a) lack of root attraction,
(b) reduced larval penetration,
(c) failure of host response to the parasite,
and (d) hypersentive tissue reactions.

(a) Lack of root attraction. Wieser (1955) working on the effect of tomato seedlings and excised roots on M. hapla showed that attractiveness of roots to

larvae of this nematode is dependent upon rate of growth and degree of maturation of the root. The apical 2 mm (root cap and meristematic region) of excised root tips appeared to be repellent to the nematode, while the next 6 mm (region of elongation) was attractive. He assumed that this difference was connected with the different anatomy and physiology of these two portions. The remaining portion of the root, up to 16 mm behind the root apex, was either neutral or slightly repellent to the nematode. Wieser (1956) also investigated the effect of excised distal portions of the roots of bean (var. Pinto), eggplant (Solanum melongena L. var. Black Beauty), and soybean (var. Bansei) on larvae of M. hapla. He found that the distal root portions of bean (1-16 mm in length, measured from the apex of root) had a repellent effect on the nematode, whereas the distal root portions of eggplant and soybean showed a random variation of their effect, some of the roots being attractive, some repellent, and some neutral. He interpreted the effect of the roots on the nematodes as the result of an interplay between a repellent and an attractive agent, the latter being present in the living plant and the former possibly coming into play with the decay or chemical breakdown of the root. Peacock (1959) investigated the resistance of L. peruvianum to M. incognita. He found that larvae of this nematode

were attracted to excised root tips of L. peruvianum a little less strongly than they were to roots of susceptible tomato (L. esculentum); the larvae also penetrated L. peruvianum root tips in lesser numbers. The larvae developed in the resistant roots at a slower rate than in the susceptible. In some resistant roots there was little or no development of the larvae and no swelling; in the swellings which did occur there was no more than one developing female as compared with up to 8 in the swellings on roots of L. esculentum.

(b) Reduced larval penetration. Dean and Struble (1953) investigated the resistance and susceptibility to root-knot nematode, M. incognita in tomato. They found that root systems of resistant L. peruvianum and L. peruvianum hybrid were invaded by fewer larvae, usually half or less, than those of susceptible Marglobe tomato. Nematodes entering resistant roots produced extensive necrosis of host tissue within 48 hours. Two weeks after inoculation most of the invading larvae had died. No larvae in a resistant root ever developed as far as the second molt.

(c) Failure of host response to the parasite. Host reaction is necessary for continued development of species of Meloidogyne. The presence of the

parasite may induce the host to produce compounds which activate enzymes of the parasite which in turn break down host compounds, thus, leading to the production of an environment suitable for development of the parasite. This is clearly demonstrated by the work of Barrons (1939), Christie (1949), and Dropkin and Nelson (1960) who showed that production of giant cells was necessary for the development of root-knot nematode females. Dropkin and Nelson (1960), using 19 different varieties of soybean exposed to M. incognita incognita, and M. incognita acrita, found that the same stimulus exerted by a larva will produce giant cells in one host and invoke necrosis in another. The authors classified the host reaction into 4 types: Type 1 refers to a hypersensitive reaction in which cells immediately around the larva die, and no further development occurs. Type 2 cells undergo moderate cell fusion and display unusually great numbers of cell inclusions of various forms. Type 3 cells are very large, with many nuclei, and with a diffuse, highly vacuolated cytoplasm. Type 4 cells consist of large, thick-walled multinucleate units with dense cytoplasm and few cell inclusions. Type 1, 2 and 3 cells are always associated with poorly developed parasites with few eggs, whereas type 4 cells are associated with rapid parasite growth and abundant egg production.

Peacock (1959) listed the following 5 ways in which an unknown chemical in a plant may be responsible for resistance of the plant to root-knot nematode attack:

1. By masking the attractant substance in the root, or by actively repelling the nematode.

2. By killing the nematode on entry, or retarding its development.

3. By neutralising the effect of nematode saliva on giant-cell formation.

4. By changing the composition of the cell-wall, so that nematode saliva is no longer effective, or the cell-wall is impenetrable to the nematode stylet.

5. By upsetting the sex-ratio of the nematode, either physiologically or by eliminating the females.

A high proportion of males has often been shown to be a characteristic of root-knot nematode populations of resistant plant varieties (Triantaphyllou, 1960). This appears to be an effect rather than a cause of resistance, but it works to the advantage of the plant.

(d) Hypersensitive tissue reactions.

Hypersensitive tissue reactions have been reported for resistance of soybean to M. incognita incognita and M. incognita acrita (Dropkin and Nelson, 1960),

and of snap bean (Phaseolus vulgaris L.) to M. incognita acrita (Fassuliotis et al., 1967). The hypersensitive cells die so quickly after attack by the nematode that the parasite is effectively walled off and injury to the host is confined to a few cells.

These few examples of the concept of resistance emphasize that no generalisation can be applied to resistance in a particular host-parasite relationship. The evidence is in favour of the work by Riggs and Winstead (1958) who attempted to transfer root-knot resistance in tomato, inoculated with M. incognita, by grafting. They found that a susceptible scion on resistant stock showed little or no root galling, but in most cases showed leaf galling; resistant scion on susceptible stock showed severe root galling but no leaf galling; intact susceptible plants showed galling of roots and leaves; and intact resistant plants showed little or no root galling and no leaf galling. They, therefore, suggested that the resistance or susceptibility factor(s) was inherent within individual cells in both the roots and tops of plants and either was not translocated or did not cross the graft union.

To produce plants resistant to root-knot nematodes, however, involves several stages in

research. Wallace (1963) listed the following 6 stages in which breeding for nematode-resistant plants could be approached:

1. The nematode must be defined as clearly as possible because different races or species may produce different symptoms in the species of plant. In any case, it is always advisable to assess resistance with several nematode populations from different localities to see if racial differences occur. The status of the plant itself must likewise be known.

2. It is necessary to ensure that laboratory or greenhouse techniques for the assessment of resistance provide maximum opportunity for the plants to react to the nematodes. Thus, physical conditions should be optimal for hatching, movement, invasion and development. Where tests have to be done initially on a field scale, the problem is more difficult, because uniformity of infection and control of the environment is almost impossible. In these cases well randomised and statistically designed plot experiments seem to be the only answer.

3. Valid criteria for the assessment of resistance must be devised which allow only the minimum of subjective appraisal. Data on nematode pathogenicity and symptoms of the infested plant provide such information and nematode population studies will indicate whether the plant is immune,

tolerant or resistant.

4. Different varieties or closely related species of the plant being studied are then tested for resistance and some idea is obtained of the genetics of inheritable resistance, so that a plant breeding programme can be started.

5. Crosses and backcrosses are made with potentially useful plant material until a variety is obtained which appears to fulfil the requirements of a nematode-resistant plant and which still has commercially desirable qualities.

6. The resistant plant is tested under field conditions in different localities and soil types to assess the influence of environment on resistance. The population dynamics of the nematode under the resistant crop should also be studied because it may not be advisable to grow the resistant types continuously if there is a danger that other races or closely related species will be selected out and multiply.

As increasing emphasis has been placed on efforts to provide acceptable varieties of agriculturally important plants that are resistant to root-knot nematode, plant breeders have, therefore, successfully incorporated resistance to root-knot nematode into various crops, including beans. Isbell (1931) found resistance in 2 strains of pole

beans (Phaseolus vulgaris L.), Alabama No.1, and Alabama No.2 to species of Meloidogyne (referred to then as H. marioni). Isbell's observations were confirmed by Barrons (1938) who further noted that, although Alabama No.1 and Alabama No.2 beans were highly resistant to H. marioni, both in the adult and seedling stage; occasional slight swellings, which never developed into galls, were formed immediately behind the root tip of these beans when grown in soil badly infested with the nematode. This observation raised the question as to whether Alabama No.1 and Alabama No.2 beans resist the entry of nematodes into their roots with the exception of occasional larvae which induce these slight swellings, or whether larvae enter but fail to induce the formation of giant cells and the resulting galls. Barrons (1939) then conducted a study on bean rootlets in order to determine whether nematode larvae actually enter root-knot resistant varieties and, if so, at what rate as compared with susceptible varieties. He found that there were no significant differences between the rate of entry in, respectively, the most resistant and susceptible Alabama No.1, and Kentucky Wonder beans in the seedling stage. Barrons (1940), therefore, initiated a bean breeding experiment designed to combine the root-knot resistance of Alabama No.1 with desirable traits possessed by other pole bean varieties. He

concluded that the resistance of root-knot in Alabama No.1 bean is inherited as a double recessive trait; it also appears probable that the inheritance is on a quantitative basis with all individuals possessing two or more dominant genes governing susceptibility to root-knot, and those with one dominant gene appearing intermediate.

After the texonomic revision of the species of the root-knot nematode by Chitwood (1949), and the adoption of the generic name, Meloidogyne, Blazey et al. (1964) re-examined bean plants for root-knot resistance. They found that, among the bean varieties tested and recognized as the most resistant to H. marioni, including Isbell's Alabama No.1, all were susceptible to M. hapla, M. javanica, M. arenaria, and M. arenaria thamesi with resistance confined only to M. incognita. They confirmed Barrons' (1940) conclusion that resistance was due to the interaction of two independent recessive genes. Fassuliotis et al. (1967) have, however, reported a new source of resistance to M. incognita acrita for use in the development of a bush type snap bean. The investigators found that selections in F4 and F5 from a hybrid (B-3864) of snap bean were highly resistant to the root-knot nematode, M. incognita acrita in both greenhouse and field evaluations. The hybrid was developed from a cross

between a susceptible bush type breeding line X PI-165426 (a resistant source from Mexico). Significantly fewer females were recovered from roots of B-3864 than from Black Valentine, a susceptible variety, or from Alabama No.1, a resistant variety. Although roots of B-3864 were readily penetrated by the larvae, and the root tips showed slight swellings at infection loci, visible external necrosis became apparent 4-6 days after infection; the majority of the larvae did not develop beyond the second stage. From histopathological studies, this was attributed to a reduced amount of syncytial development and/or to a hypersentive reaction of tissues surrounding the head of the nematode. Recently, Fassuliotis et al. (1970) have also reported another source of resistance to M. incognita. They found that, from their initial screening tests which included over 1100 Plant Introductions, cultivars, and breeding lines, PI-165426 was selected as having the greater potential as a source of resistance to the root-knot nematode, M. incognita. Although Alabama No.1 has a high level of resistance to M. incognita, PI-165426 was considered a better parent because it also had resistance to fungus, R. solani reported by McLean et al. (1968).

Fassuliotis et al. (1970) also noted that

temperature was an important factor to be considered when breeding for resistance. They found that resistance decreased with an increase in temperature from 16 to 28°C, although the number of females recovered at the highest temperature did not reach that of the susceptible cultivars. They did not, however, assess whether higher temperatures under field conditions would significantly increase susceptibility in their resistant lines. They also encountered two major problems in breeding snap beans for nematode resistance. They found that (1) host resistance and galling response were apparently controlled by separate genetic mechanisms, and (2) bean plants were very sensitive to desturbances of the roots. They stated that the first problem was not as serious as the second, but if its existence was not recognized, it could cause delays in the breeding programme. Plants were found that were completely free of galls, although they were very heavily infested with nematodes. Selection of these plants resulted in progeny which did not form galls, but which were nevertheless susceptible. When susceptible, non-galling plants were used as parents, their progeny segregated for galling, but they were uniformly susceptible to infection. Selection of non-galling plants rapidly eliminated the gall response, but did not increase resistance. Selection of susceptible, non-galling plants was avoided by

examining the roots after 30 days which allowed enough time for females to mature and form egg masses. In order for them to see the egg masses and mature females readily, it was necessary to remove nearly all adhering soil, which created a severe shock to the month-old plant and, in a sense, created the second problem. They observed that, in contrast to tomato which can be uprooted, examined, and replanted at almost any stage and still be vigorous enough to use in a crossing programme, beans which are uprooted after more than a few days of growth are seriously stunted and will produce only a few seeds. This factor makes it difficult to make crosses to a resistant parent, so crosses are usually made to plants whose parents were considered resistant.

Although the use of resistant crop varieties could offer the best and most economical method of root-knot nematode control on beans in Kenya, it appears from the discussion that breeding for resistance to the nematode is, however, complex and sometimes may necessarily proceed in conjunction with breeding for resistance to other pathogens. To be of value, a variety must be resistant to the nematode species prevalent in the region concerned, and, in addition, have acceptable agronomic qualities, yield well, produce food of good quality, and in general be more profitable for the farmer than the

standard varieties. The production of nematode-resistant varieties must be, therefore, a cooperative effort between nematologists, plant breeders, agronomists, and pathologists. The closest cooperation between these scientific disciplines should be encouraged in this country if bean varieties resistant to the species of root-knot nematodes present are to be developed. If resistance to the nematode species cannot be found, it might be possible to develop a nematode-tolerant bean variety which can produce a good crop where the susceptible varieties can only produce a poor crop.

It is suggested that further research on the control of root-knot nematodes on beans in Kenya is both desirable and necessary. Experiments should be sited in several places in both low (Coastal Region) and intermediate altitude areas (1,500 m, and below) to screen all available bean germplasm for resistance to species of root-knot nematodes present. It is suggested that resistance in beans be evaluated by the "root-knot index" method, that is, by observing the incidence of gall formation on plants grown to maturity in soil heavily infested with root-knot nematodes. Infection categories for recording data should be similar to those used by Smith (1941): Class 0 = all roots without visible galls, Class 1 = 1 to 25% of roots with galls, Class 2 = 26 to 50%,

Class 3 = 51 to 75%, and Class 4 = 76 to 100% of roots with galls. Continued testing of resistance of beans and other food or cash crops should provide valuable data on the habits and locations of the various races of root-knot nematodes. It would also provide information of great practical value for the planning of rotations in Kenya.

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APPENDIX 1

Analysis of variance of effects of D-D and EDB application on numbers of *Meloidogyne* larvae; Kenya Canners Plantation, Thika, 1971.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Replicates	4	1.846820	0.461705	4.41**
Varieties	2	0.885359	0.442680	4.22*
Treatments	4	5.344950	1.336238	12.75***
V X T	8	12.142046	1.517756	14.48***
Error	56	5.867931	0.104784	
Total	74	26.087106		

* Significant at P = 0.05

** Significant at P = 0.01

*** Significant at P = 0.001

APPENDIX 2

Analysis of variance of effects of D-D and EDB application on mean yields (kg/plot) of Kikara and Mexico 142; Kenya Canners Plantation, Thika, 1971.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Replicates	4	9.544812	2.386203	5.11**
Varieties	1	1.936512	1.936512	4.15*
Treatments	4	7.422812	1.855703	3.97**
V X T	4	1.974908	0.493727	1.06 N.S.
Error	36	16.818348	0.467176	
Total	49	37.697392		

* Significant at P = 0.05

** Significant at P = 0.01

N.S. = Not significant

APPENDIX 3

Analysis of variance of effects of D-D application on mean yields (kg/plot) of Mexico 142; Kuraiha Estate, Thika, 1971-72.

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Replicates	5	0.8330	0.1666	1.58 N.S.
Treatments	1	1.8019	1.8019	17.09**
Error	5	0.5272	0.1054	
Total	11	3.1621		

** Significant at P = 0.01

N.S. = Not significant

APPENDIX 4

Analysis of variance of percentage penetration of *Meloidogyne incognita* and *M. javanica* larvae in roots of Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon and Premier bean varieties. (*M. incognita* inoculum consisted of larvae derived from egg masses; *M. javanica* inoculum consisted of larvae derived from soil).

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Replicates	4	831.77	207.94	1<
Varieties	5	480.35	96.07	1<
Treatments	1	19332.15	19332.15	80.82***
V X T	5	111.95	22.39	1<
Error	44	10524.63	239.20	
Total	59	31280.85		

*** Significant at P = 0.001

APPENDIX 5

Analysis of variance of percentage penetration of *Meloidogyne incognita* and *M. javanica* larvae in roots of Kikara, Mexico 142, Canadian Wonder, Masterpiece, Marathon and Premier bean varieties. (Inoculum of both species derived from egg masses).

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Squares	F
Replicates	4	543.10	135.78	1<
Varieties	5	2127.55	425.51	1.602 N.S.
Treatments	1	6678.15	6678.15	25.145***
V X T	5	1437.95	287.59	1.083 N.S.
Error	44	11686.10	265.59	
Total	59	22472.85		

*** Significant at P = 0.001

N.S. = Not significant

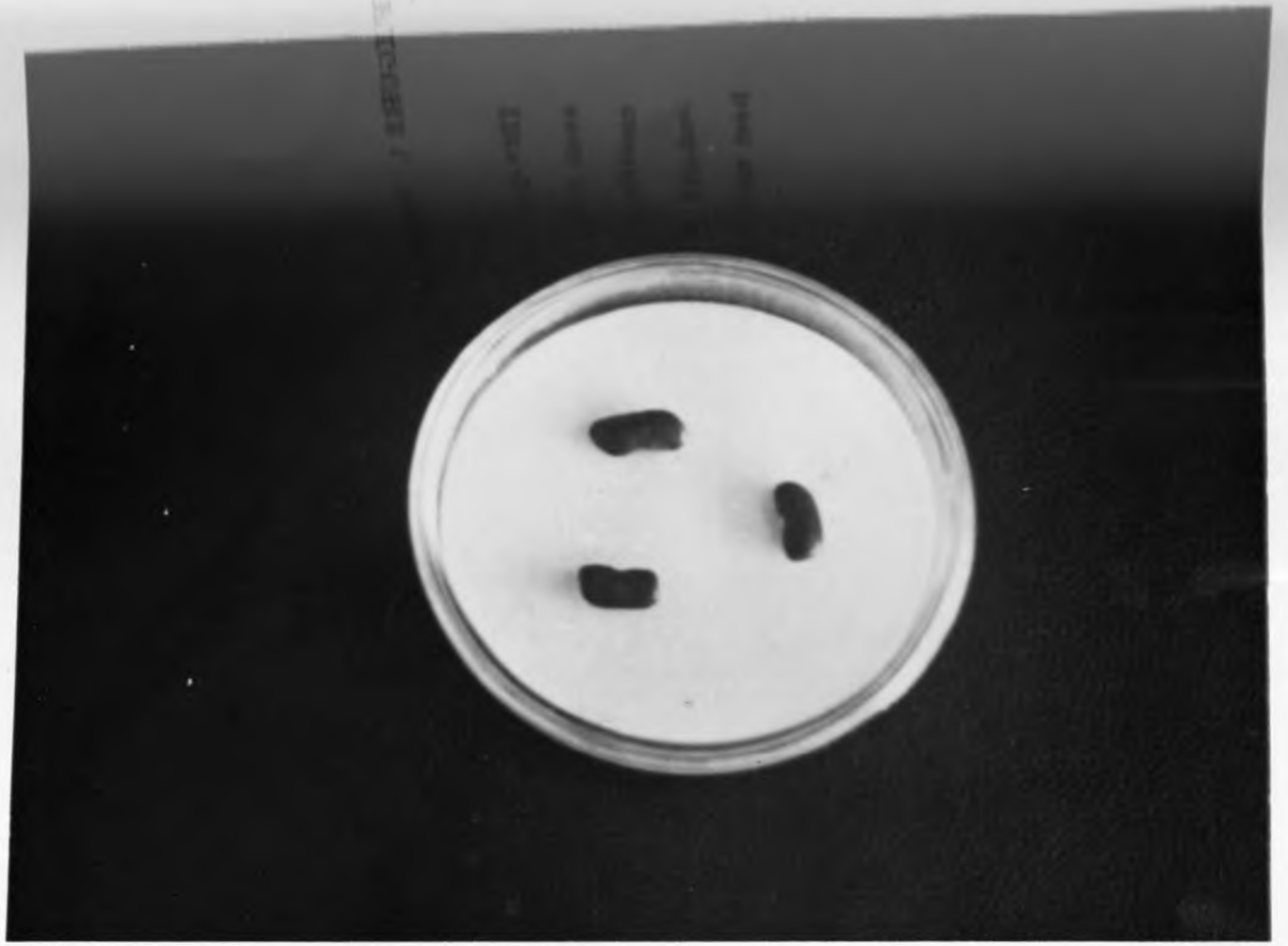


FIGURE 1. DISINFESTED BEAN SEEDS

Three disinfested Canadian Wonder bean seeds in a sterilized petri dish containing a filter paper, ready for incubation. (Representative of all bean varieties used in this study).

FIGURE 2. BEAN SEEDLINGS

Bean seedlings transplanted into sterilized sandy soil in paper cups after pregermination for 60 hours, and kept in a growth chamber with a thermograph for recording temperature.

FIGURE 3. BEAN NEMATICIDE EXPERIMENT

A bean nematicide experiment at Kenya Canners Plantation, Thika: foreground, Mexico 142 untreated plot; background, Mexico 142 plot treated with D-D broadcast (336.8 l/ha).

FIGURE 4. BEAN ROOTS FROM NEMATICIDE EXPERIMENT

Bean roots from the nematicide experiment at Kuraiha Estate, Thika: left, healthy root systems of Kikara dug from a plot treated with D-D in the row; right, heavily infested root systems of Kikara with root-knot nematode, M. incognita dug from an untreated plot.

FIGURE 5. KIKARA BEAN PLANTS

Thirty-day-old Kikara bean plants (greenhouse pot test): left, stunted plant grown in soil heavily contaminated with a mixed population of the root-knot nematodes, M. incognita and M. javanica; right, healthy plant grown in sterilized soil.

FIGURE 6. KIKARA BEAN ROOT SYSTEMS

Thirty-day-old Kikara bean root systems (greenhouse pot test): left, healthy roots grown in sterilized soil; right, stunted and galled root systems grown in soil heavily infested with a mixed population of the root-knot nematodes, M. incognita and M. javanica.

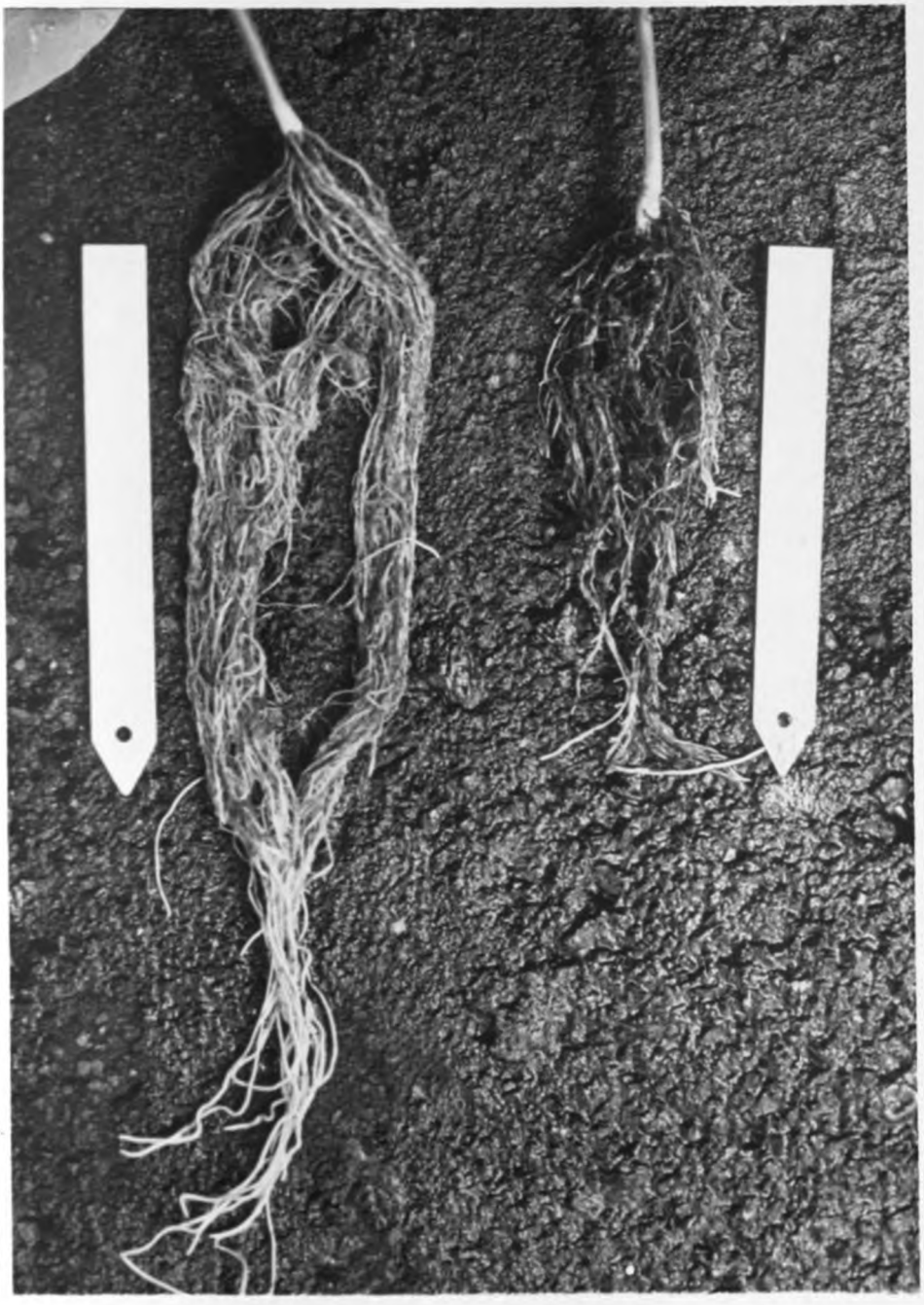


FIGURE 7. BEAN ROOT SYSTEM

Mexico 142 root system,
3-4 cm long, after incubation
at 25°C for 60 hours.

FIGURE 7. BEAN ROOT SYSTEM

Mexico 142 root system,
3-4 cm long, after incubation
at 25°C for 60 hours.

FIGURE 8. BEAN ROOT SYSTEM

Kikara root system,
2-2.5 cm long, representing
a typical root system, after
incubation at 25°C for 60 hours.

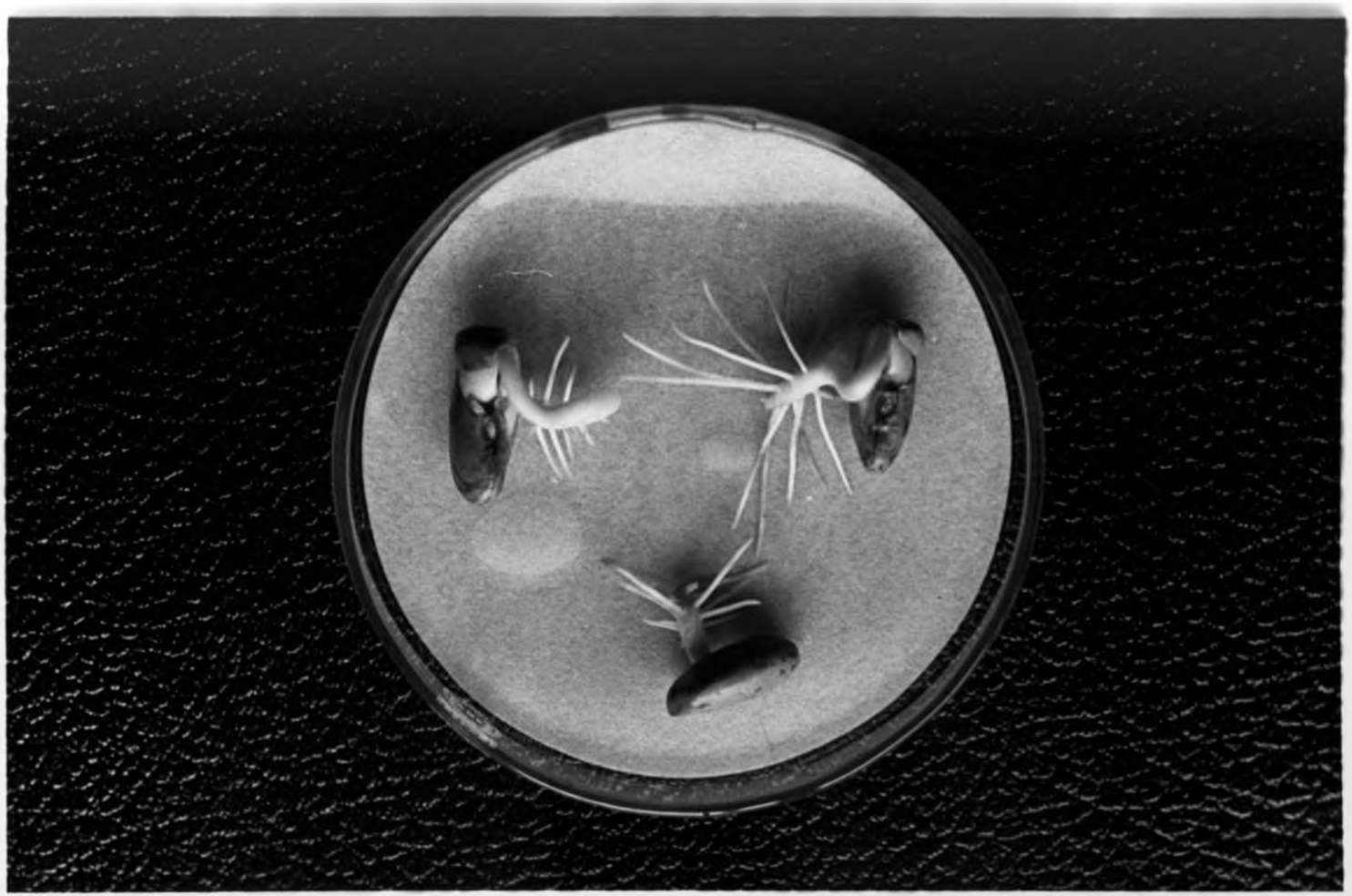


FIGURE 9. NON-INFECTED BEAN ROOT SECTION

Longitudinal section of a non-infected root of a Canadian Wonder bean plant 20 days after germination (X 88).

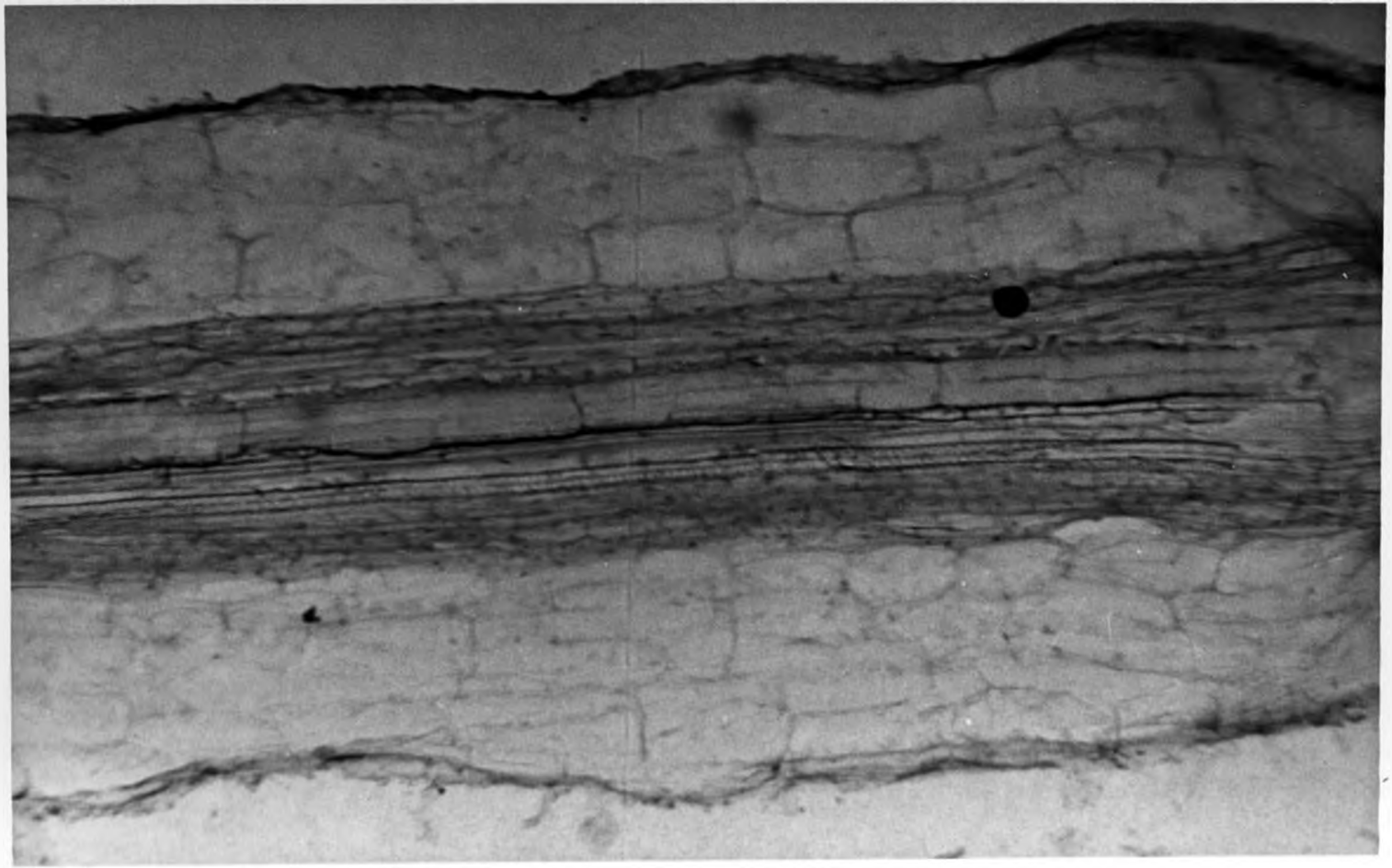


FIGURE 10. NON-INFECTED BEAN ROOT SECTION

Transverse section of a non-infected root of a Mexico 142 bean plant 30 days after germination (X 88).

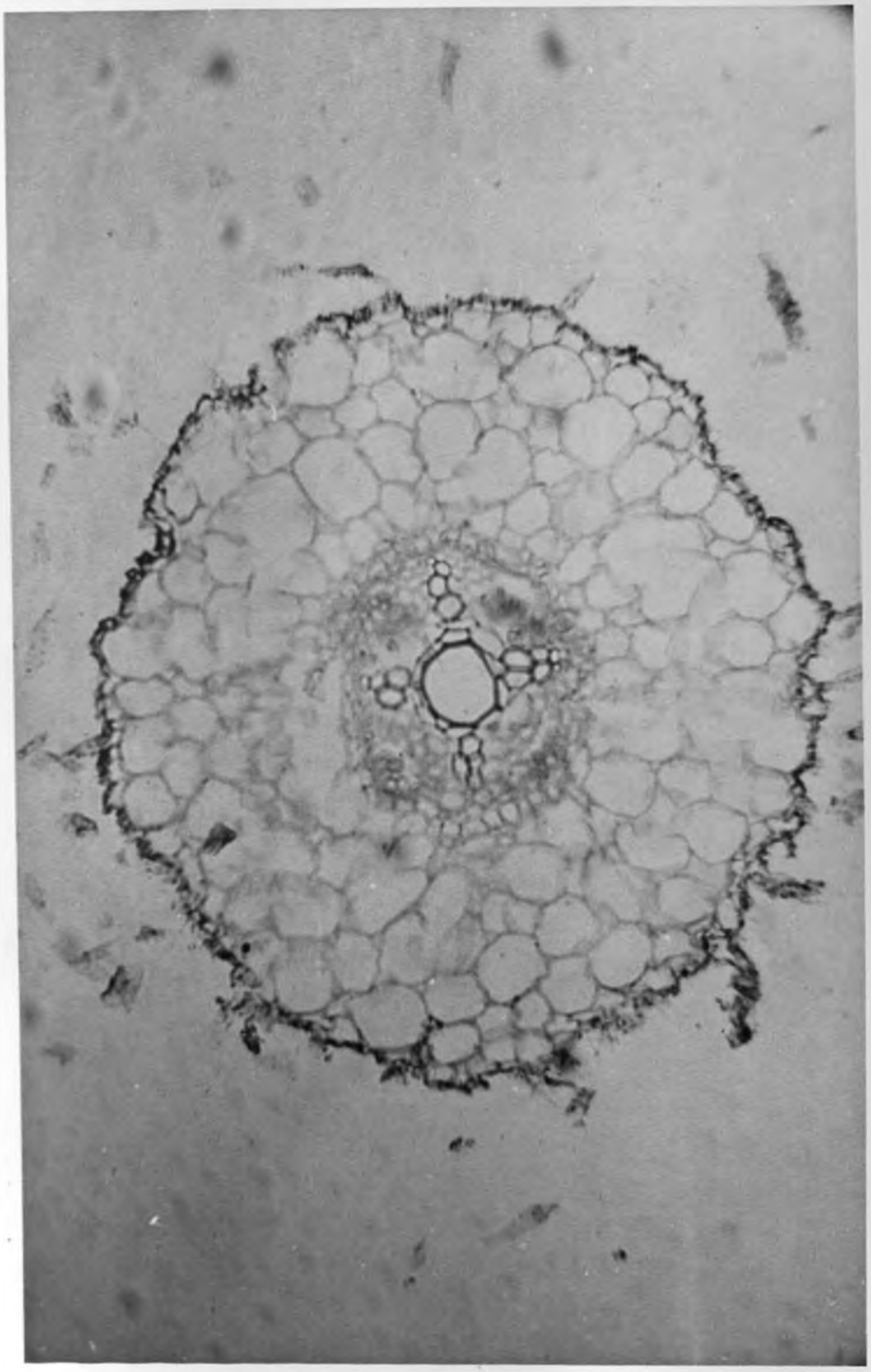


FIGURE 11. INFECTED MEXICO 142 BEAN ROOT SECTION

Longitudinal section of a root of a Mexico 142 bean plant 10 days after inoculation showing early second-stage larva of M. incognita migrating, after penetration, both intercellularly through the cortex, and intracellularly to the stele of the root (X 88).

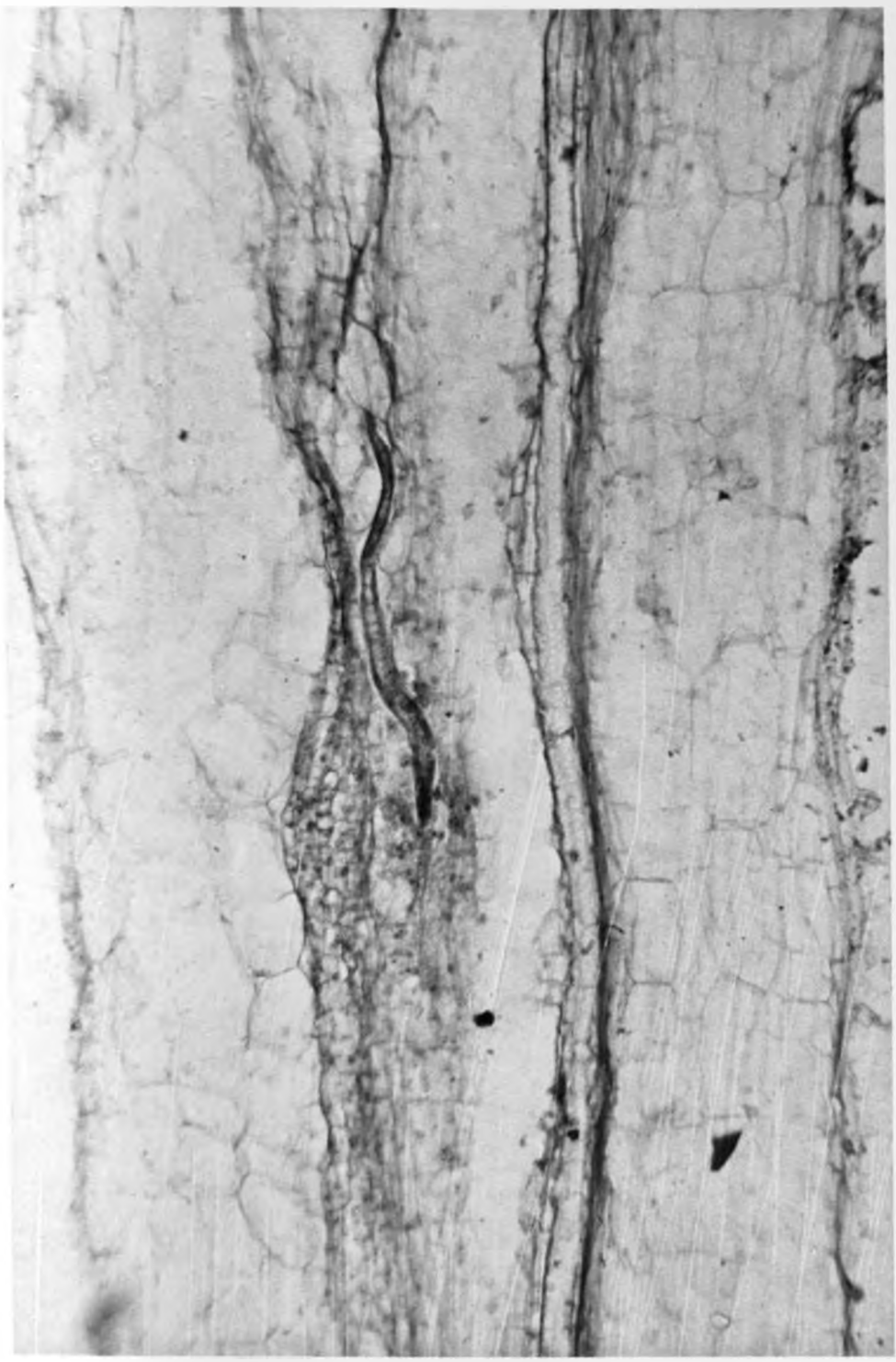


FIGURE 12. INFECTED PREMIER BEAN ROOT SECTION

Transverse section of a root of a Premier bean plant 10 days after inoculation showing: head of a late second-stage larva of *M. javanica* inserted into the vascular system, with the remaining sausage-shaped portion of the larva located in the cortical cells; giant cells around the head of the larva in the phloem and xylem parenchyma cells; and hypertrophy of the cortical and hyperplasia of the phloem and xylem parenchyma cells (X 88).

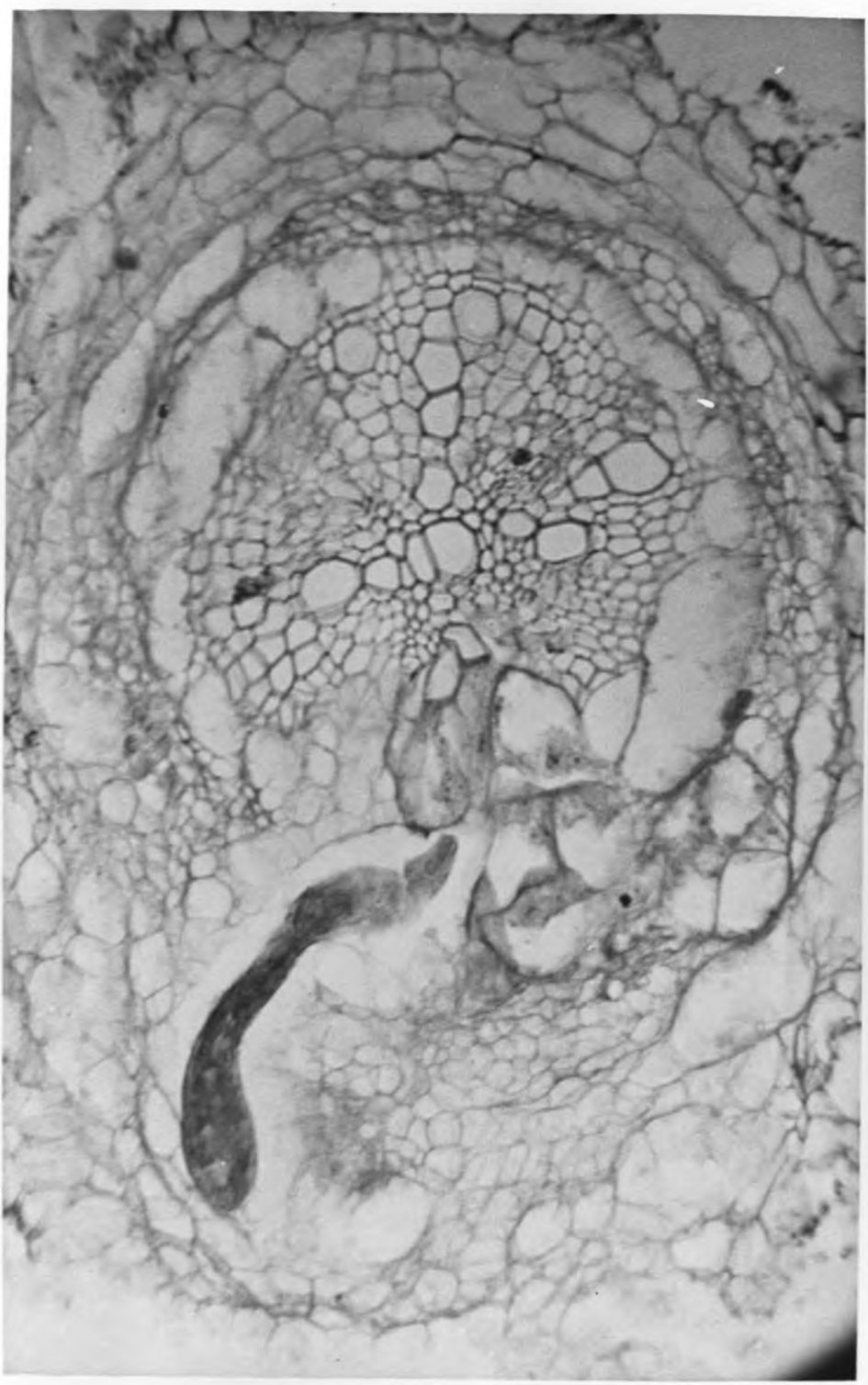


FIGURE 13. INFECTED MASTERPIECE BEAN ROOT SECTION

Longitudinal section of a root of a Masterpiece bean plant 20 days after inoculation showing: head of an adult female of *M. javanica* inserted into the vascular system; coalesced phloem and xylem cells, with the stele more or less completely transformed into dense giant cells; and disrupted cortical cells caused by the increase in size of the mature female nematode (X 88).

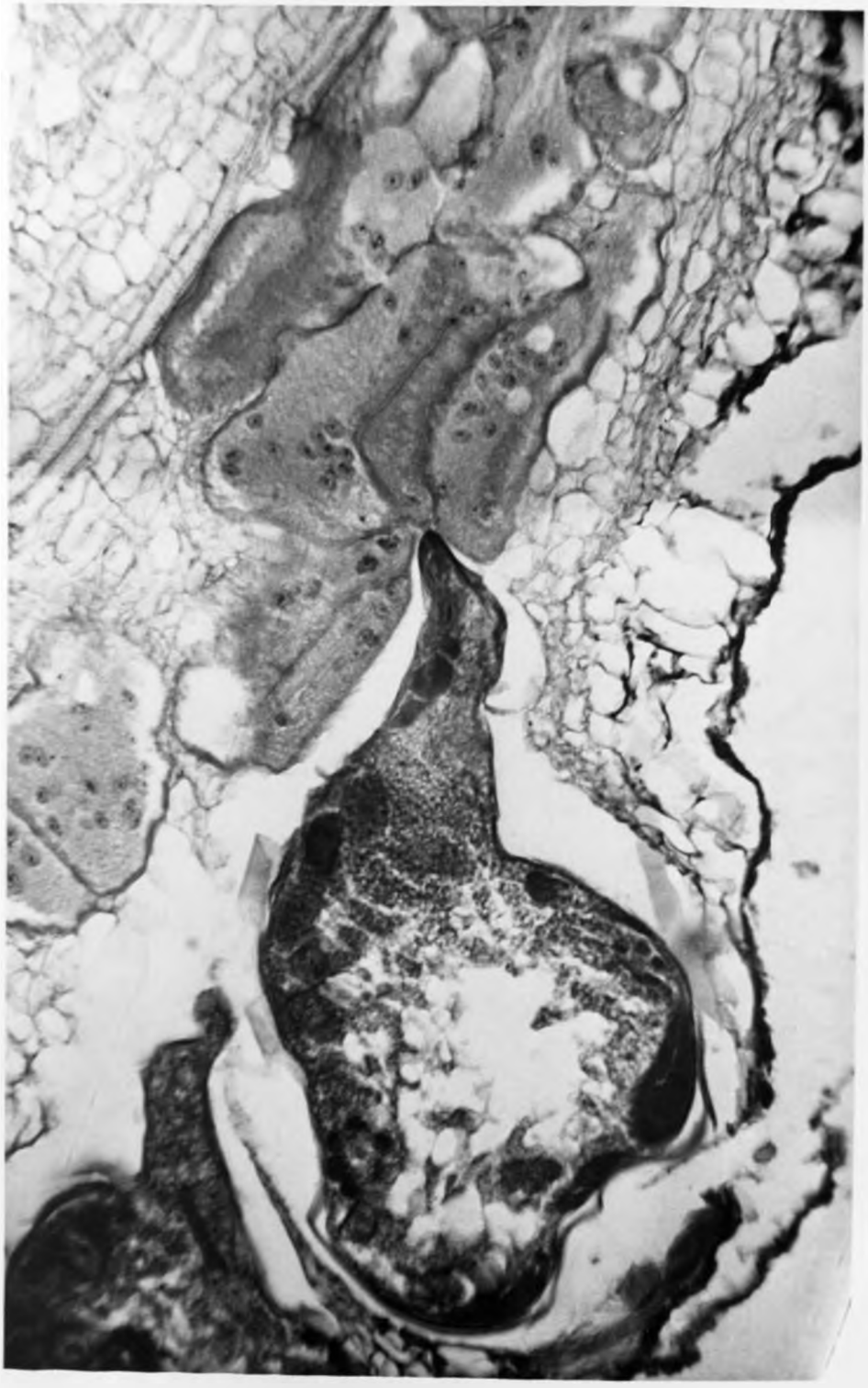


FIGURE 14. INFECTED KIKARA BEAN ROOT SECTION

Transverse section of a root of a Kikara bean plant 30 days after inoculation showing: head of an adult female of M. incognita inserted into the vascular system, with giant cells around the head of the female nematode; and collapsed epidermal and disrupted cortical cells forming a cavity caused by the increase in size of the mature female and its egg masses (X 88).

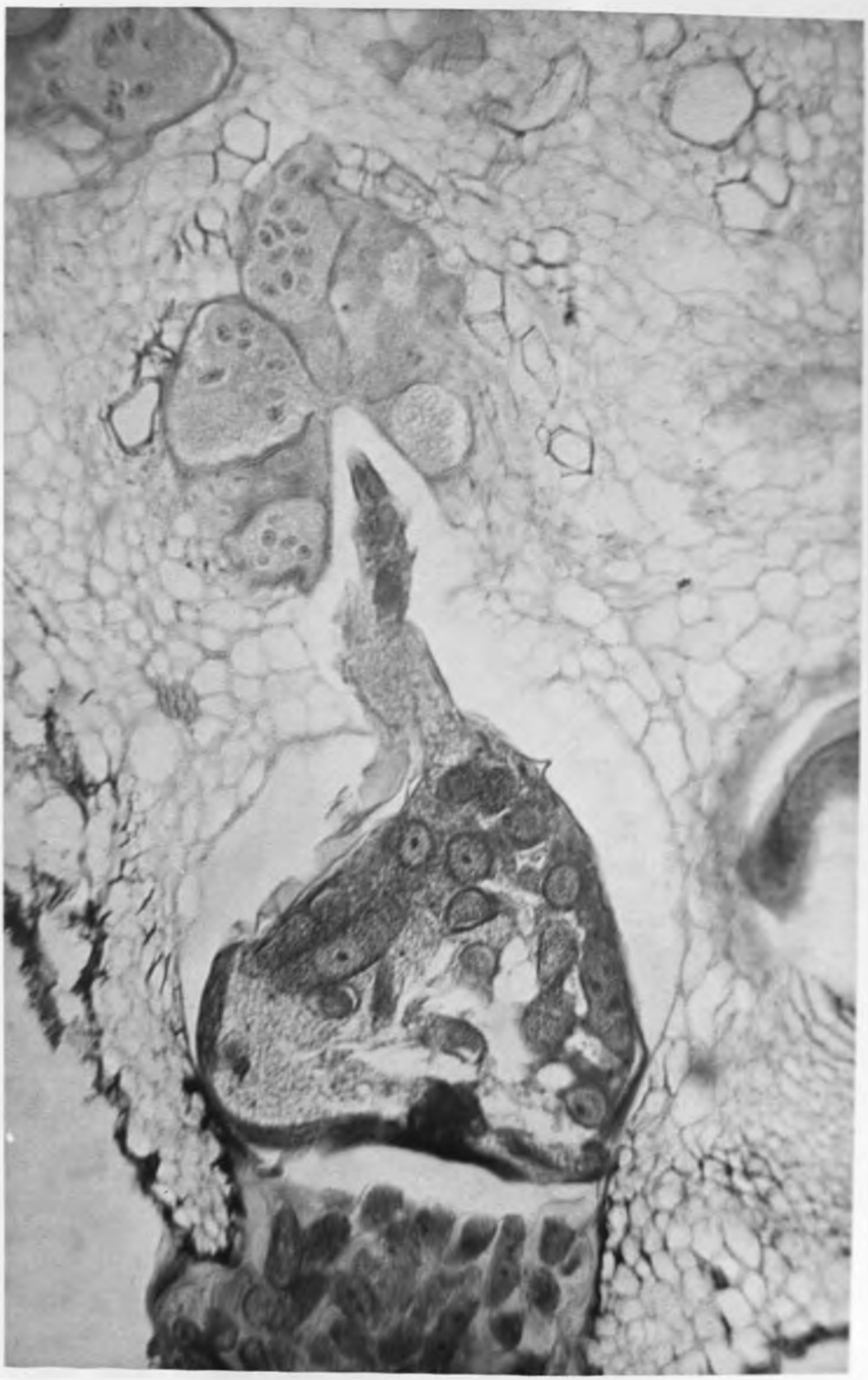


FIGURE 15. INFECTED MEXICO 142 BEAN ROOT SECTION

Transverse section of a root of a Mexico 142 bean plant 40 days after inoculation showing collapsed epidermal and disrupted cortical cells forming a cavity caused by the increase in size of an adult female of M. javanica and its egg masses (X 88).

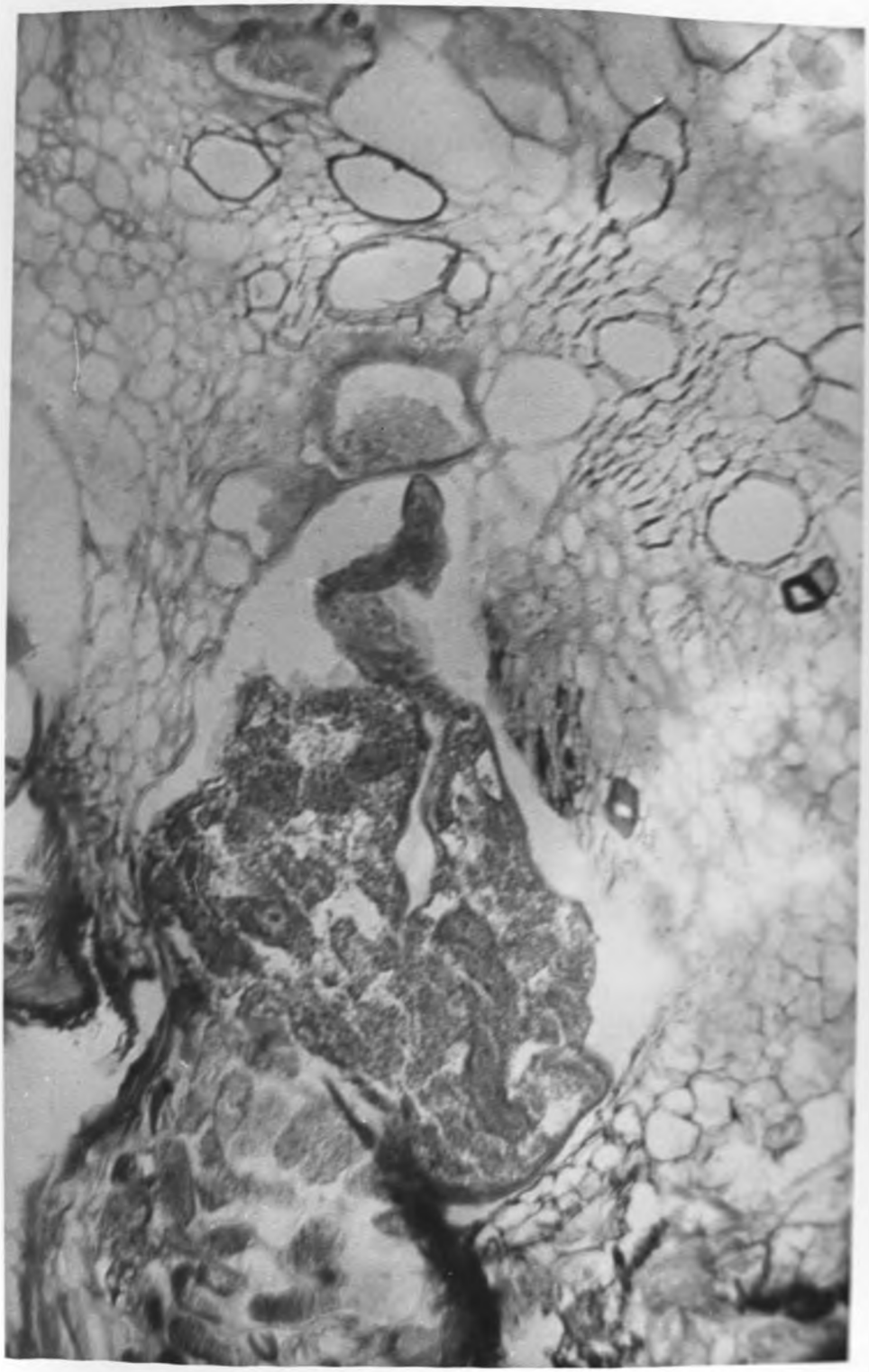


FIGURE 16. INFECTED MARATHON BEAN ROOT SECTION

Transverse section of a root of a Marathon bean plant 10 days after inoculation showing growth of lateral roots in the proximity of galls, induced by an infective larva of *M. javanica* (X 88).

Power

Start

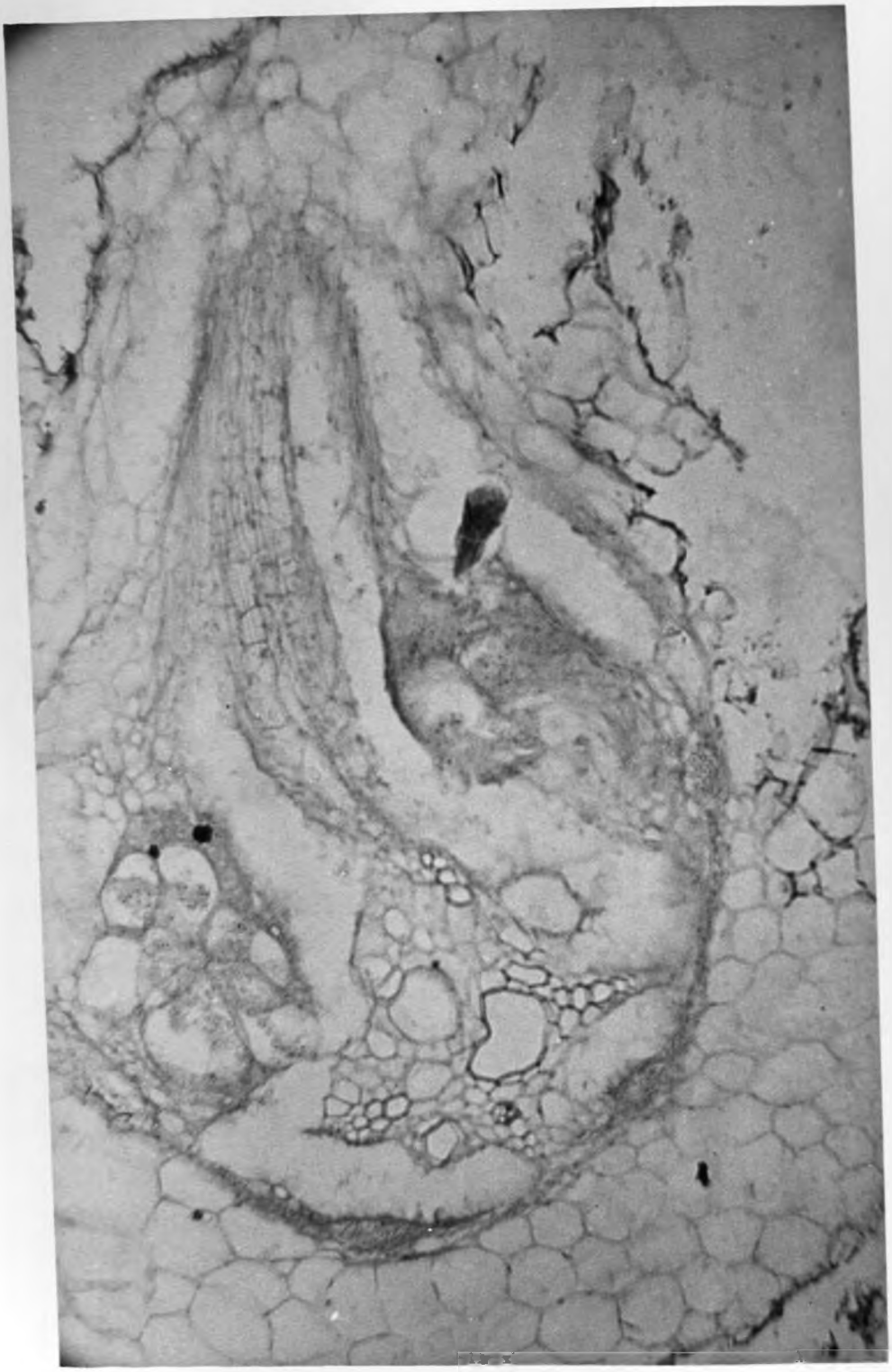


FIGURE 17. INFECTED CANADIAN WONDER BEAN ROOT SECTION

Longitudinal section of a root of a Canadian Wonder bean plant 10 days after inoculation showing growth of lateral roots in the proximity of galls, induced by an infective larva of *M. incognita* (X 88).

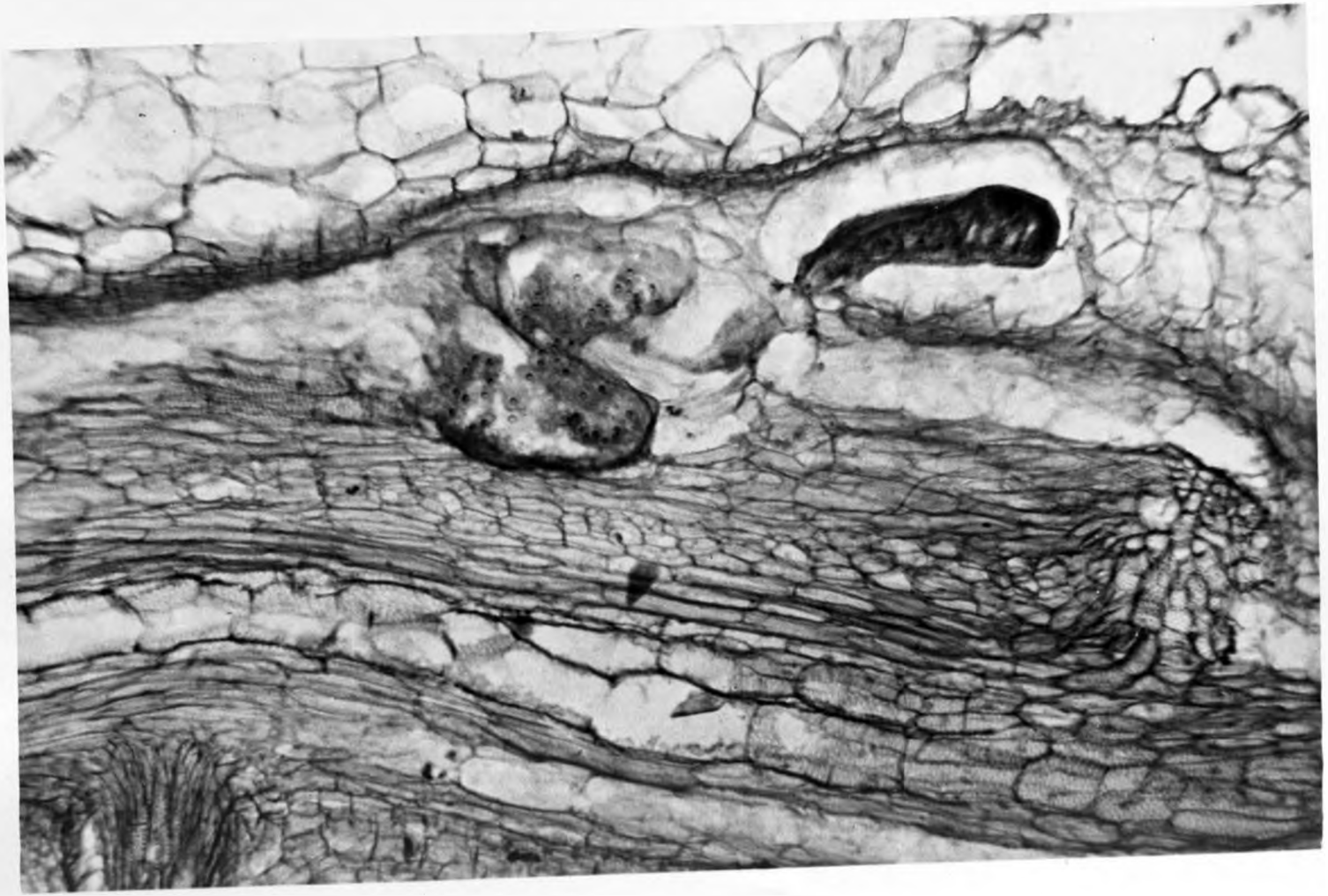


FIGURE 18. INFECTED MARATHON BEAN ROOT SECTION

Transverse section of a root of a Marathon bean plant 20 days after inoculation showing coalesced phloem and xylem cells, with the stele more or less completely transformed into giant cells induced by the feeding of an adult female of *M. incognita* (X 88).

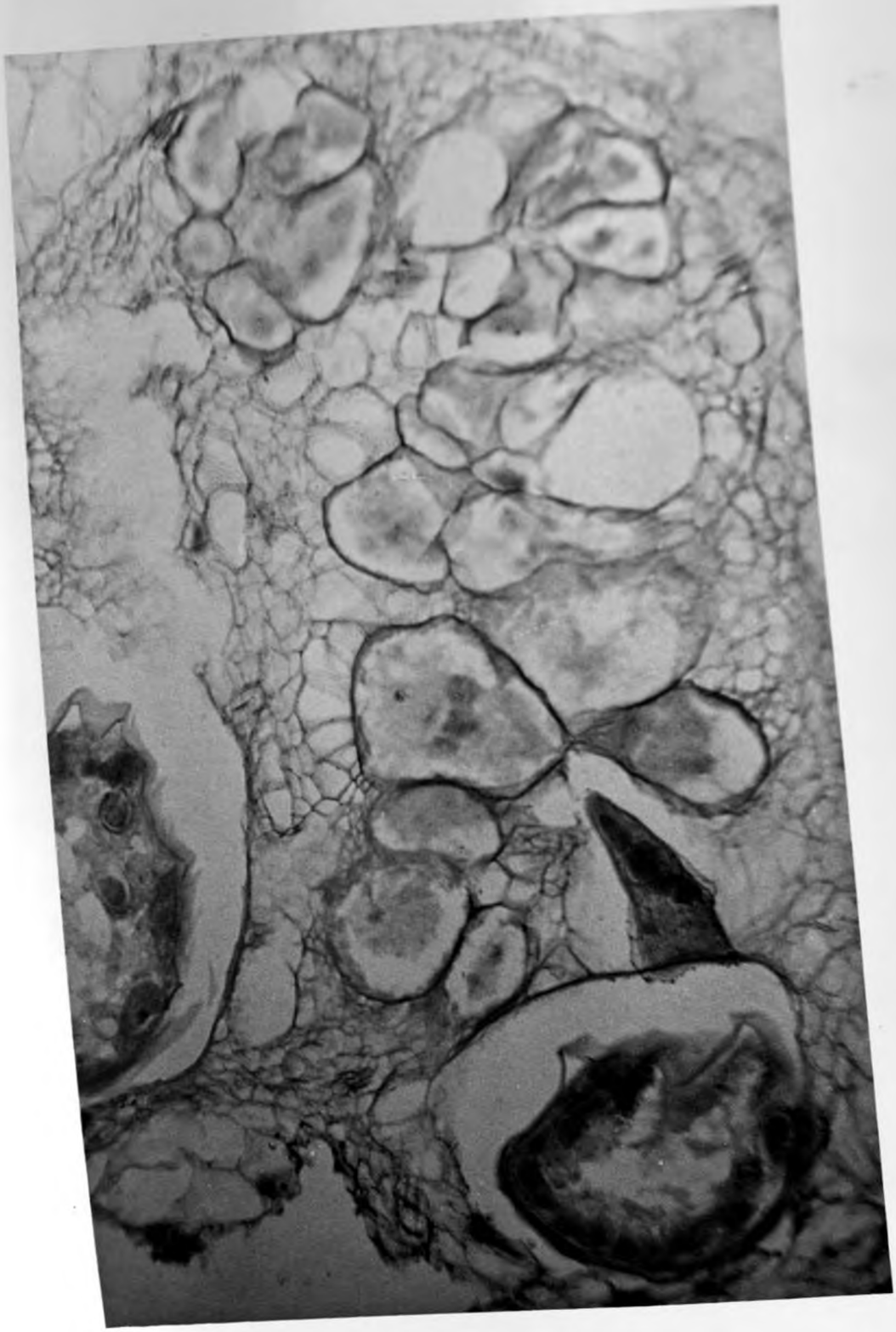
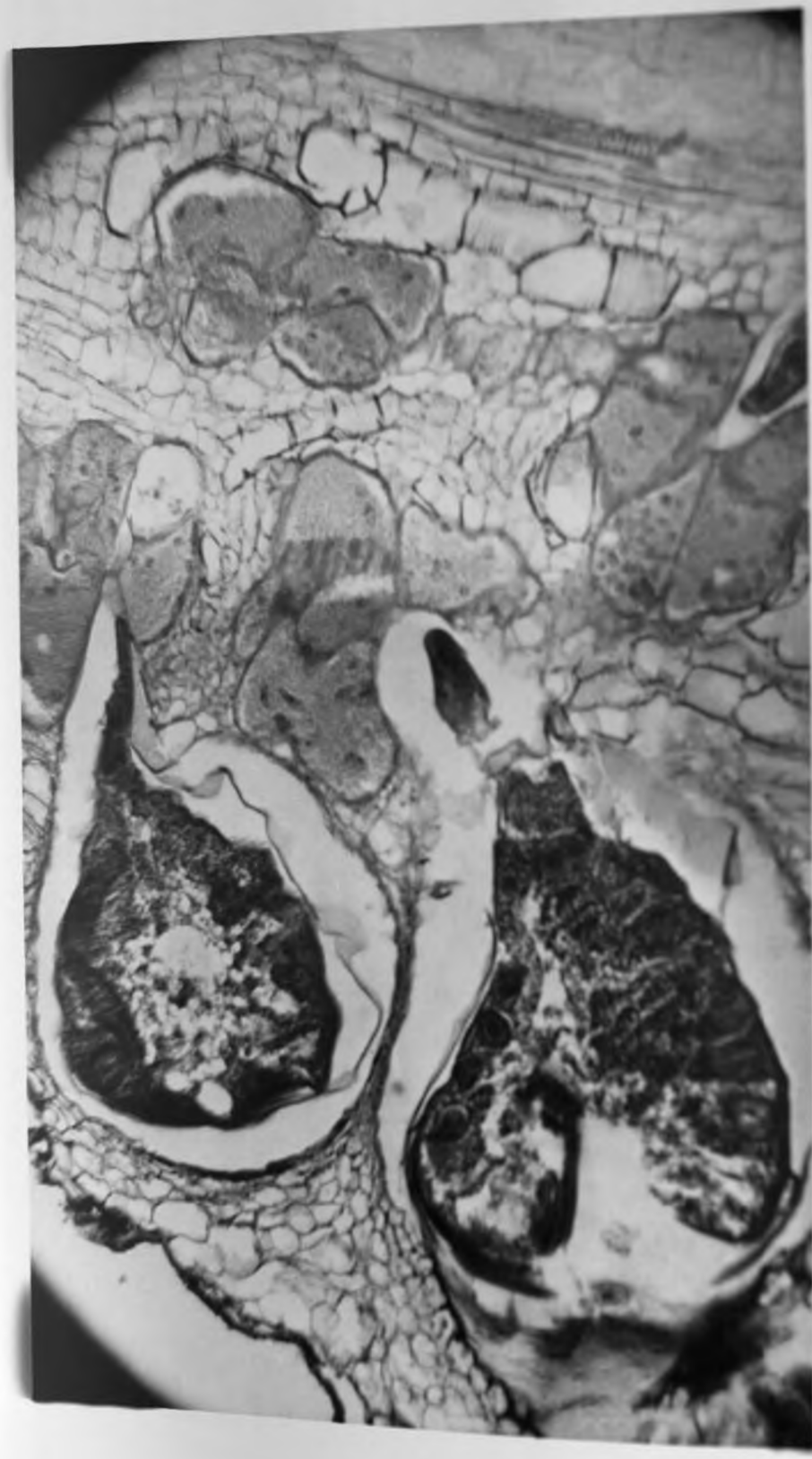


FIGURE 19. INFECTED CANADIAN WONDER BEAN ROOT SECTION

Longitudinal section of a root of a Canadian Wonder bean plant 20 days after inoculation showing two adult nematode females of M. javanica feeding in the vascular tissues in close proximity, resulting in the extensive formation of giant cells (X 88).



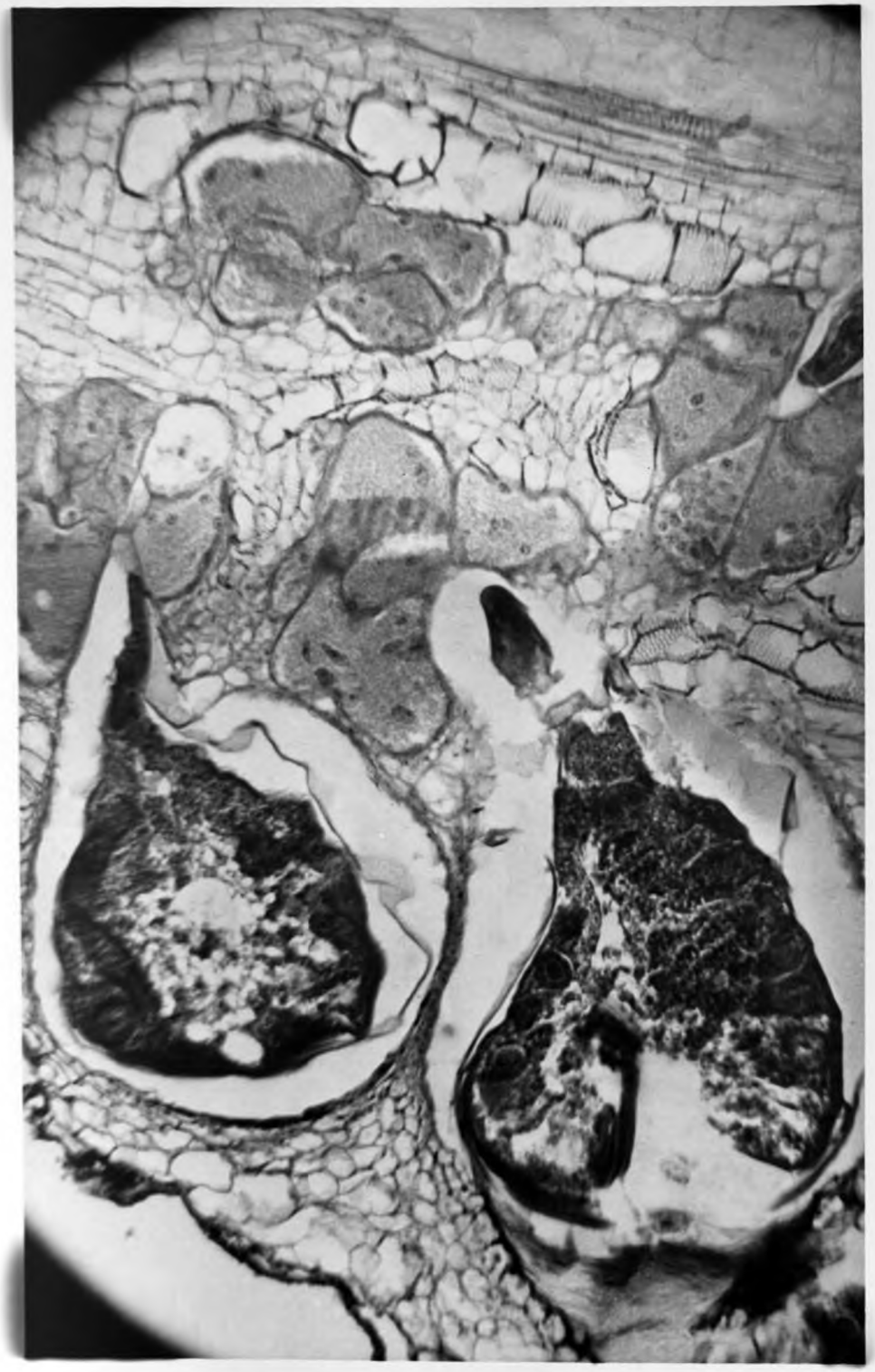


FIGURE 20. INFECTED MEXICO 142 BEAN ROOT SECTION

Transverse section of a root of a Mexico 142 bean plant 30 days after inoculation showing three adult nematode females of M. javanica feeding in the vascular tissues in close proximity, resulting in the extensive formation of giant cells (X 88).

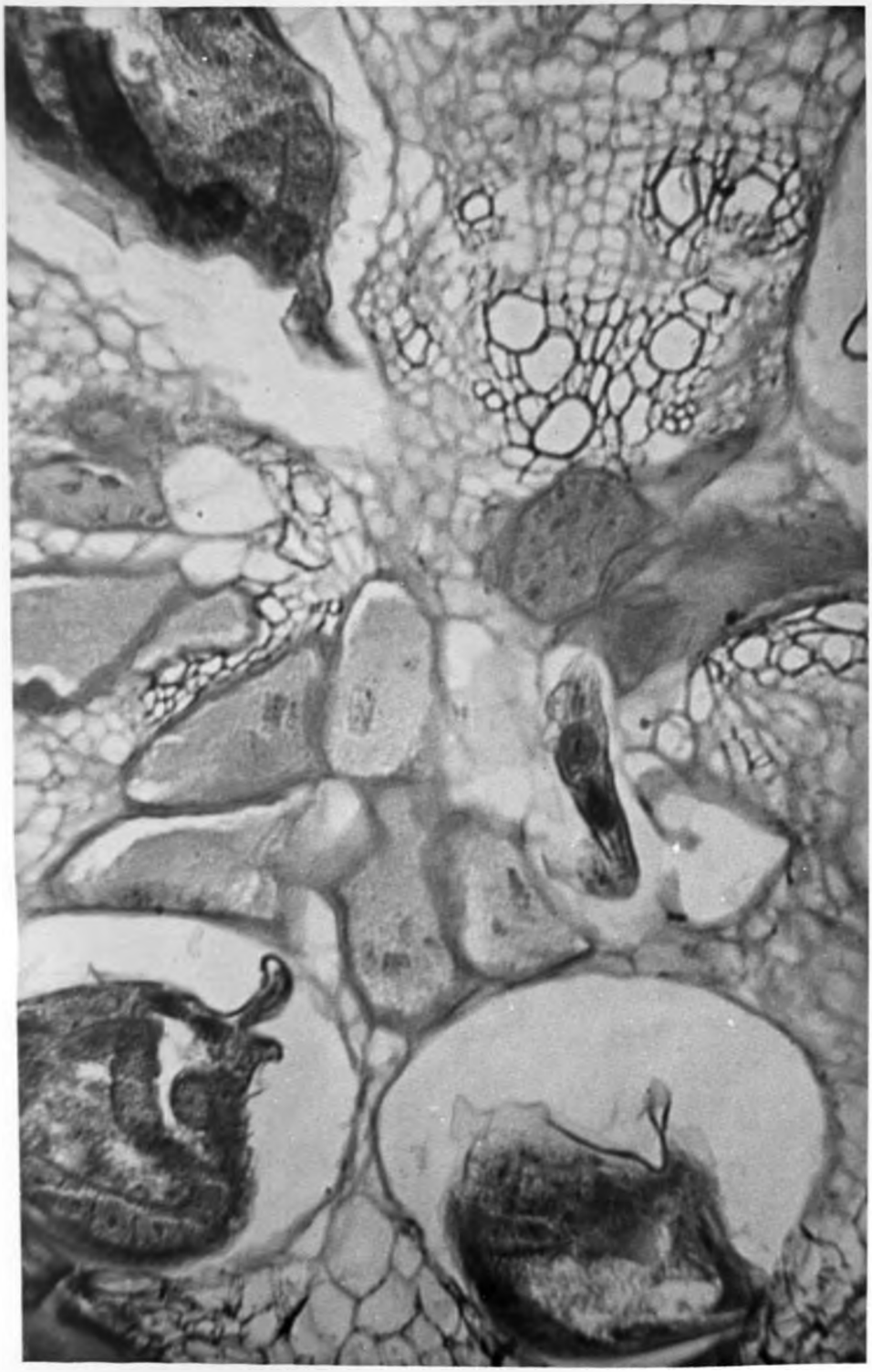


FIGURE 21. INFECTED CANADIAN WONDER BEAN ROOT SECTION

Transverse section of a root of a Canadian Wonder bean plant 10 days after inoculation showing "abnormal xylem", which is characterized by secondary wall thickenings of annular, or reticulate types, formed from xylem parenchyma associated with giant cells, induced by M. incognita (X 175).

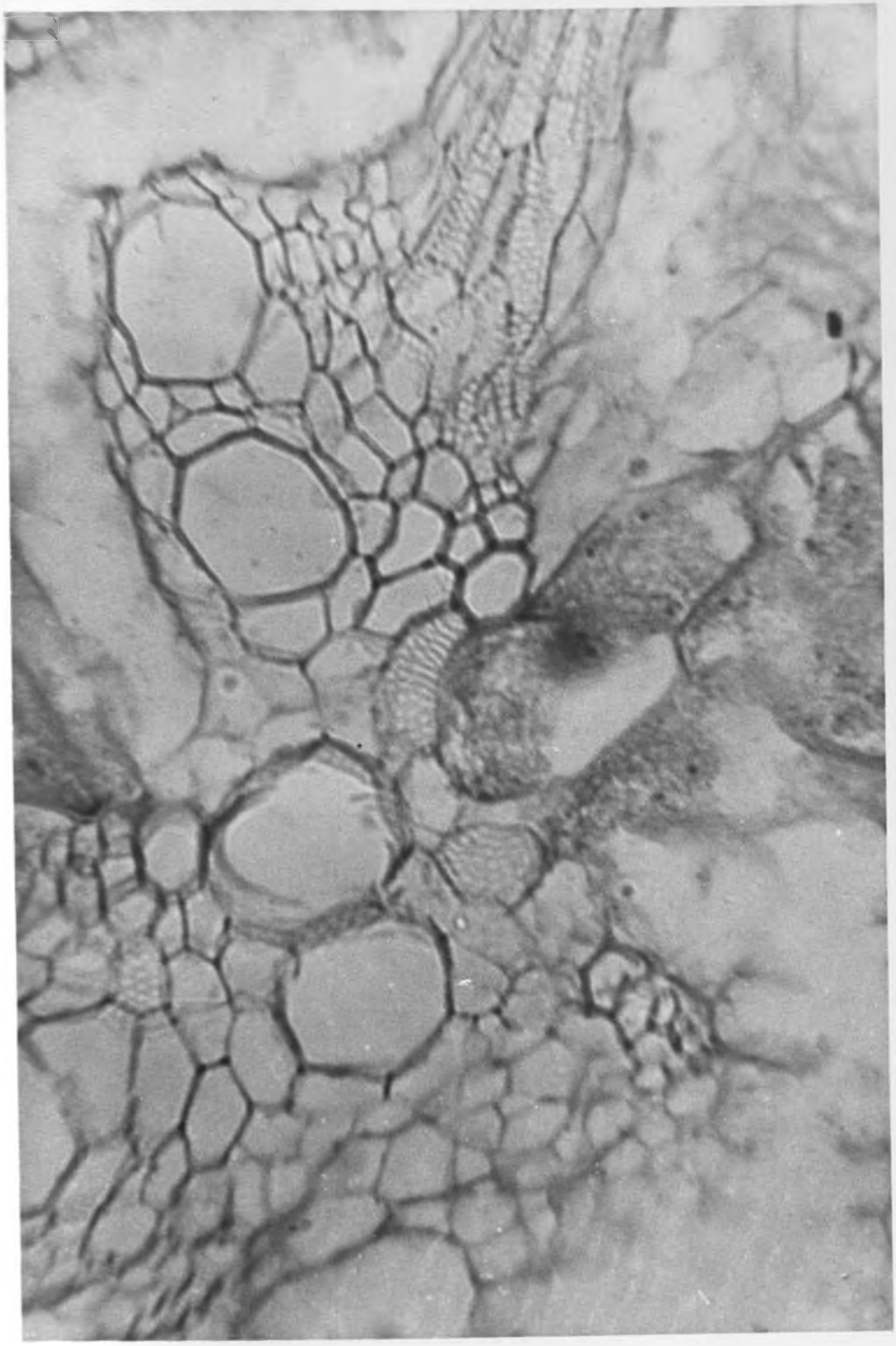


FIGURE 22. INFECTED KIKARA BEAN ROOT SECTION

Transverse section of a root of a Kikara bean plant 20 days after inoculation showing abnormal xylem in xylem parenchyma cells, associated with giant cells, induced by M. incognita (X 175).

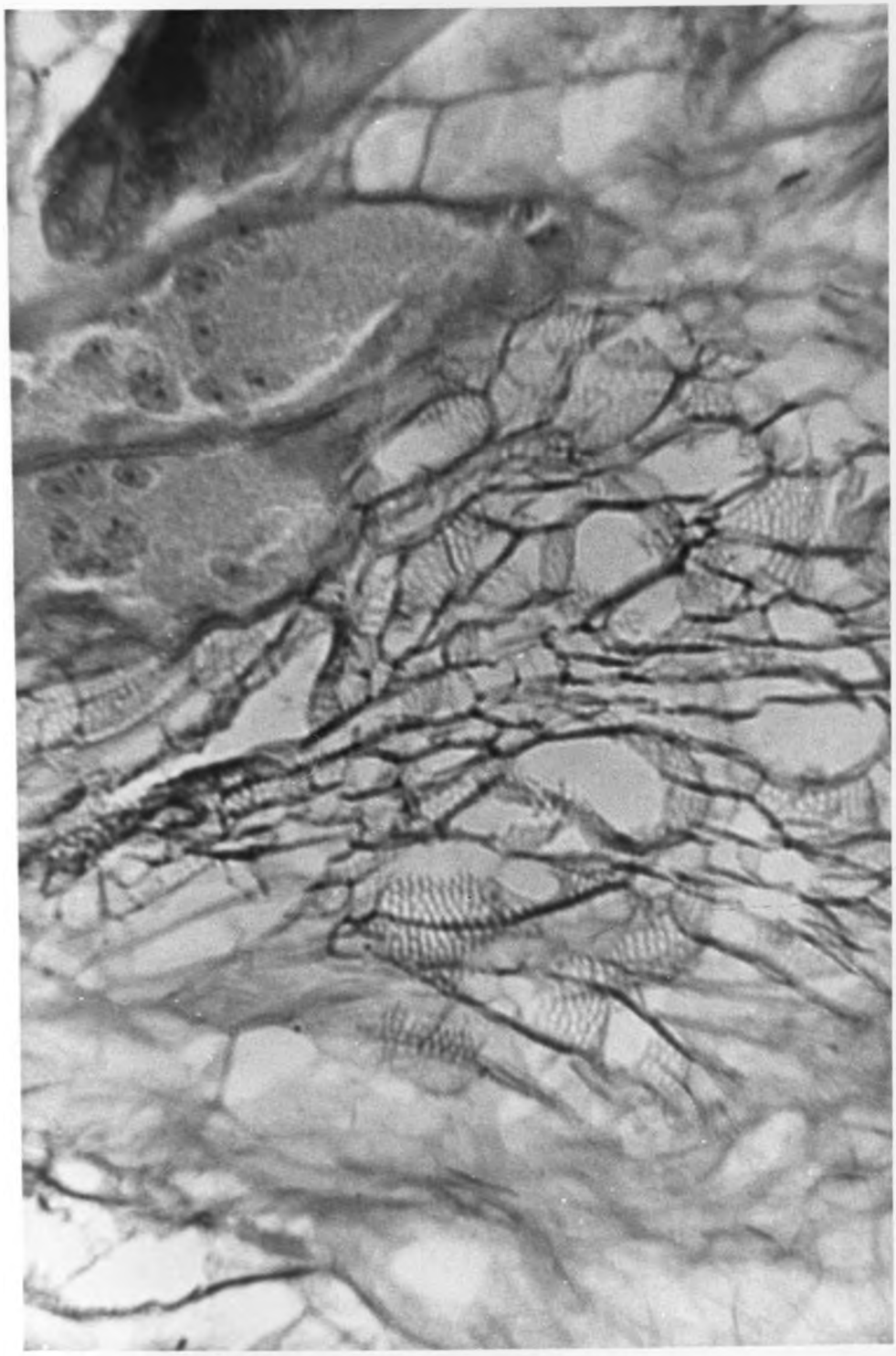


FIGURE 23. INFECTED MEXICO 142 BEAN ROOT SECTION

Transverse section of a root of a Mexico 142 bean plant 30 days after inoculation showing abnormal xylem in xylem parenchyma cells, surrounded by giant cells, induced by M. javanica (X 175).

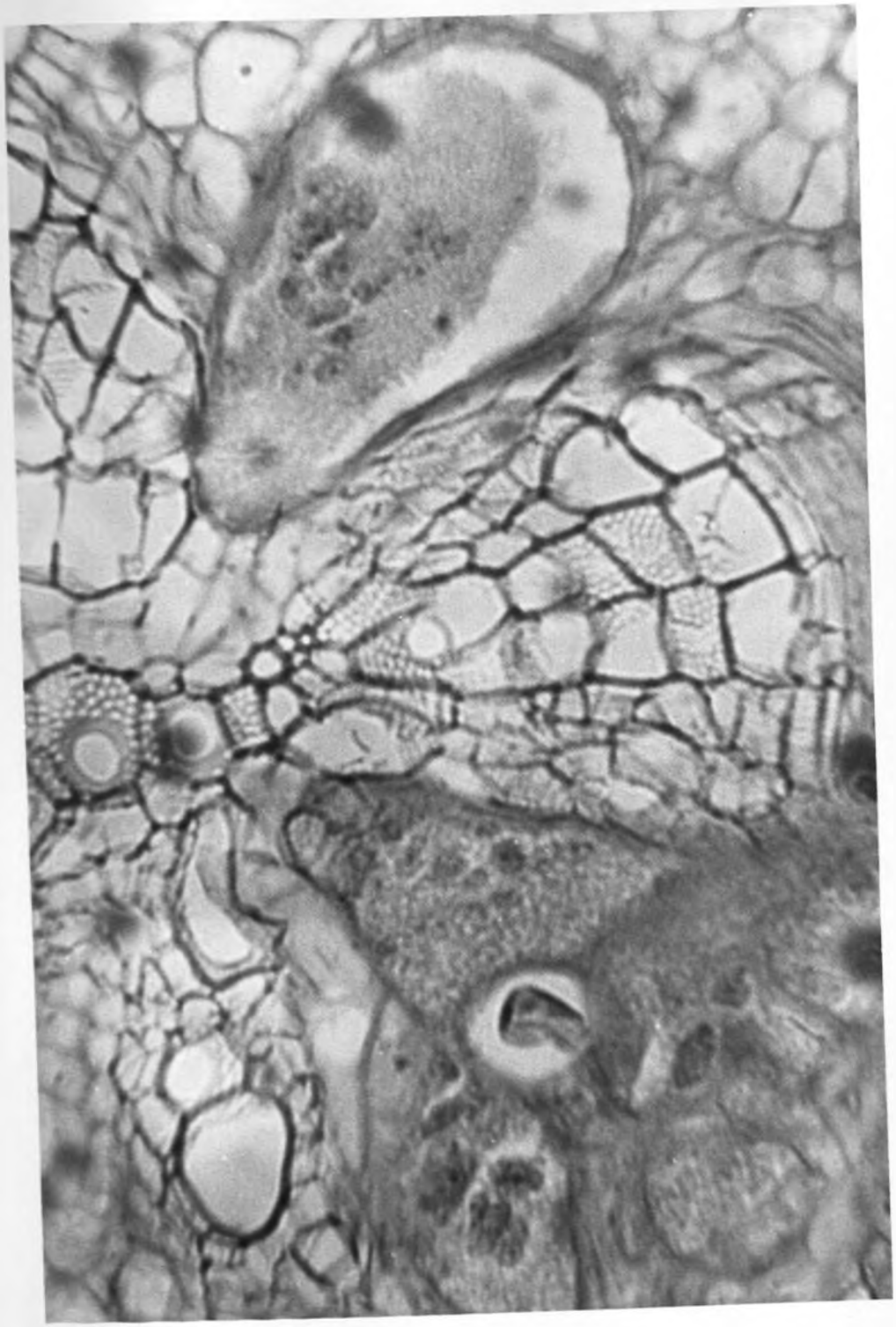


FIGURE 24. INFECTED MEXICO 142 BEAN ROOT SECTION

Transverse section of a root of a Mexico 142 bean plant 10 days after inoculation showing giant cells in the cortex, induced by M. incognita (X 88).

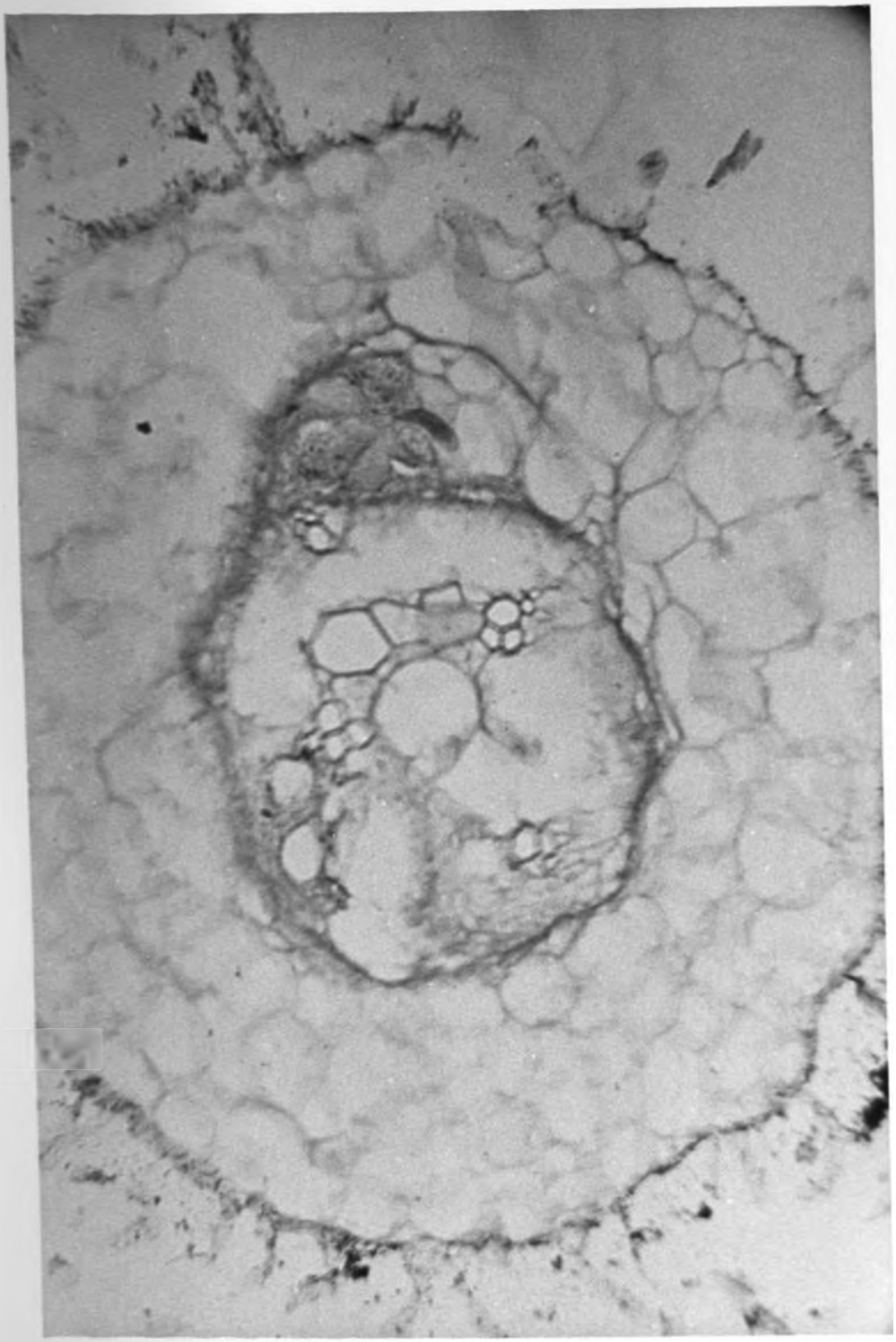


FIGURE 25. INFECTED CANADIAN WONDER BEAN ROOT SECTION

Transverse section of a root of a Canadian Wonder bean plant 10 days after inoculation showing giant cells in the cortex, induced by *M. incognita* (X 88).

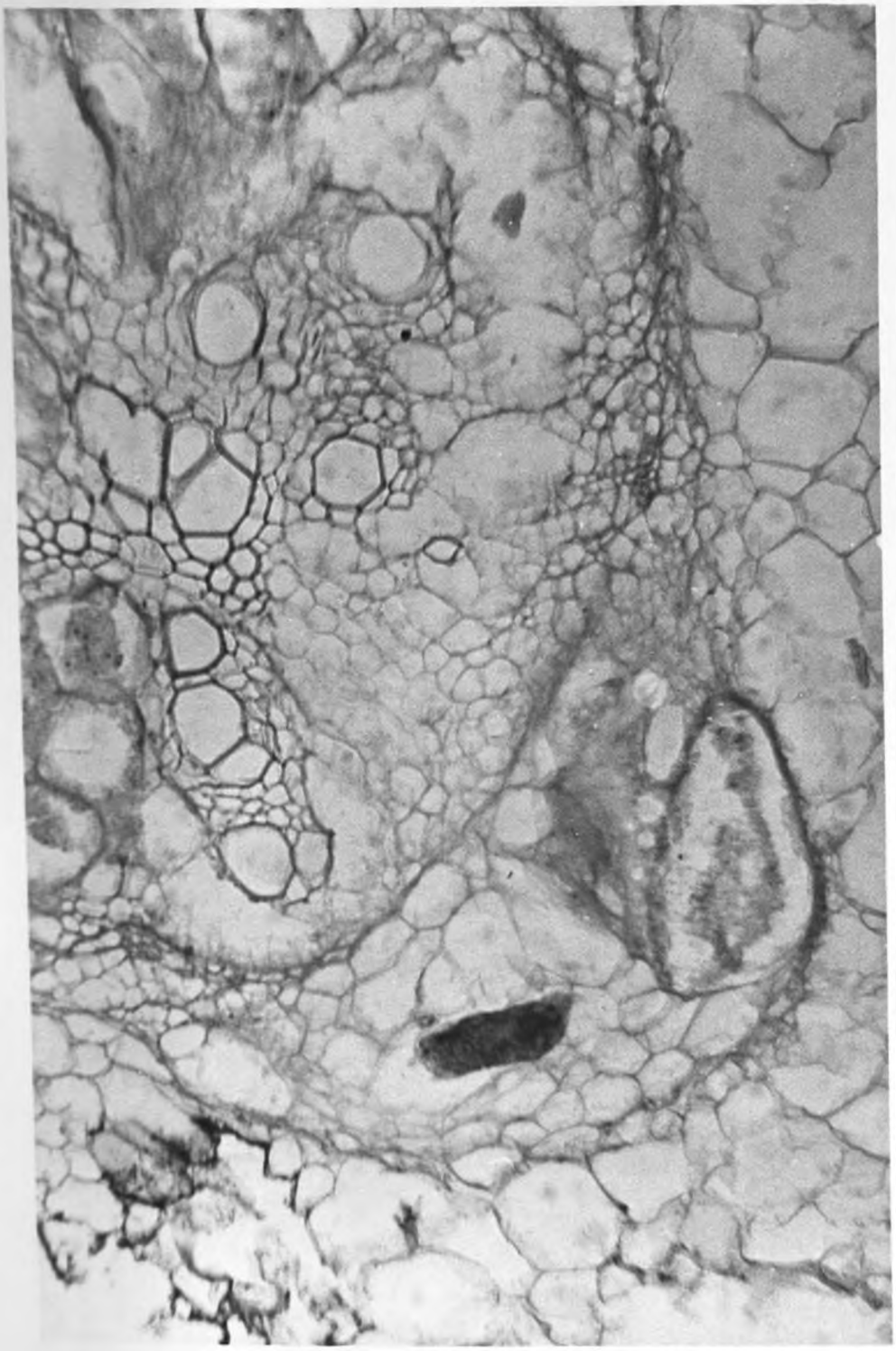


FIGURE 26. INFECTED PREMIER BEAN ROOT SECTION

Transverse section of a root of a Premier bean plant 10 days after inoculation showing giant cells in the cortex, induced by M. javanica (X 88).

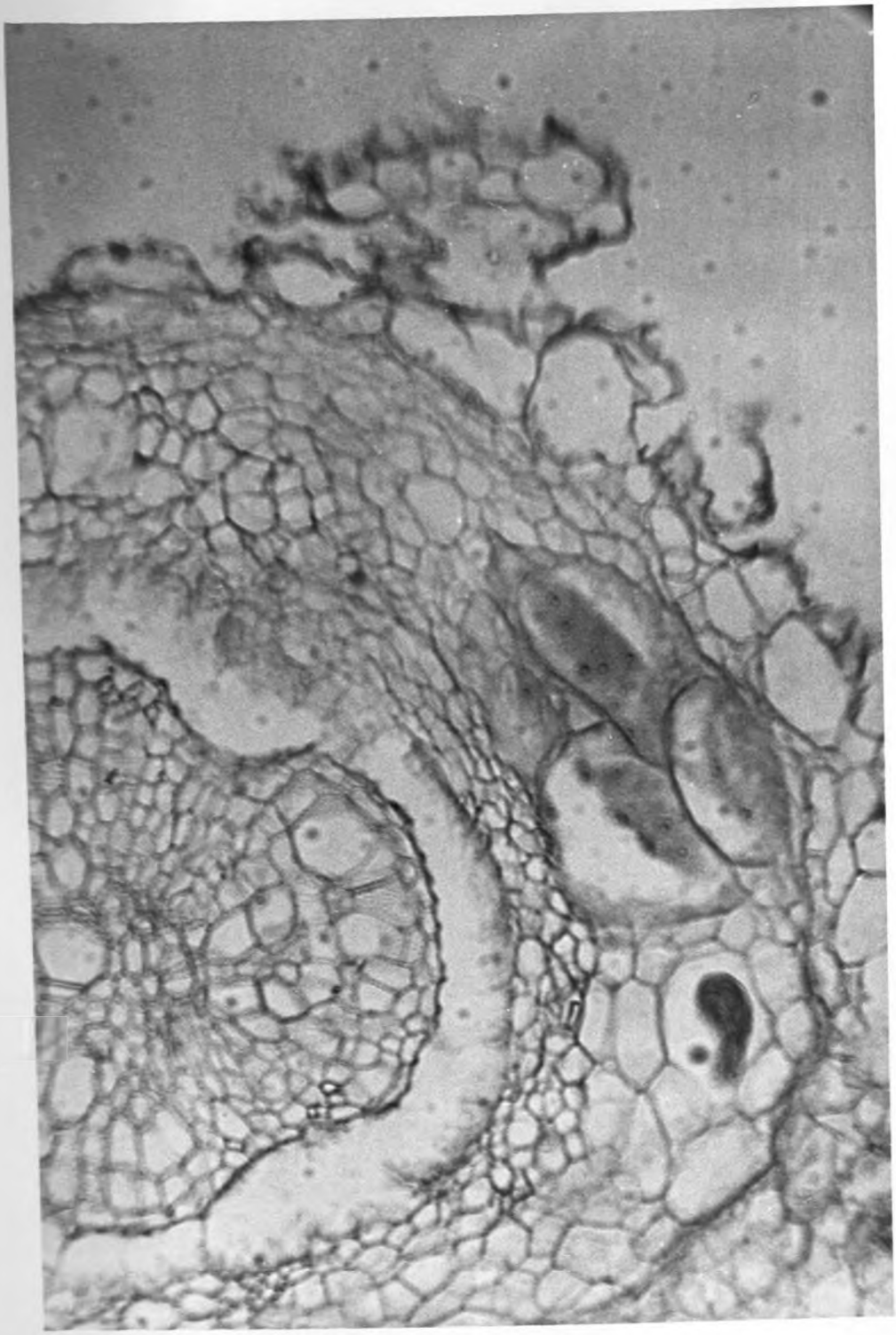


FIGURE 27. INFECTED CANADIAN WONDER BEAN ROOT SECTION

Transverse section of a root of a Canadian Wonder bean plant 20 days after inoculation showing: secondary thickening of giant cell walls irregular in pattern; and highly granular and dense cytoplasm of the giant cells, induced by M. incognita (X 88).

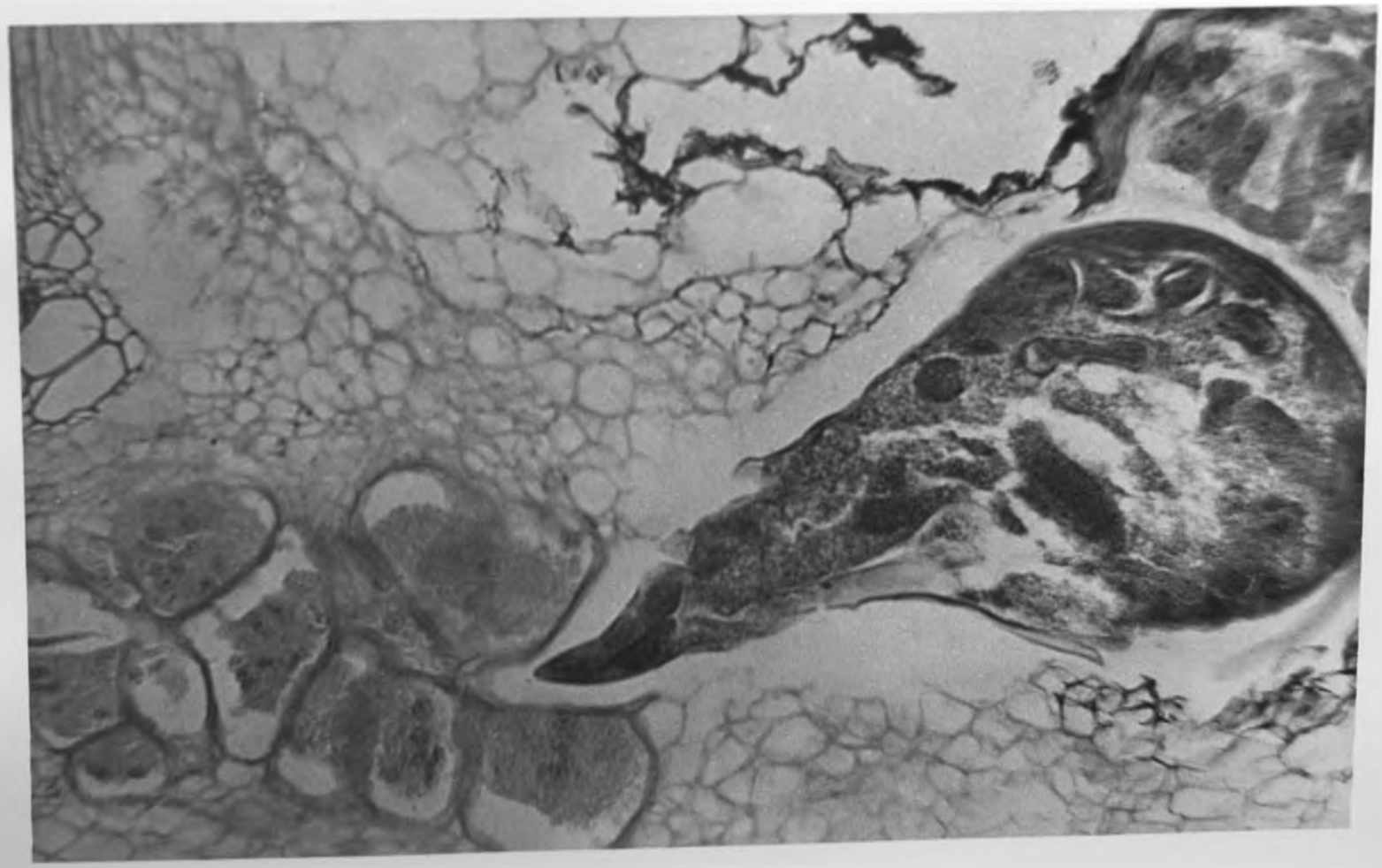


FIGURE 28. INFECTED MARATHON BEAN ROOT SECTION

Longitudinal section of a root of a Marathon bean plant 20 days after inoculation showing: highly multinucleate giant cells; and hypertrophied nuclei and nucleoli aggregating somewhat toward the centre of the giant cells, induced by M. javanica (X 88).

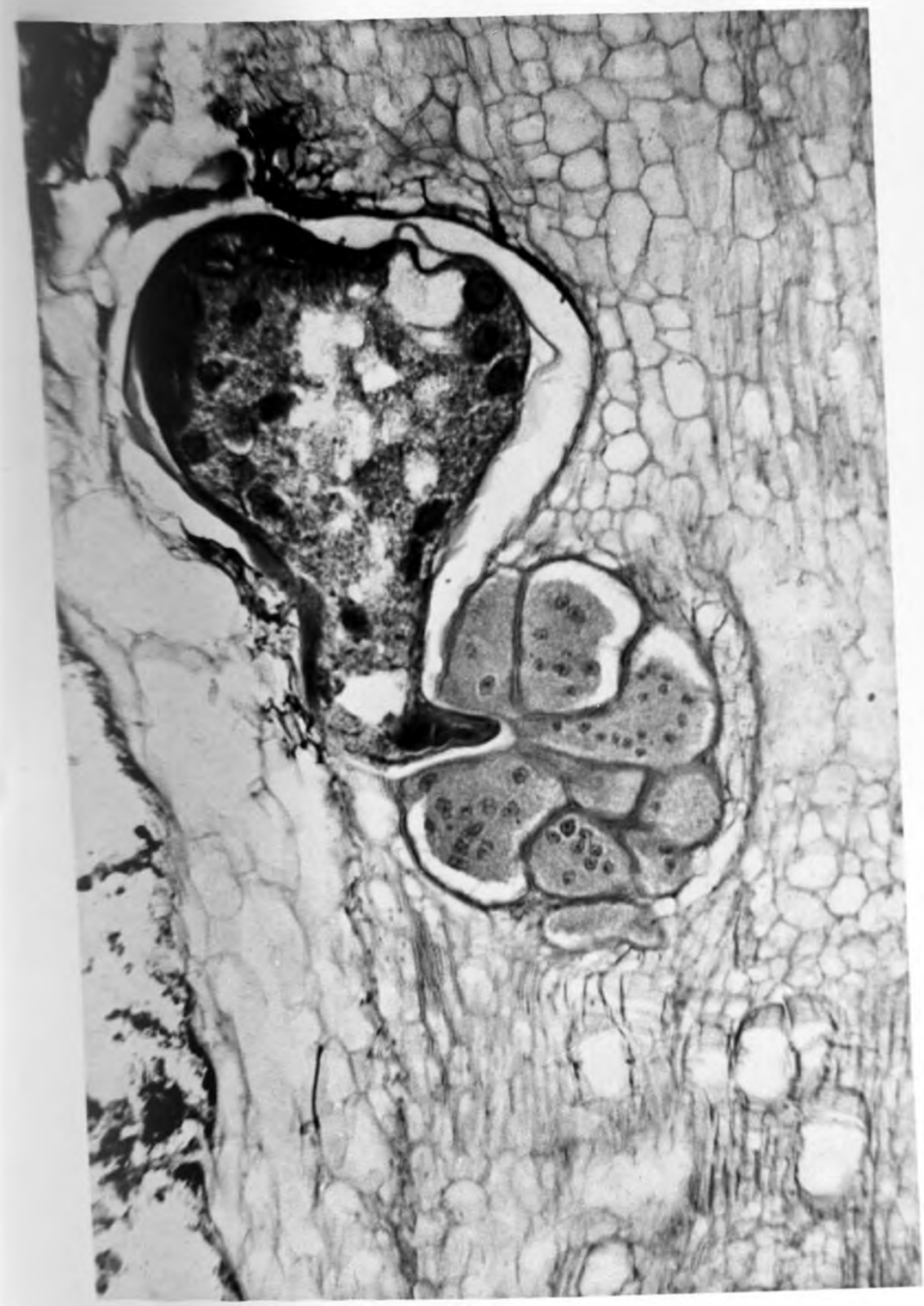


FIGURE 29. INFECTED PREMIER BEAN ROOT SECTION

Transverse section of a root of a Premier bean plant 30 days after inoculation showing: highly vacuolated cytoplasm of the giant cells, induced by M. incognita; and degenerated giant cell nuclei with their contents diffused into the cytoplasm (X 88).

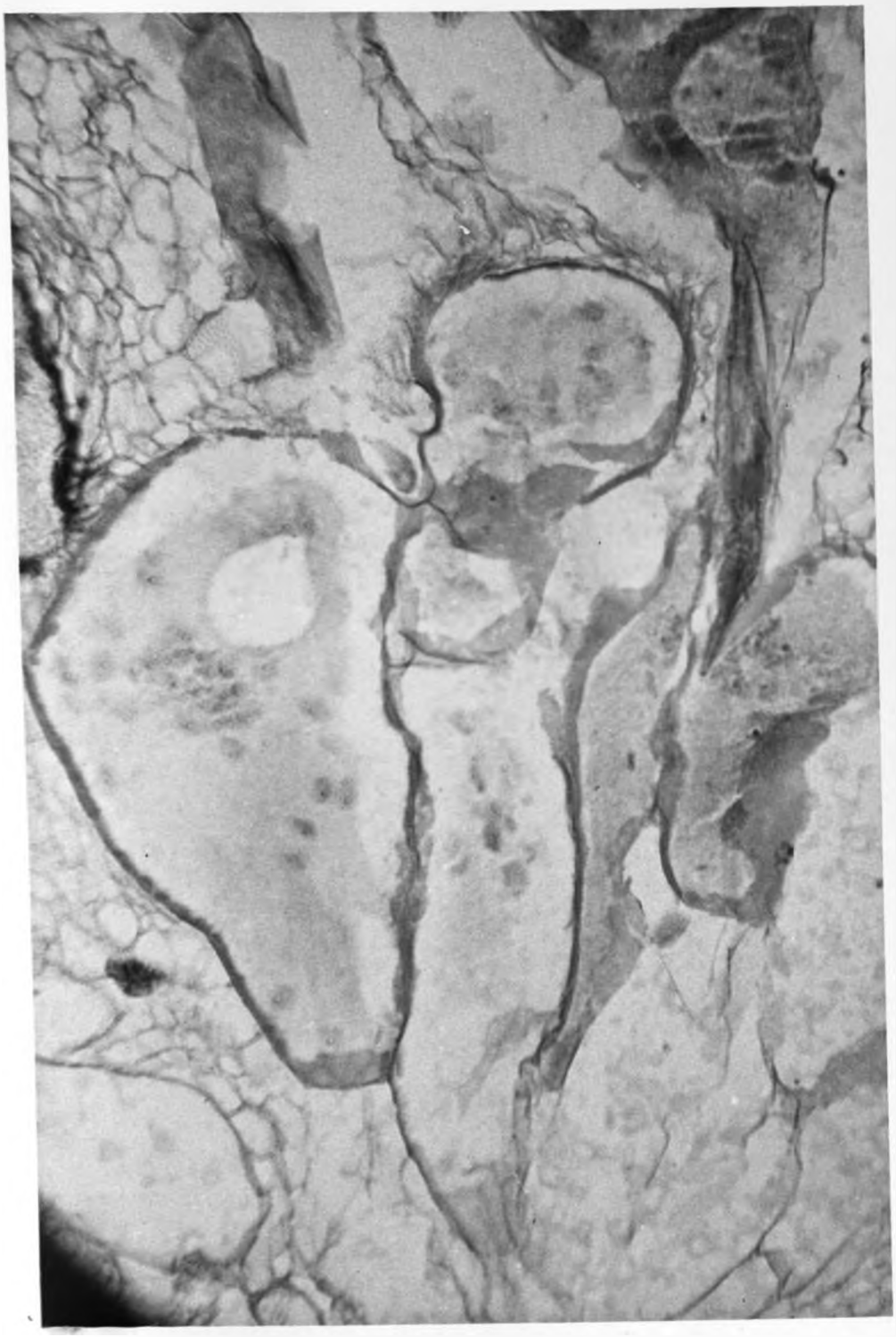


FIGURE 30. INFECTED KIKARA BEAN ROOT SECTION

Longitudinal section of a root of a Kikara bean plant 40 days after inoculation showing completely degenerated giant cell cytoplasm, which had been induced by M. incognita, and the disappearance of nuclei and nucleoli (X 88).

FIGURE 31. EARLY SECOND-STAGE LARVA

Camera lucida drawing of an infective early second-stage root-knot nematode larva, penetrating a root of a Marathon bean plant, at a point in the elongating region close behind the root tip (X 102).

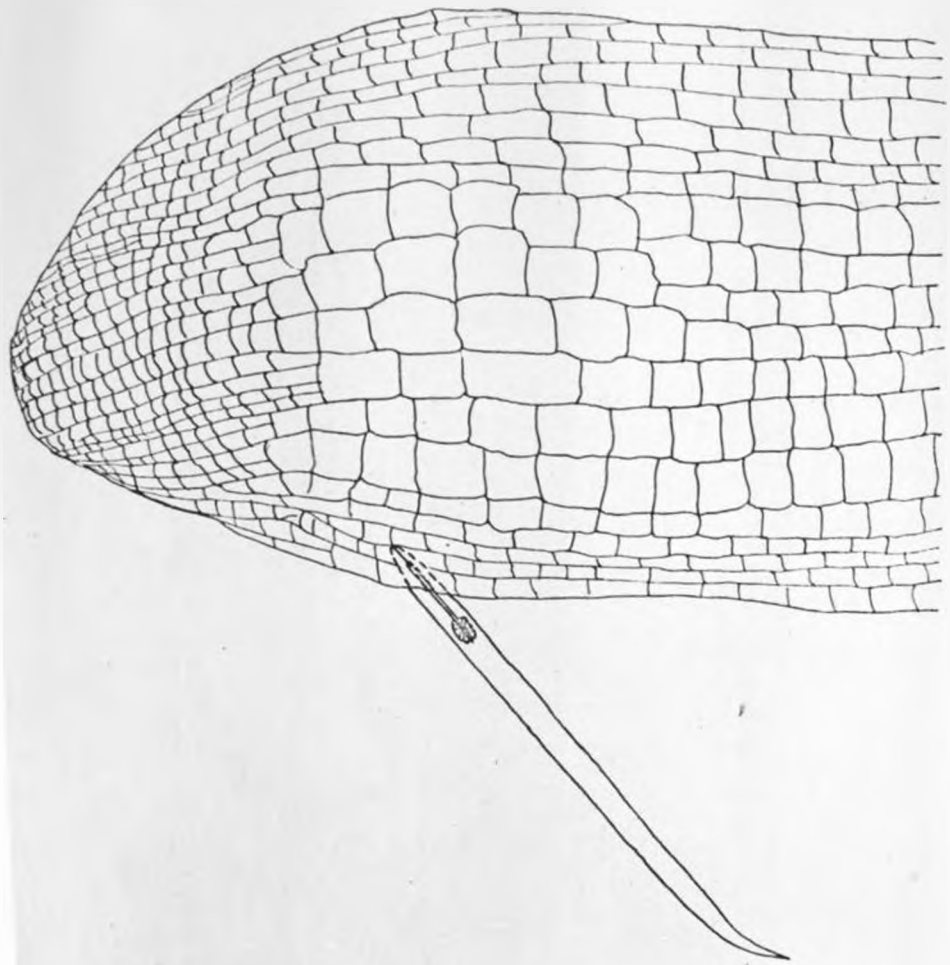
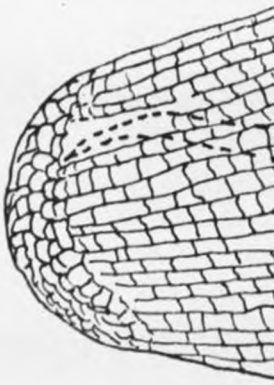


FIGURE 32. NEMATODE POSITION IN BEAN ROOT TISSUES

Camera lucida drawing of an infective early second-stage root-knot nematode larva with its head in the vascular system, after penetration, lying parallel to the long axis of a root of a Kikara bean plant (X 102).



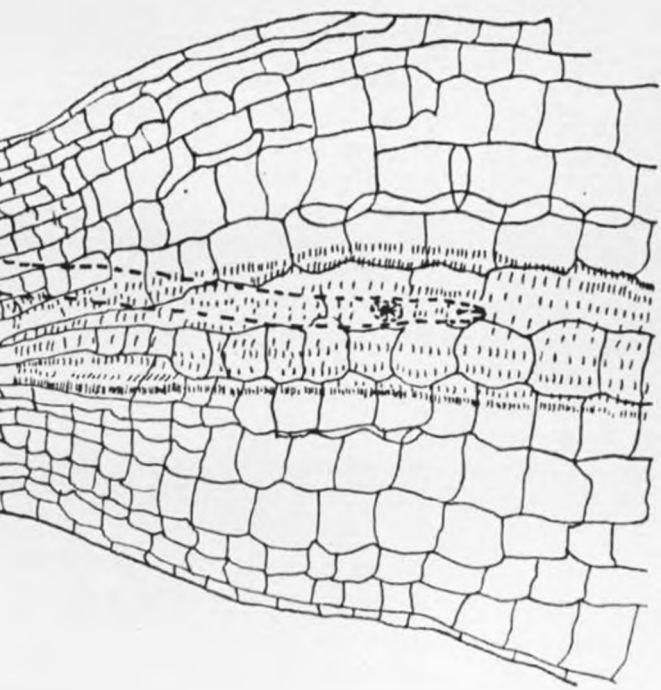


FIGURE 33. LATE SECOND-STAGE LARVA IN BEAN ROOT TISSUES

Camera lucida drawing of a late second-stage root-knot nematode larva, at the time of the 2nd moult, with a characteristic spiked tail, in a root of a Kikara bean plant (X 102).

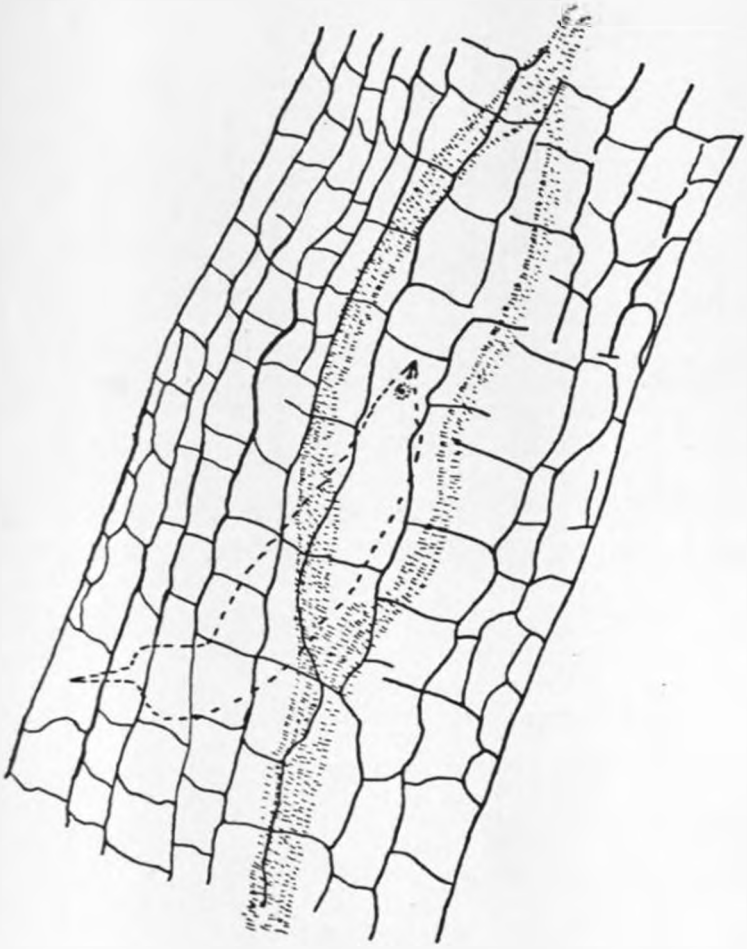


FIGURE 34. YOUNG ADULT FEMALE ROOT-KNOT NEMATODE

Camera lucida drawing of a young adult female root-knot nematode that has not yet laid eggs, after the 2nd, 3rd, and 4th moults, in a root of a Marathon bean plant, with its posterior end protruding on the root surface (X 102).

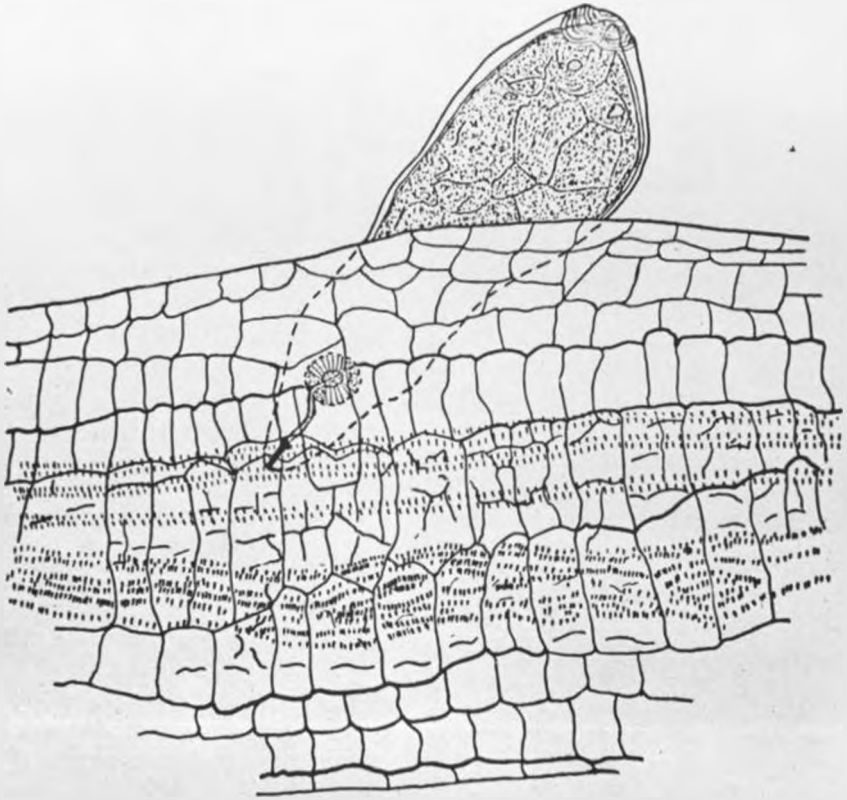


FIGURE 35. ADULT FEMALE ROOT-KNOT NEMATODE

Camera lucida drawing of an adult female root-knot nematode, M. incognita, in a root of a Mexico 142 bean plant with its posterior end, attached to its egg mass, protruding on the root surface (X 102).

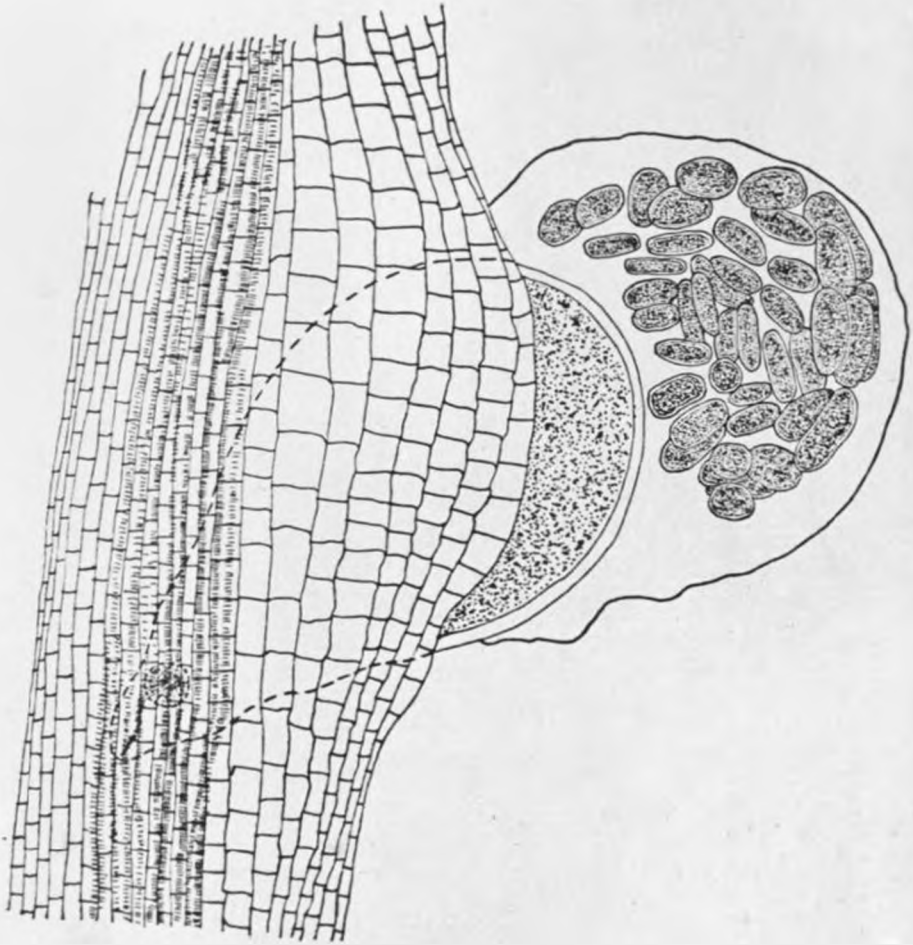


FIGURE 36. INFECTED MEXICO 142 BEAN ROOT SECTION

Transverse section of a root of a Mexico 142 bean plant 20 days after inoculation showing M. incognita feeding on a nucleus in a giant cell (X 175).