Development and Pathogenesis of a Root-Knot Nematode, *Meloidogyne javanica*

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ABSTRACT: Histological studies showed that the soybean variety N. C. Hampton was a favorable host for the root-knot nematode, *Meloidogyne javanica.* Infective second-stage larvae invaded young roots within 24 hr after inoculation. Most abundant penetration of the host occurred in the meristematic tissues, but the regions of cell enlargement and differentiation also were invaded; as many as 30 larvae were observed feeding on a single root tip. Larval penetration was both intercellular and intracellular and passage of larvae through the cortex caused distortion and necrosis of nearby cells. Nematodes were found feeding in cortex, endodermis, pericycle, and stele and this feeding resulted in hypertrophy, hyperplasia, and giant cell formation in tissues immediately surrounding the head of the nematode. Giant cells were associated with the development of *M. javanica* and were more abundant in the stelar tissue than in the cortex. Nematode females oviposited 30 days after inoculation and the life cycle was completed in about 35 days.

Root-knot nematodes are noted for their ability to incite marked morphological and anatomical changes in host roots (Bird, 1972; Dropkin, 1959; Dropkin and Nelson, 1960; Endo, 1971; Ibrahim et al., 1972). Dropkin (1959) demonstrated that host–parasite interactions between soybean varieties and *Meloidogyne* spp. could be used as bioassay procedures to distinguish races of root-knot nematodes. Dropkin and Nelson (1960) reported that giant cells formed by these nematodes on soybeans could be arranged into four morphological types depending on the host–parasite interactions.

The life cycle of *Meloidogyne* spp. has been the subject of numerous investigations. Tarjan (1952) studied four species of *Meloidogyne* infecting snapdragon roots and found no basic differences between the nematode species in regard to their development. However, life cycles of root-knot nematodes have manifested host effects based on species (Godfrey and Oliveira, 1932; Tarjan, 1952), nutrition (Bird, 1970; Oteifa, 1953), and ambient temperature (Bird and Wallace, 1965; Bryant and Wyllie, 1968; Dropkin, 1963).

The purposes of the present study were to describe the development of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, in the roots of the soybean variety N. C. Hampton and the histological changes which occur in the infected root tissues.

Materials and Methods

The nematode inoculum used in this study was originally obtained from soybean roots infected with *M. javanica.* A single egg mass of an identified female was isolated and the hatched larvae were then reared on tomato plants to obtain the required inoculum.

Soybean seeds of the variety N. C. Hampton were planted in steamed sandy loam soil in 24 clay pots, 15 cm diam. After emergence, the seedlings were thinned to two per pot, and then inoculated with 1,000 second-stage larvae 7 days after sowing. Pots were kept outdoors (day temperature 30 ± 2 C, night temperature 18 ± 2 C) and watered every other day. Seedlings of two pots were pulled gently at 1, 2, 4, 6, 8, and 10 days after inoculation and then at 5-day intervals up to the end of the experiment which proceeded for 40 days.

Galled parts of the infected roots were killed and fixed in FAA. For study of the nematode life cycle, parts of the fixed roots were stained with a modified Flemming's formula recommended by Oteifa and El Gindi (1956). The stained materials were washed in running water for 3 hr, then dehydrated in an aqueous ethyl alcohol series. Finally, the dehydrated root materials were cleared in clove oil and mounted in Canada balsam.

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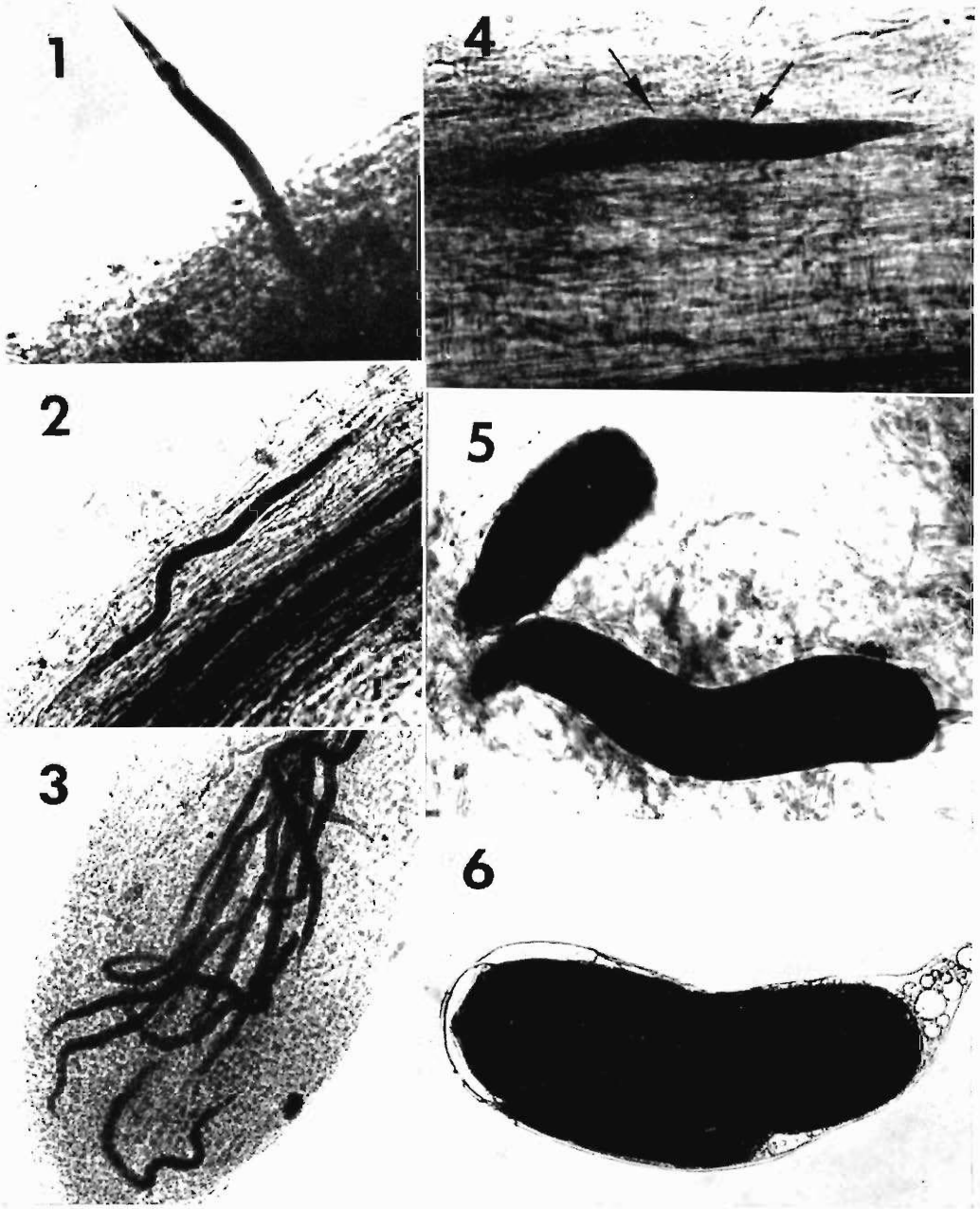
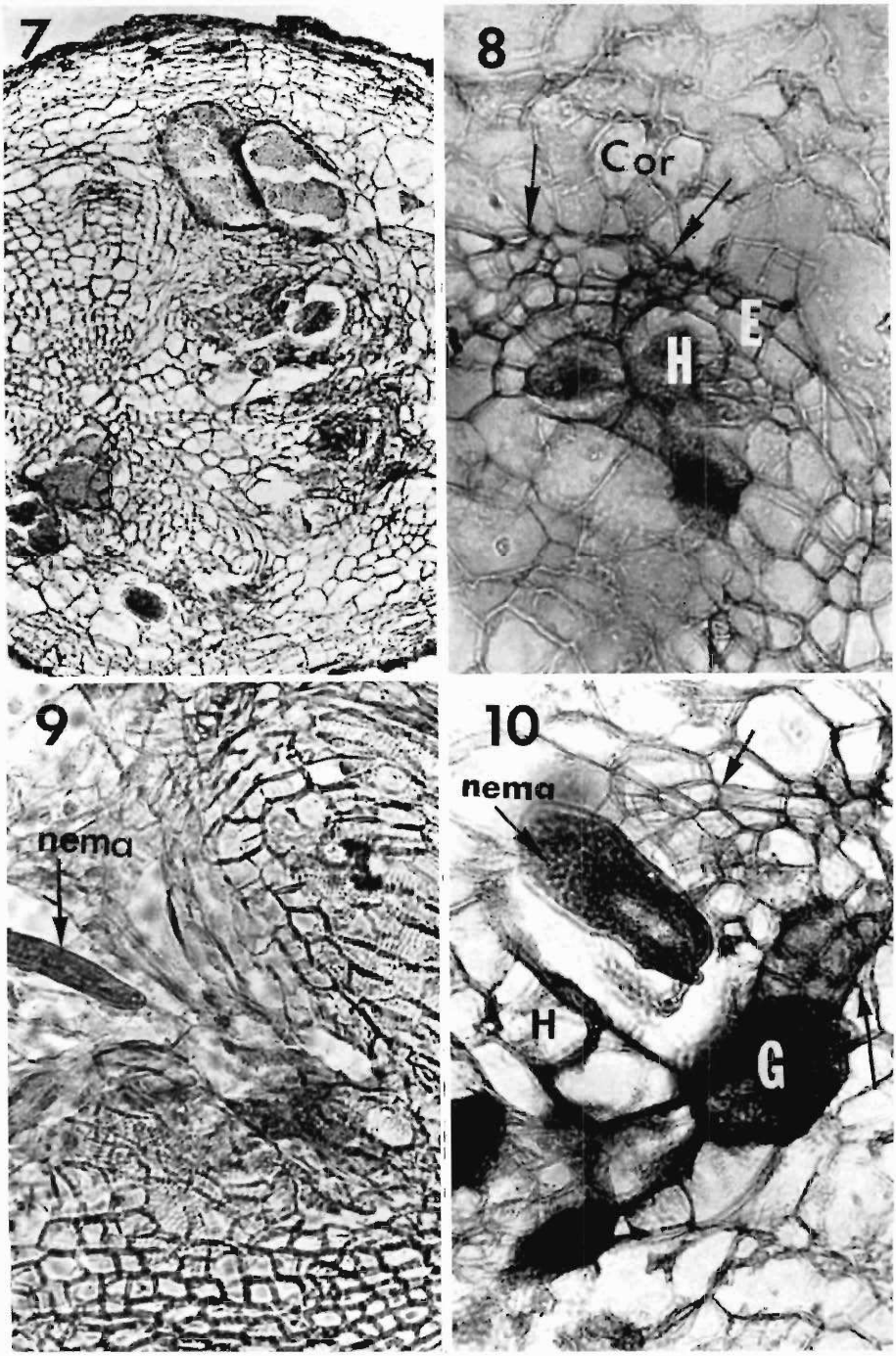


Figures 1–6. Penetration and development of *Meloidogyne javanica* larvae in soybean roots. 1. Infective second-stage larva penetrating the root tip. 2. Second-stage larva migrating in the cortex. 3. Group of second-stage larvae in the root tip. 4. Third-stage larva in the cortex (arrow). 5. Fourth-stage larvae feeding on the vascular tissue. 6. Isolated fourth-stage male larva.



In a histopathological study, the fixed infected roots were dehydrated in an ethyl and butyl alcohol series after which they were embedded in paraffin wax, sectioned, and stained with safranin and light green.

All sections were examined under a light microscope.

Results

Microscopic observations of stained soybean roots revealed the gross morphology of infected root tissues and the different developmental stages of *M. javanica* (Figs. 1–10). One day after inoculation, infective second-stage larvae were found penetrating the roots at or near the root tips (Fig. 1). Larval passage through the root tissues was intercellular and intracellular. Direct larval penetration of the epidermis and cortex distorted and killed nearby cells. At 2 days, second-stage larvae were observed feeding on meristematic and cortical tissues and as many as 30 larvae were seen feeding on a single root tip (Figs. 2, 3). Larvae were oriented in various directions, but more were arranged parallel to the longitudinal axis of the root with their posterior ends extended toward the root tip (Fig. 2). Within 2 days, hypertrophy of cortical parenchyma and endodermal cells occurred about the larval head. In 4 days, cells immediately surrounding the hypertrophied cells and nematode mouth were stimulated to divide and form hyperplastic tissue (Fig. 8). In 6 days, third-stage larvae were observed mostly in the inner cortex feeding on tissues of the endodermis, pericycle, and outer stele. Hypertrophied and hyperplastic cells surrounding the larval head marked the beginning of giant cell formation. Some of the affected cells contained granular materials and their cell walls were partially thickened and darkly stained (Fig. 9). In 8-day infections, walls between enlarged divided cells started to break down. Within 8 to 10 days, the majority of larvae had reached the third stage of development (Fig. 4). In 15 days, fourth-stage larvae were observed feeding on the pericyclic and stelar tissues, especially protoxylem poles (Figs. 5, 10). As many as seven giant cells were found around the head of the fourth-stage larvae. Abnormal xylem vessels, crushed and necrotic cells adjacent to developing females, and giant cells were seen in the roots. In 20 days, cell wall dissolution of adjoined giant cells was seen and resulted in the formation of openings between adjacent giant cells.

The fourth-stage male larva appeared typically as an elongated thread whereas the preadult female stage had an elongated, round body (Figs. 5, 6). In 20 and 25 days following inoculations, respectively, young and mature males and females were observed in infected root tissues. Nematode females oviposited 30 days after inoculation. Eggs of *M. javanica* collected from egg masses were observed to hatch in water at room temperature after 4–6 days. Second-stage larvae of the second generation appeared in the roots 35 days after inoculation.

Discussion

The results showed that roots of soybean seedlings became infected with second-stage larvae of *M. javanica* within 24 hr after inoculation. The seedling root tips were the most favorable parts for nematode infection. Similar results were found by Christie (1936), who reported that invasion by root-knot nematode larvae was limited mainly to root tip regions with comparatively undifferentiated tissues. The results also indicated that progressive development of the nematode larvae and presence of adults were associated with anatomical changes of the root tissues, especially in the formation of giant cells. The observed cellular disorganization of infected roots could be related to migration, feeding, and development of the infecting nematode. The histopathological changes described in this paper were

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Figures 7–10. Cross sections of soybean roots infected with *M. javanica*. 7. Heavily infected root, 35 days after inoculation. 8. Cellular hypertrophy (H) and hyperplasia (arrow) in the cortex (Cor), endodermis (E), and stelar tissue. 9. Third-stage larva feeding on the pericycle and the vascular tissue. Note cellular disorganization around the nematode (nema) head. 10. Infected root, 15 days following inoculation, showing the formation of hypertrophy (H), hyperplasia (arrow), and giant cells (G) around the nematode feeding site. Note dark-stained granular materials inside the giant cell.

similar to those reported previously (Christie, 1936; Davis and Jenkins, 1960; Dropkin and Nelson, 1960; Sasser and Taylor, 1952).

It is evident that nematode development and the subsequent degeneration of root tissues often resulted in the formation of large areas of necrotic cells and vacant areas, especially in the cortex. These vacant areas were not invaded by a growth of rejuvenated parenchyma cells.

Literature Cited

Bird, A. F. 1970. The effects of nitrogen deficiency on the growth of *Meloidogyne javanica* at different population levels. Nematologica 16: 13–21.

———. 1972. Cell wall breakdown during the formation of syncytia induced in plants by root knot nematodes. Intern. J. Parasitol. 2: 431–432.

———, **and H. R. Wallace.** 1965. The influence of temperature on *Meloidogyne hapla* and *M. javanica*. Nematologica 11: 581–589.

Bryant, W. E., and T. D. Wyllie. 1968. Effect of temperature and variety on nematode development and gall production on soybeans infected with *Meloidogyne incognita acrita*. Phytopathology 58: 1045 (Abstr.).

Christie, R. A. 1936. The development of root-knot nematode galls. Phytopathology 26: 1–22.

Davis, R. A., and W. R. Jenkins. 1960. Histopathology of gardenia (*Gardenia jasminoides veitchi*) infected with three species of *Meloidogyne*. Nematologica 5: 228–230.

Dropkin, V. H. 1959. Varietal response of soybeans to *Meloidogyne*. A bioassay system for separating races of root-knot nematodes. Phytopathology 49: 18–23.

———. 1963. Effect of temperature on growth of root-knot nematodes in soybeans and tobacco. Phytopathology 53: 663–666.

———, **and P. E. Nelson.** 1960. The histopathology of root-knot nematode infections in soybeans. Phytopathology 50: 442–447.

Endo, B. Y. 1971. Nematode-induced syncytia (giant cell). Host-parasite relationships of Heteroderidae. *In*: Plant Parasitic Nematodes (edited by Zuckerman, B. M., W. F. Mai, and R. A. Rohde), Vol. II, p. 91–117. Academic Press, New York.

Godfrey, G. H., and J. Oliveira. 1932. The development of root-knot nematode in relation to root tissues of pineapple and cowpea. Phytopathology 22: 325–348.

Ibrahim, I. K. A., I. A. Ibrahim, and S. I. Massoud. 1972. Induction of galling and lateral roots on five varieties of soybeans by *Meloidogyne javanica* and *M. incognita*. Plant Dis. Reptr. 56: 882–884.

Oteifa, B. A. 1953. Development of the root-knot nematode *Meloidogyne incognita* as affected by potassium nutrition of the host. Phytopathology 43: 171–174.

———, **and D. M. El Gindi.** 1956. Studies on root-knot nematodes *Meloidogyne* spp. in Giza, Egypt. II. Developmental behavior of the root-knot nematode *M. javanica*. Fac. Agr. Cairo Univ., Bull. No. 101.

Sasser, J. N., and A. L. Taylor. 1952. Studies on entry of larvae of root-knot nematodes into roots of susceptible and resistant plants. Phytopathology 42: 474 (Abstr.).

Tarjan, A. C. 1952. Comparative studies of some root-knot nematodes infecting the common snapdragon, *Antirrhinum majus*. Phytopathology 42: 641–644.