

Histopathology of Nodular Tissues of Legumes Infected with Certain Nematodes

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ABSTRACT

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The histopathology of nodules on greenhouse-grown soybean, garden pea, and peanut infected with each of *Meloidogyne incognita* (*M. hapla* on peanut), *Pratylenchus penetrans*, and *Belonolaimus longicaudatus* was investigated. *Meloidogyne* spp. developed more readily within nodules of soybean than within those of the other legumes. *Meloidogyne incognita* was generally found inside vascular bundles in soybean nodules; this parasite did not alter the structural integrity of soybean nodules, but bacteroids did not develop adjacent to nematodes and infected nodules deteriorated earlier than noninfected nodules. Many giant cells in soybean nodules and all giant

cells in Wando pea tissues had the morphological characteristics of transfer cells. Nodule development was suppressed on Wando pea, and many deteriorated prematurely. Few *M. hapla* were observed in nodules of peanut, and little damage was evident when nematodes invaded tissue adjacent to nodules. *Pratylenchus penetrans* was limited to the cortex of soybean and peanut nodules, but readily entered nodules of Wando pea. Damage caused by *B. longicaudatus* on soybean and peanut was limited mainly to cortical tissues. This pest caused premature senescence of nodules on garden pea.

Additional key words: *Rhizobium japonicum*, *Rhizobium leguminosarum*, *Pisum sativum*, *Glycine max*, *Arachis hypogaea*.

Heterodera glycines and certain other *Heterodera* species greatly inhibit nodulation and N₂ fixation on their leguminous hosts. The most damaging of these interactions is that between *H. glycines* and *Rhizobium japonicum* on soybean (2, 6, 11). Race 1 of this nematode was found to reproduce very little in the nodular tissue and often induced a hypersensitive cell reaction (1). In contrast, Taha and Raski (15) showed that *Meloidogyne javanica* and *Heterodera trifolii* reproduced readily in nodules of white clover and altered the numbers of nodules only by suppressing root growth. Nodular tissues were not disrupted by infection, and giant cells formed in vascular bundles. Wardojo et al. (18) showed that *Pratylenchus penetrans* inhibited nodulation on white clover. *Heterodera goettingiana* may interfere with nodulation on pea, depending on cultural practices (8, 13).

Other types of microorganisms, including viruses (16, 17), fungi (12), and microbivorous nematodes (19) affect nodule development on certain legumes. Jatala et al. (7) showed that the microbivorous nematode *Pristionchus lheritieri* may serve as a carrier for *R. japonicum*. However, the microbivorous nematode, *Acrobeloides buetschlii* was found to penetrate nodules of Wando pea, where it reproduced and inhibited N₂-fixation (19). Tu et al. (17) reported that soybean infected with soybean mosaic virus (SMV) is less suitable for *Rhizobium* than healthy plants, and in later work Tu (16) found that SMV

infectivity in nodules was consistently higher than in root tissues. *Fusarium oxysporum* also is antagonistic to *R. japonicum* on soybean (12).

Since a major source of nitrogen is that fixed by *Rhizobium* spp. and certain nematodes interfere with the biological processes that provide this nitrogen, histological investigations were conducted on soybean, Wando pea, and peanut to determine the influence of nematodes with different feeding habits on nodule development and morphology. A preliminary report of this work has been published (4).

MATERIALS AND METHODS

Nematodes of three feeding habits were used in this study: the sedentary endoparasites, *Meloidogyne incognita* (Kofoid & White) Chitwood on soybean and Wando pea, and *M. hapla* Chitwood on peanut; and the migratory endoparasite, *Pratylenchus penetrans* (Cobb) Filipj. & Schuurm.-Stekh. and the migratory ectoparasite, *Belonolaimus longicaudatus* Rau on all three hosts.

Four greenhouse experiments were conducted to provide tissues for this investigation. Seeds of soybean (*Glycine max* 'Lee'), peanut (*Arachis hypogaea* 'Florigiant'), and pea (*Pisum sativum* 'Wando') were germinated in vermiculite, and transplanted singly to 1-liter plastic pots containing 578- μ m (35-mesh) and 245-

μm (65-mesh) silica sand (1:1, v/v) at time of inoculation. Plants were inoculated simultaneously with 200 mg of the appropriate commercial preparation of *Rhizobium* (Nitragin Company, Milwaukee, Wis.) and with the given

nematode species. Control plants were inoculated only with *Rhizobium*.

Eggs of *M. hapla* and of *M. incognita* were extracted from 50-day-old galled tomato roots with 1% sodium

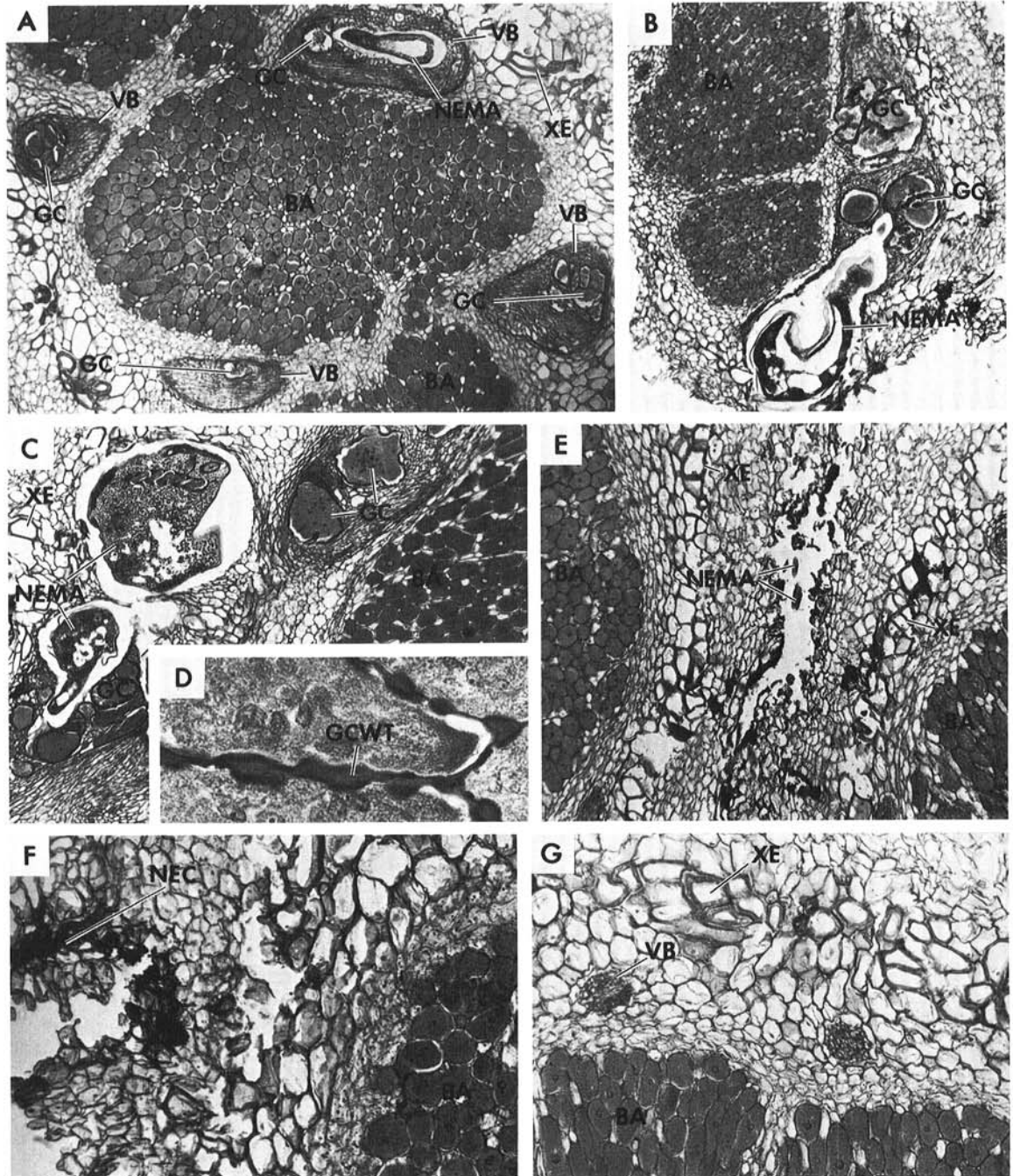


Fig. 1—(A to G). Responses of nodular tissues of soybean to infection by nematodes (BA = bacteroids; XE = xylem element). **A)** *Meloidogyne incognita* (NEMA) and associated giant cells (GC) in vascular bundles (VB). **B)** Adult female of *M. incognita* (NEMA) and giant cells (GC) occupying a sector of a nodule. **C)** Portion of a nodule developing on a root gall induced by *M. incognita*. **D)** Giant-cell wall thickenings (GCWT) induced by *M. incognita*. **E)** Limited necrosis around *Pratylenchus penetrans* (NEMA) in cortical tissues between two nodules. **F)** Necrosis (NEC) that resulted from feeding by *Belonolaimus longicaudatus* in cortical tissue of nodule. **G)** Portion of control nodule showing ring of xylary elements and vascular bundles (VB).

hypochlorite (3) and added at the rates of 10,000 and 7,000 per pot, respectively. *Pratylenchus penetrans*, extracted from soybean roots in a mist chamber, was used at the rate of 6,000/pot. *Belonolaimus longicaudatus*, added at the rate of 2,000/pot, was cultured on soybean and extracted by mixing the soil with tap water containing 12.5 $\mu\text{g/ml}$ Separan®, followed by decanting and sieving. All plants received half-strength Hoagland's nutrient solution at the time of transplanting. After 2 weeks the same nutrient solution minus N was provided as needed. Pots were flushed semiweekly with tap water. Supplemental light (8,600 lx from GE mercury and Lucalox lamps) was used to provide a 16-hour day.

Two replicates of each experiment were harvested 50-60 days after inoculation for histological studies. Root segments with nodules were fixed in formalin-alcohol-acetic acid, dehydrated with t-butyl alcohol series, and embedded in Tissuerep (Fisher Scientific Co.,

Pittsburgh, Pa.). Sections (12- to 16- μm thick) were cut with a rotary microtome, mounted with Haupt's adhesive and 4% formalin, and stained in Conant's quadruple stain (Triarch, Inc., Ripon, Wis.).

RESULTS

Interactions of nematodes with nodules on soybean.—*Meloidogyne incognita* developed and reproduced in nodular tissue of soybean (Fig. 1-A to C). In some instances, all vascular bundles contained giant cells and/or female nematodes (Fig. 1-A). Nematodes and giant cells developed in sectors of a given nodule without disrupting bacteroids in adjacent sectors (Fig. 1-B). Nodules frequently developed on galls caused by *M. incognita* (Fig. 1-C). The walls of giant cells in nodular tissue frequently had thickened areas (Fig. 1-D).

Damage to nodular tissue on soybean inoculated with

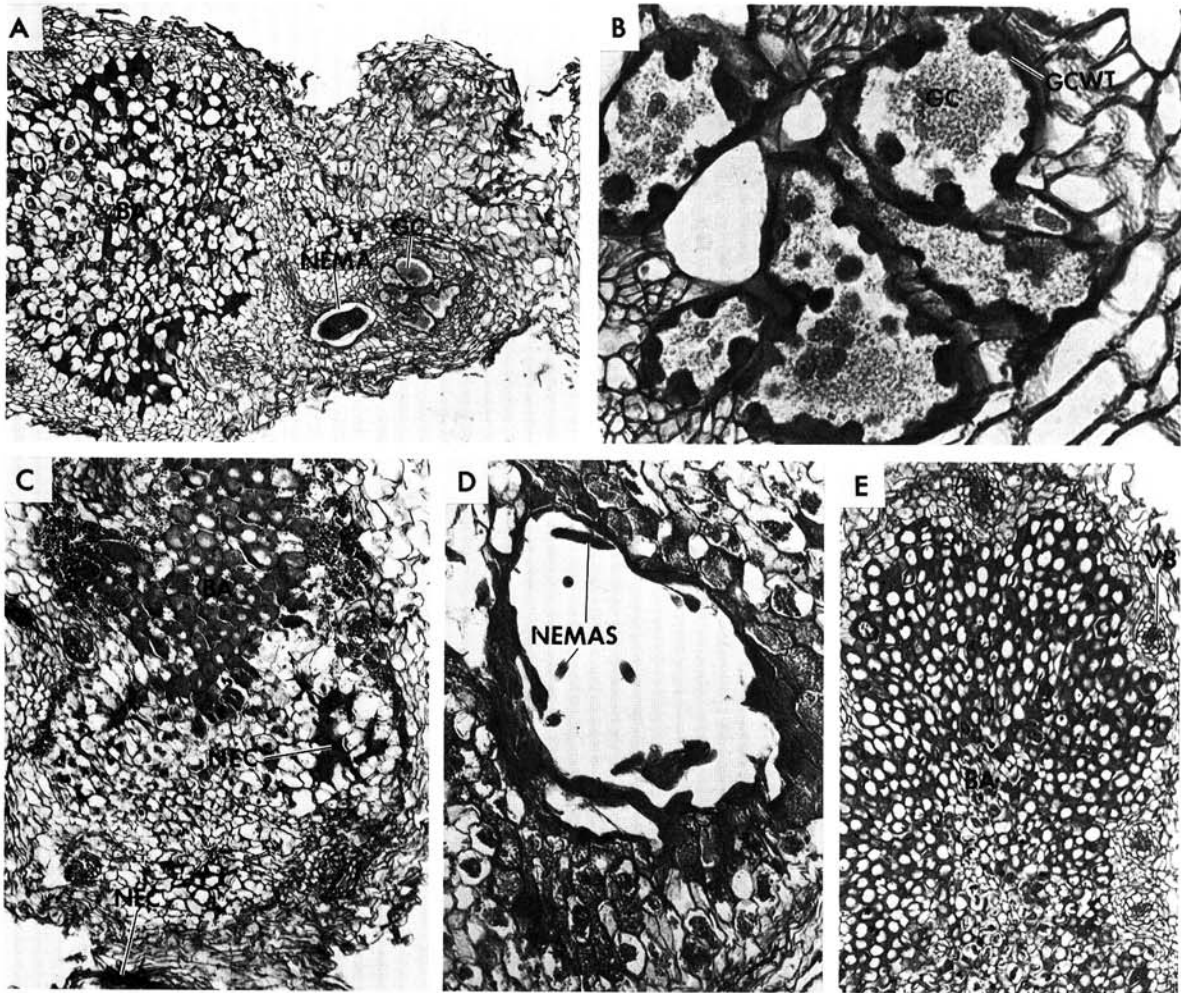


Fig. 2-(A to E). Responses of nodular tissues of Wando pea to feeding by nematodes (BA = bacteroids; GC = giant cell). A) Deterioration of bacteroids after penetration and feeding of a single *Meloidogyne incognita*. B) Wall ingrowths (GCWT = giant cell wall thickenings) associated with giant cells (GC) induced by *M. incognita* in Wando pea. C) Premature senescence of nodule, apparently resulting from feeding by *Belonolaimus longicaudatus* (NEC = necrosis). D) Cavity and specimens of *Pratylenchus penetrans* (NEMAS) in center of nodule (some of these nematodes may be microbivorous forms). E) Portion of control nodule with vascular bundles (VB = vascular bundle).

P. penetrans was limited to the cortical tissue outside the ring of xylary elements where cavities bordered by necrotic cells were formed (Fig. 1-E). A similar type of necrotic response, but without cavities, also was observed with *B. longicaudatus* on soybean (Fig. 1-F). Xylary elements (Fig. 1-G) around the periphery of the nodule apparently serves as a barrier to penetration by *P. penetrans* and feeding by *B. longicaudatus*.

Interactions of nematodes with nodules on Wando pea.—*Meloidogyne incognita* induced large galls and suppressed nodule formation on Wando pea. Nodules of Wando pea generally were less suitable for development and reproduction of *M. incognita* than those of soybean. Where nematodes fed, surrounding nodular tissue deteriorated (Fig. 2-A). Striking wall ingrowths were evident in giant cells in all tissues (Fig. 2-B).

Considerable damage was caused by *B. longicaudatus* even though it fed primarily on cortical tissues. Nodules tended to break down after feeding by this long-styled nematode (Fig. 2-C).

In contrast to the interaction on soybean, *P. penetrans* often penetrated Wando pea nodules (Fig. 2-D). The absence of a ring of xylary elements surrounding the bacteroids (Fig. 2-E) may have resulted in nodules of this plant being susceptible to penetration and/or feeding by *P. penetrans* and *B. longicaudatus*. The inoculum for *P.*

penetrans was not pure, and some microbivorous nematodes also penetrated nodular tissues.

Interactions of nematodes with nodules on peanut.—*Meloidogyne hapla* was not detected in nodules of peanut, and had no apparent effect on nodules that developed on galls (Fig. 3-A). *Pratylenchus penetrans* was limited to cortical tissues of nodules (Fig. 3-B). Necrotic tissues often surrounded invading nematodes and may reflect a degree of hypersensitive resistance of these tissues to *P. penetrans*.

Damage of nodules by *B. longicaudatus* also usually was limited to cortical tissues. However, extensive feeding sometimes caused much necrosis and occasionally deterioration of bacteroids (Fig. 3-C). The absence of a distinct ring of xylary elements appeared to allow this pest to cause more damage on peanut nodules (Fig. 3-D) than it did on soybean.

DISCUSSION AND CONCLUSIONS

The highly compatible interaction of *M. incognita* with nodular tissues of soybean partially explains the limited effects of this nematode on N_2 fixation on this host (5). The results of Taha and Raski (15), however, indicate that such interactions may cause some damage in the form of premature senescence of bacteroids and nodules. The

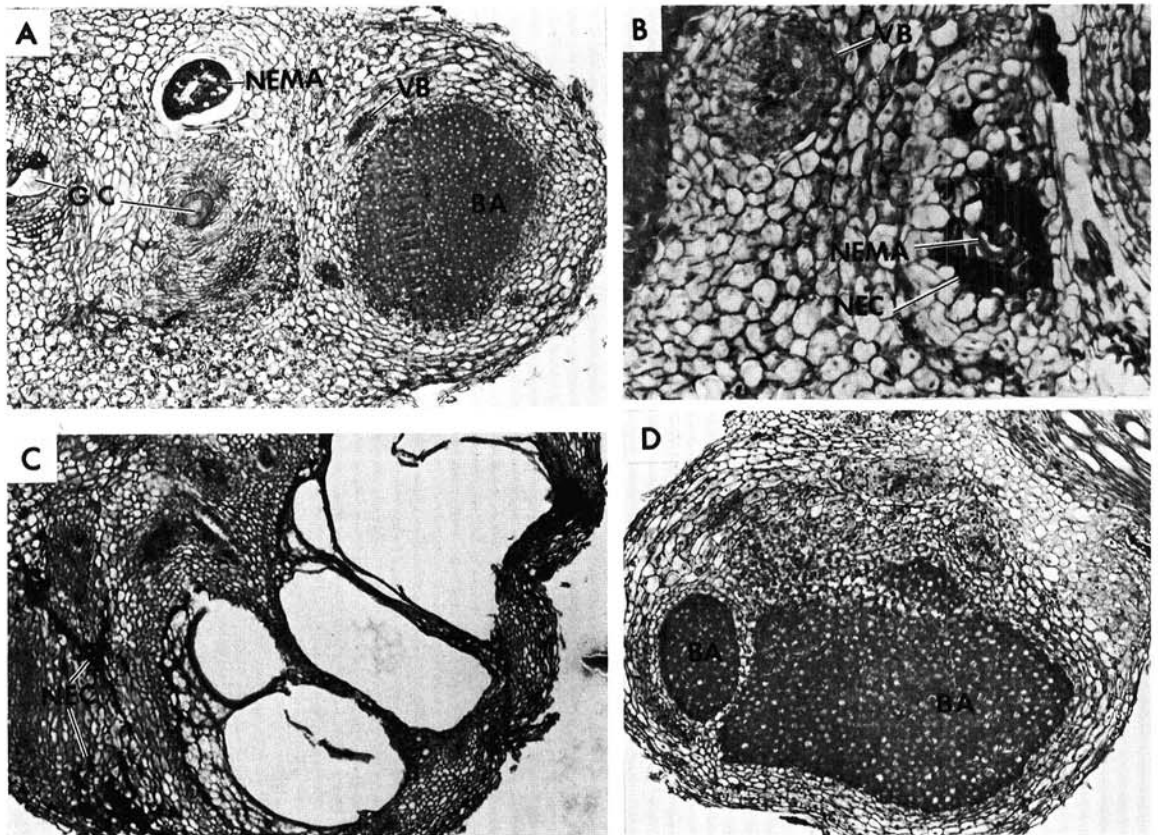


Fig. 3-(A to D). Responses of nodular tissues of peanut to feeding by nematodes (BA = bacteroids; GC = giant cell; VB = vascular bundle). A) Nondamaged nodule developing on a root gall induced by *Meloidogyne hapla*. B) Necrotic cortical tissue (NEC) surrounding *Pratylenchus penetrans* (NEMA). C) Unusual deterioration and necrosis (NEC) of nodule, apparently resulting from feeding by *Belonolaimus longicaudatus* (necrosis generally limited to outer cortex). D) Portion of a nodule from control.

explanation of how second-stage larvae of *Meloidogyne* species penetrate the ring of xylary elements around the periphery of soybean nodules when the other nematode species cannot, remains unknown. The thickened areas of many giant cells in soybean nodules apparently enables them to function as "transfer cells" as described by Jones and Northcote (9, 10) and Pate and Gunning (14). The type of wall ingrowths found in the giant cells in Wando pea also are typical of transfer cells which are thought to function in solute transport across the cell walls (4).

The severe damage normally encountered in the field on soybean and peanut parasitized by *B. longicaudatus* apparently results from a suppression of root growth rather than damage to nodular tissues which does occur with *H. glycines* on soybean.

The only nodules penetrated by the migratory endoparasite *P. penetrans* were those on Wando pea. Minimal damage to soybean and peanut nodules may be related to that species colonizing only cortical tissues. However, the capacity of this nematode to penetrate nodules of Wando pea and its association with microbivorous nematodes such as *Acrobeloides buetschlii* may result in considerable damage in field situations on this and certain other legumes. As indicated earlier, *A. buetschlii* does have the capacity to penetrate, reproduce, and inhibit nodulation in Wando pea (19).

Although all of these nematodes may suppress numbers of nodules that develop and N_2 fixation to a limited extent (5), there was a lack of the general hypersensitive or antagonistic interaction caused by *H. glycines* in soybean nodular tissues (1). Nodules of Wando pea were more susceptible than those of soybean or peanut to penetration and/or damage by any of the nematodes tested. Although direct damage of soybean and peanut nodules by these nematode species generally was minimal, the necrosis and wounding induced by these nematodes may predispose nodular tissues to penetration by bacteria, fungi, and microbivorous nematodes (such as *Acrobeloides*) in the field. Thus, disease complexes may occur in nodules.

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