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Histopathology of Root-knot Nematode (*Meloidogyne incognita*) Infection on White Yam (*Dioscorea rotundata*) Tubers[1]

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Abstract: White yam tissues naturally and artificially infected with root-knot nematodes were fixed, sectioned, and examined with a microscope. Infective second-stage juveniles of *Meloidogyne incognita* penetrated and moved intercellularly within the tuber. Feeding sites were always in the ground tissue layer where the vascular tissues are distributed in the tubers. Giant cells were always associated with xylem tissue. They were thin walled with dense cytoplasm and multinucleated. The nuclei of the giant cells were only half the size of those found in roots of infected tomato plants. Normal nematode growth and development followed giant cell formation. Females deposited eggs into a gelatinous egg mass within the tuber, and a necrotic ring formed around the female after eggs had been produced. Second-stage juveniles hatched, migrated, and re-infected other areas of the tuber. No males were observed from the tuber.

Key words: giant cell, histopathology, *Meloidogyne incognita,* root-knot nematode, yam.

Root-knot nematodes, *Meloidogyne* spp. (Kofoid & White) Chitwood, are important pests of edible yams, *Dioscorea* spp., wherever they are grown (1,4,8). If preplant populations of root-knot nematodes are high enough, plant growth and tuber yield are severely affected (1,3,8). Infected tubers are galled and flaky and may develop abnormal rootlets, especially after a period of storage (3,6,8). The deformity and consequent unattractive appearance of infected tubers reduce their market value (3,8). More important, however, root-knot nematode-infected tubers in storage lose weight rapidly and are highly prone to secondary infection by fungi such as *Aspergillus niger* van Teigh and *Penicillium sclerotigenum* Yamomoto (2,8). This results in a considerable reduction in the edible portion of the tubers or, in severe cases, the total loss of the stored tubers. Infected tubers also contain fewer pharmaceutically useful steroids such as diosgenin.

There is limited information on root-knot nematode histopathology on yams. Jenkins and Bird (6) reported the formation of "stone cells" in infected storage tubers which were composed of a mature female and an egg mass enclosed in wound periderm. They concluded that this reaction inhibited or drastically limited the ability of the root-knot nematode to reproduce within the tuber. This conclusion has led many reviewers (4) to underestimate the importance of root-knot nematodes as storage pests of yams. However, root-knot nematodes complete their life cycle in the yam tuber and eggs remain viable even after a 3–4-month storage period (8).

The objective of this investigation was to study in detail the histopathology of yam tuber tissues naturally and artificially infected with *Meloidogyne incognita* to better understand the activities and effects of the nematode on edible yams.

Materials and Methods

Galled and nongalled tubers of white yam, *Dioscorea rotundata* Poir ex-Nigeria, were purchased from a local grocery store in Reading, England. Adult *Meloidogyne* females were dissected out of the galled tubers and stained with acid fuchsin in lactophenol, and perineal patterns were prepared for identification.

Pieces containing galls were cut from infected tubers and fixed in 0.05 M phosphate-buffered 3% glutaraldehyde at 4 C for 16 hours. They were rinsed in six changes of buffer and dehydrated in a graded ethanol series (35, 50, 75, 90, and

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100%). The dehydrated pieces were passed through daily changes of 100% butanol, 1:1 butanol:xylene, 100% xylene, 1:1 xylene:paraplast, and 100% paraplast.

The last two changes were made on a hot plate (60 C). The pieces were embedded in paraplast. Sections 15 μm thick were cut with a microtome, affixed to a glass slide, rehydrated by passing through a descending ethanol series (100, 95, 70, and 50%), stained in safranin, and counterstained in fast green. All sections were mounted in Canada Balsam prior to examination with a compound microscope.

Several 3-mm-thick 20-mm-d tissue discs were obtained from the proximal end of peeled nongalled tubers using a cork borer. The discs were immediately rinsed in sterile distilled water and kept for 24 hours on moist sterile filter paper in sterile petri dishes at 23 C for conditioning. Conditioning is necessary because toxic metabolites may occasionally be released from cut surfaces of storage tissues, and they may inhibit parasite penetration (10). Conditioned discs were each inoculated with 100 second-stage juveniles (J2) of *Meloidogyne incognita*. The juveniles were obtained by extracting eggs from *M. incognita*-infested tomato (*Lycopersicon esculentum* Mill cv. Moneymaker) roots by the NaOCl method (5) and allowing the eggs to hatch at room temperature. Newly hatched J2 were collected daily and stored at 5 C for 3–4 days prior to use.

At daily intervals for 7 days, five inoculated tissue discs were fixed and processed for histological observations as previously described. Galled Moneymaker tomato roots were processed for histopathology as described for galled yam tissues.

Results

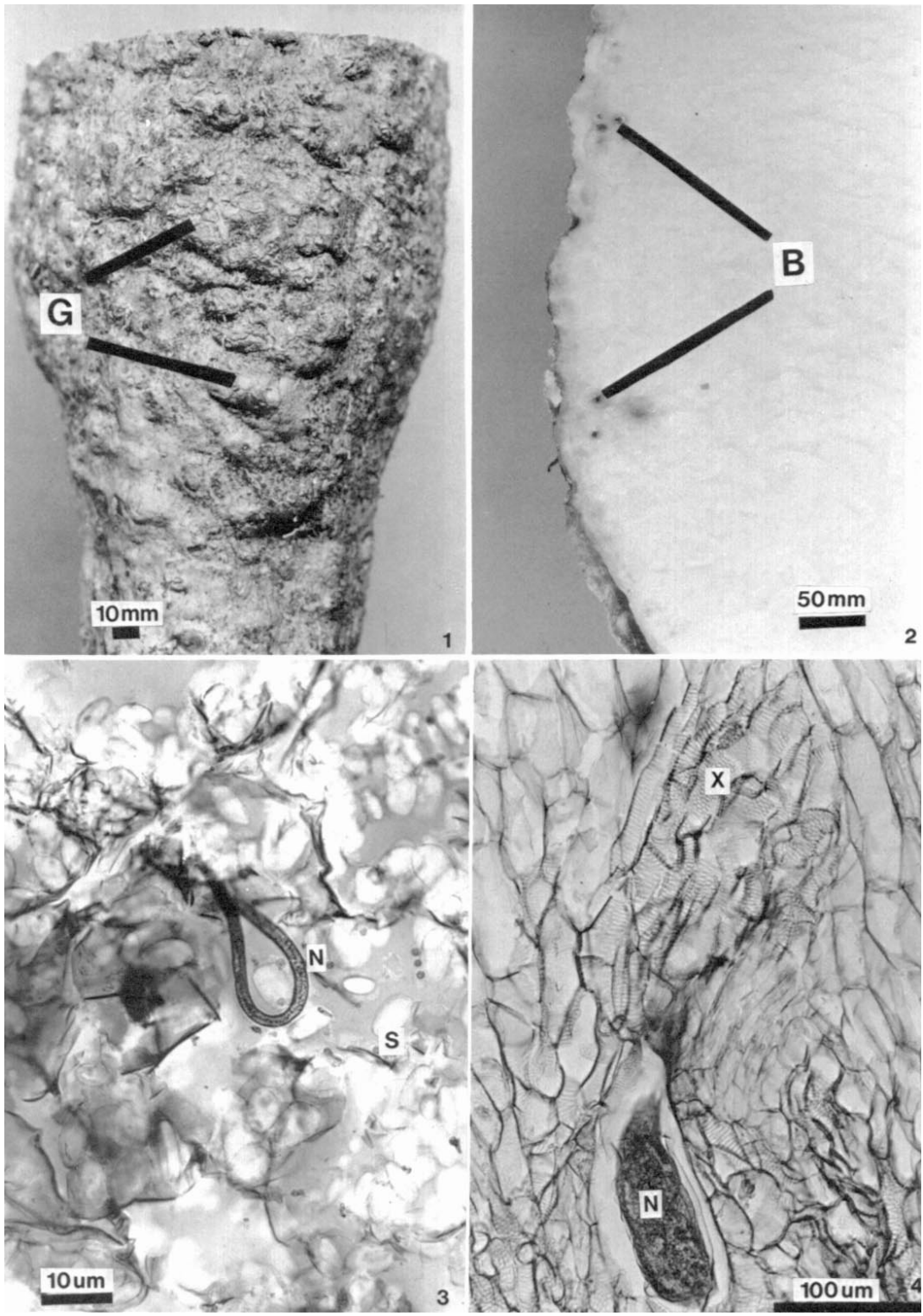
Galls on the tubers were large and often coalesced (Fig. 1). Necrosis in the ground tissue was associated with many of the galls (Fig. 2). The perineal patterns confirmed that the galled tubers were infected with *Meloidogyne incognita*.

Results of artificial infection indicated that the infective J2 moved intercellularly within the yam tissues (Fig. 3) until they reached the vascular tissues where they established feeding sites. Feeding sites were established only in the innermost ground tissue layer and never in the outer cork periderm, cortex, or meristematic layers. Normal growth and development of the nematodes followed the establishment of feeding sites (Figs. 4, 5). Giant cell formation was induced within the tubers (Figs. 5, 6). The number of giant cells that could be observed near the head of the nematode ranged from one to three, but only one giant cell was most common. The giant cell in the yam tuber was strikingly thin walled, but it had dense cytoplasm and was as multinucleate as one formed in the tomato roots (Fig. 7). The nuclei of the giant cell in the yam tuber were only half the size of those observed in tomato. No giant cell had been induced in the tissue discs 7 days after inoculation.

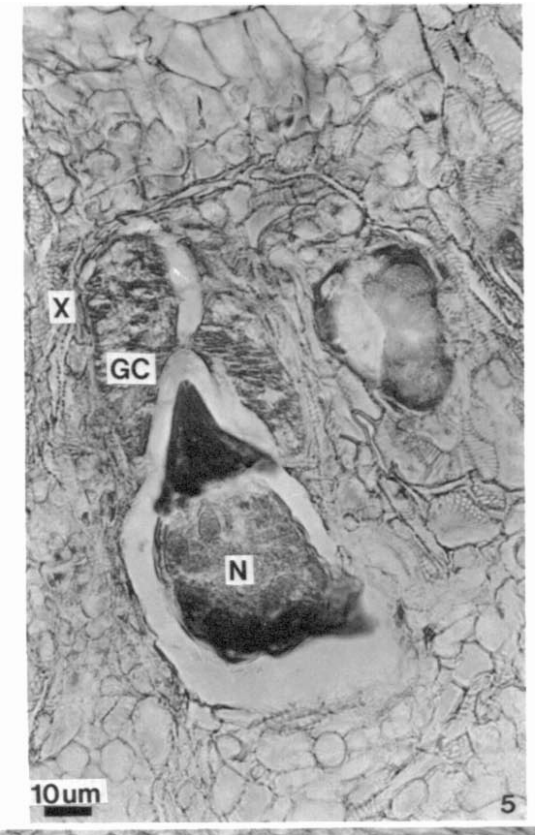
Fully developed females which had produced eggs were usually surrounded by a ring of necrotic tissue. The onset of necrosis was always associated with the production of the gelatinous matrix by the root-knot nematode female (Fig. 8). Eggs and J2 could be found within the necrotic ring (Figs. 9, 10). Juveniles were also observed in cells adjacent to the ring of necrotic tissue.

Discussion

The results of this study indicate that the life cycle of root-knot nematodes within yam tubers, allowing for yam tissue anatomy, is similar to that in roots of other susceptible crop hosts such as tomato. This is the first report of the formation of giant cells in yam tubers, although their presence has been assumed by various workers. These giant cells differ from those in roots of susceptible crops, such as tomato, by their thin walls and smaller and fewer nuclei. These differences may or may not be significant in the growth and development of root-knot nematodes in yam tubers. Since the nematodes depend on the giant cells, this may explain why adult root-knot nematodes are usually found concentrated



FIGS. 1–4. *Dioscorea rotundata* tubers infected with *Meloidogyne incognita*. 1) Galling (G) and deformity of tubers. 2) Transverse section showing browning (B) associated with nematode galls. 3) Section with infective juveniles (N) in tuber and starch grain (S). 4) Fourth-stage juvenile in vascular tissue. X = xylem.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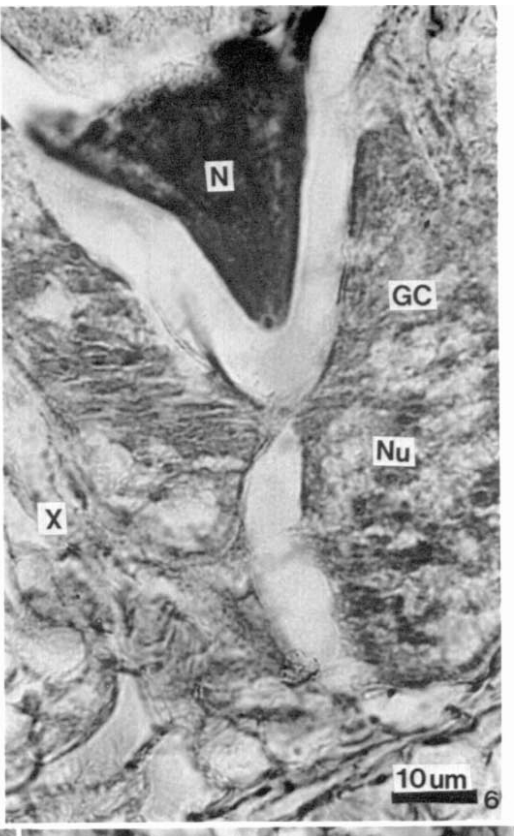
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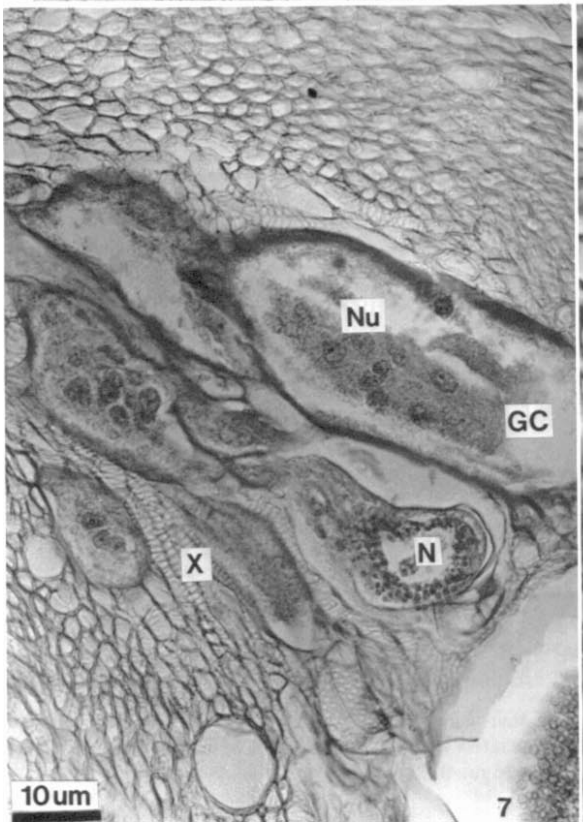
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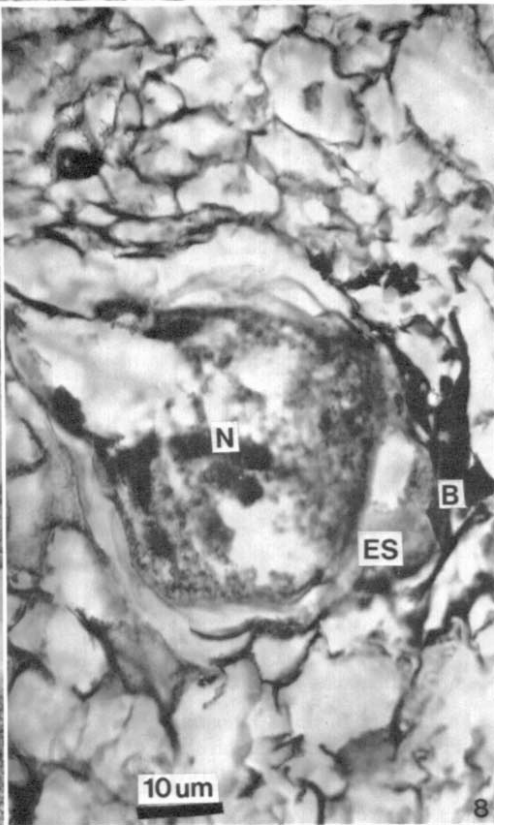
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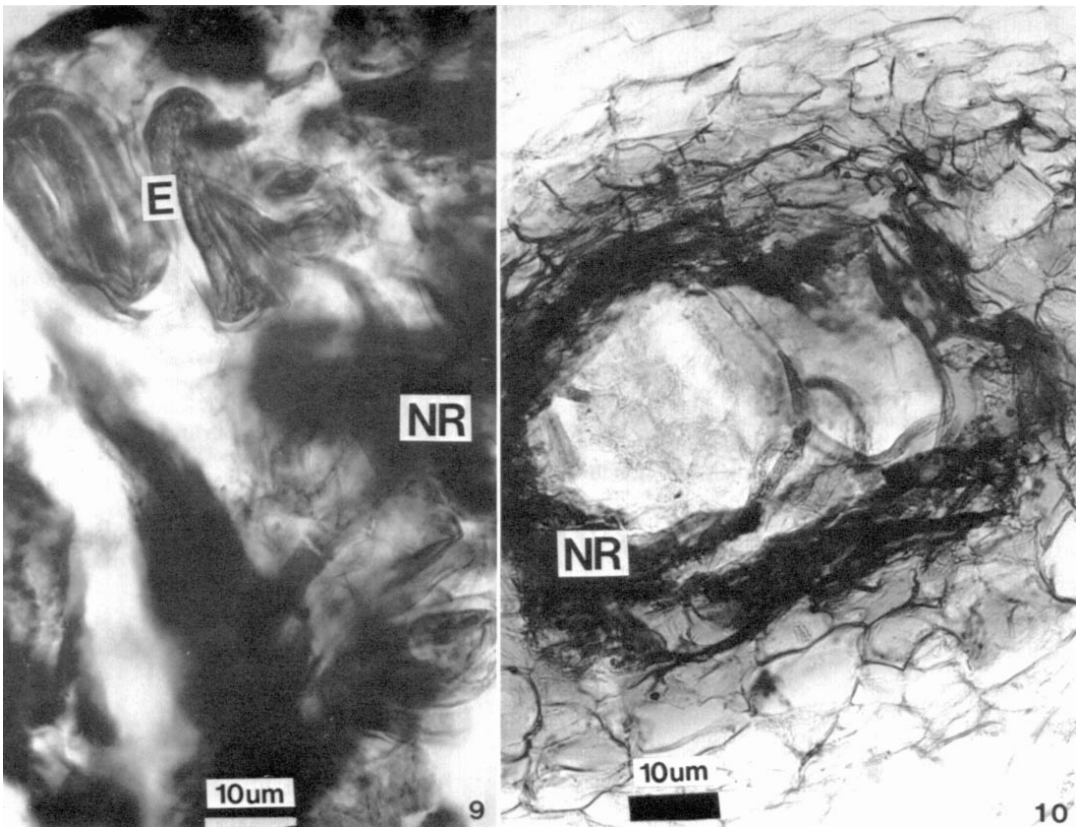

FIGS. 9, 10. Eggs and second-stage juveniles of *Meloidogyne incognita* inside ring of necrotic tissue (NR) of *Dioscorea rotundata* tuber. 9) Eggs (E). 10) Juveniles.

in the 4–6-mm layer of *D. rotundata* tubers (8). This is the area of the tuber where the first set of vascular bundles are located.

In these studies, no wound-healing response (6) was observed. Characteristic wound-healing responses include the formation of callus-like tissues or wound periderm at the wound site; intense suberization; the production of resins, gum, latex, or callose in the wound area; and the use of damaged cells and their precipitated constituents as a barrier (7,9). A wound-healing response is more likely to occur in a noncompatible yam–nematode interaction.

A necrotic ring was observed around female root-knot nematodes. It appeared only after the gelatinous matrix was produced and was confined initially to the tissues in the perineal region (Fig. 8). Contrary to the reports of other workers (4,6) this necrotic reaction does not appear to be damaging or restrictive. Second-stage juveniles of the root-knot nematode were observed in cells adjacent to the necrotic ring, thus indicating that the J2 were able to hatch and move out of the necrotic areas to cause new infection in the tuber. Previous results (8) have also shown that viable eggs and J2 were extracted from yam tubers following

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FIGS. 5–8. Giant cells produced by *Meloidogyne incognita* in *Dioscorea rotundata* or tomato. 5) *D. rotundata* tuber with two giant cells (GC) adjacent to nematode (N) in xylem tissues (X). 6) *D. rotundata* tuber with thin-walled giant cells (GC) and small nuclei (Nu). N = nematode. X = xylem. 7) Tomato root with thick-walled giant cell (GC) and large nuclei (Nu). N = nematode. X = xylem. 8) *D. rotundata* tissues and female (N) with egg sac (ES). Note necrosis (B).

a 4-month storage period. Thus, they may serve as primary inoculum when infected tubers are used as planting materials.

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