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# Histopathology of *Ditylenchus destructor* on Peanut

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*Abstract:* The time and mode of entry, and development of *Ditylenchus destructor* in peanut were studied in field and greenhouse experiments. Few nematodes were present in the cortex of the roots. At 90–120 days after planting, *D. destructor* was observed in the exocarp at the base of the pod near the point of connection with the peg. The peg was invaded from this primary infection site. The endocarp of the hull was usually penetrated through openings at the base of the mesocarp and sometimes at the pod apex. Numerous *D. destructor* were present in the testa and the vascular bundles. Nematodes were found in the embryo but not in the cotyledons. The histopathology of *D. destructor* closely resembles that of the peanut testa nematode, *Aphelenchoides arachidis* Bos.

*Key words: Arachis hypogaea, Ditylenchus destructor,* histopathology, peanut, potato rot nematode.

The potato rot nematode, *Ditylenchus destructor* Thorne, is an important pest of peanut (*Arachis hypogaea* L.) in the Republic of South Africa (5,8). It is widespread in all major peanut-producing areas, and approximately 40–60% of the pods can be affected in heavily infested fields (5). Infected hulls have brown necrotic tissue at the point of connection with the peg, and the longitudinal veins become black. Infected seeds are usually shrunken and their testae and embryos become yellow, brown, or black (5,8). The unattractive appearance of infected seeds reduces their market value.

There is limited information on the histopathology of nematode infestations of peanut. The histopathology of *Pratylenchus brachyurus* (Godfrey) Filipjev in roots, pegs, and hulls was described (7). This nematode was not found in the seeds from infected hulls (7). The histopathology of the peanut testa nematode, *Aphelenchoides arachidis* Bos, in roots, hulls, and seeds also has been described (3), and many nematodes were found in the testa.

The present study was undertaken to investigate in detail the histopathology of peanut tissues naturally and artificially infected with *Ditylenchus destructor.*

## Materials and Methods

The time and mode of entry, and development of *D. destructor* in roots, pegs, hulls, and seeds of 'Sellie' peanut were studied in the field and in the greenhouse.

*Field observations:* A field of sandy soil in the Schweizer-Reneke district, heavily infested with *D. destructor,* was selected for the study. Four single-row plots, each 5 m long, were planted with nematode-free peanut seeds on 21 October 1987. From the time of emergence until 4 February 1988, 20 plants were removed at 10-day intervals and thereafter at 30-day intervals until harvest on 4 May. For histological examination, roots from young plants and roots, pegs, hulls, and seeds from older plants were fixed in formalin–alcohol–acetic-acid (F.A.A.) for 24 hours, hand-sectioned, mounted in lactophenol, and examined with phase contrast microscopy.

*Greenhouse observations:* Nematode-free peanut seeds were planted in 48 20-cm-d plastic pots filled with 4 $dm^3$ steam sterilized sandy soil (93% sand, 4% silt, 3% clay). The plants were thinned to one per pot after emergence. The plants were fertilized by irrigation with tap water in which Chemicult (6.5% N, 2.7% P, 13% K) was dissolved. Pots were maintained at 17–27 C with a 13-hour photoperiod. One week after planting, 24 pots were inoculated with *D. destructor* obtained from groundnut callus tissue cultures (9). A suspension of 4,000 nematodes of mixed life stages was pipet-

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ted into holes in the soil around the roots. Uninoculated plants were used as controls. Two plants were removed at 7-day intervals for the first 21 days after emergence and at 14-day intervals thereafter until harvest at 126 days after planting. Roots from young plants and roots, pegs, hulls, and seeds from older plants were fixed in F.A.A., dehydrated in a series of alcohol solutions, cleared in propanol and butanol, and then embedded in paraplast (6). Transverse and longitudinal sections, 12 μm thick, were stained with Mallory's triple-stain (1), mounted in D.P.X. (BDH Ltd, Broom Rd, Poole, Dorset BH12 4NN, England), and examined with brightfield and Nomarski interference microscopy (6).

## Results

*Field observations: Ditylenchus destructor* was first observed in peanut tissue 85 days after planting. The nematodes were 3–5 cell layers deep in the exocarp at the base of the pods, near the point of connection with the peg. The surface of the primary infection site was indicated by brown, spongy cork cells. From this site, the peg was invaded as the nematodes fed on the contents of parenchyma cells causing cellular collapse (Fig. 1A, B). Empty cells were pushed aside, creating passages through which the nematodes moved. Once established in the peg, the nematodes either further penetrated the exocarp by feeding on the parenchyma cells surrounding the vascular bundles (Fig. 1C) or migrated to the base of the mesocarp through the natural opening at the point of peg attachment (Fig. 1D). The sclerenchymatous mesocarp layer delayed penetration of the endocarp. Eighty-five days after planting, no nematodes were found beyond the mesocarp; however, mesocarp cells in contact with the nematodes had become discolored (Fig. 1E). Ninety-six days after planting, mesocarp cells had broken down, creating an opening to the endocarp (Fig. 1F). A similar opening was formed through the mesocarp at the apex of the pods. Although the endocarp was already invaded at 96 days after planting, the only signs of infection were necrosis at the junction of the peg with the pod and a slight corkiness around the peg. *Ditylenchus destructor* entered the seed through the micropyle and invaded the embryo (Fig. 1H). Nematodes were observed in the testa (Fig. 1G) and on the surface of the cotyledons (Fig. 1I) but not inside the cotyledons. Eighty-five days after planting, only 9% of all plants examined were infected with *D. destructor.* At 106, 139, 178, and 193 days after planting, 7, 33, 64, and 92% of the pods per plant were infected. *Ditylenchus destructor* was not observed in the roots.

*Greenhouse observations:* The time and mode of entry, and development of *D. destructor* in peanut tissues were similar to those observed in the field. Twenty-one days after planting, a few nematodes were observed in the root cortex. Ninety-eight days after planting, *D. destructor* was present in the pegs (Fig. 2A), hulls, and seeds. Entry into the pod was most often at the base of the pod, at the point of peg attachment (Fig. 2B), but occasionally the pod was also entered at the apex. At 119 days after planting, numerous nematodes were present in the exocarp, usually feeding on the parenchyma cells surrounding the vascular bundles (Fig. 2C, E), in the endocarp, and in the testa (Fig. 2D, F). In the testa, *D. destructor* was observed in the vascular bundles (Fig. 2G).

## Discussion

The histopathology of *D. destructor* on peanut closely resembles that of *Aphelenchoides arachidis* (2–5). Both species are endoparasitic and feed on tissues of the roots, pegs, hulls, and seeds. Developing pods are invaded after fruiting pegs have penetrated the soil. Most nematodes occur in the hulls and the seeds. In the testa, many nematodes feed on the tissues near or in the vascular bundles causing discoloration of vascular strands within the seed coat. No nematodes were observed in the cotyledons. *Aphelenchoides arachidis* occurs in greater numbers in roots than does *D. destructor* and rapidly invades the developing pods. Like *D. destructor, A. arachidis* was not

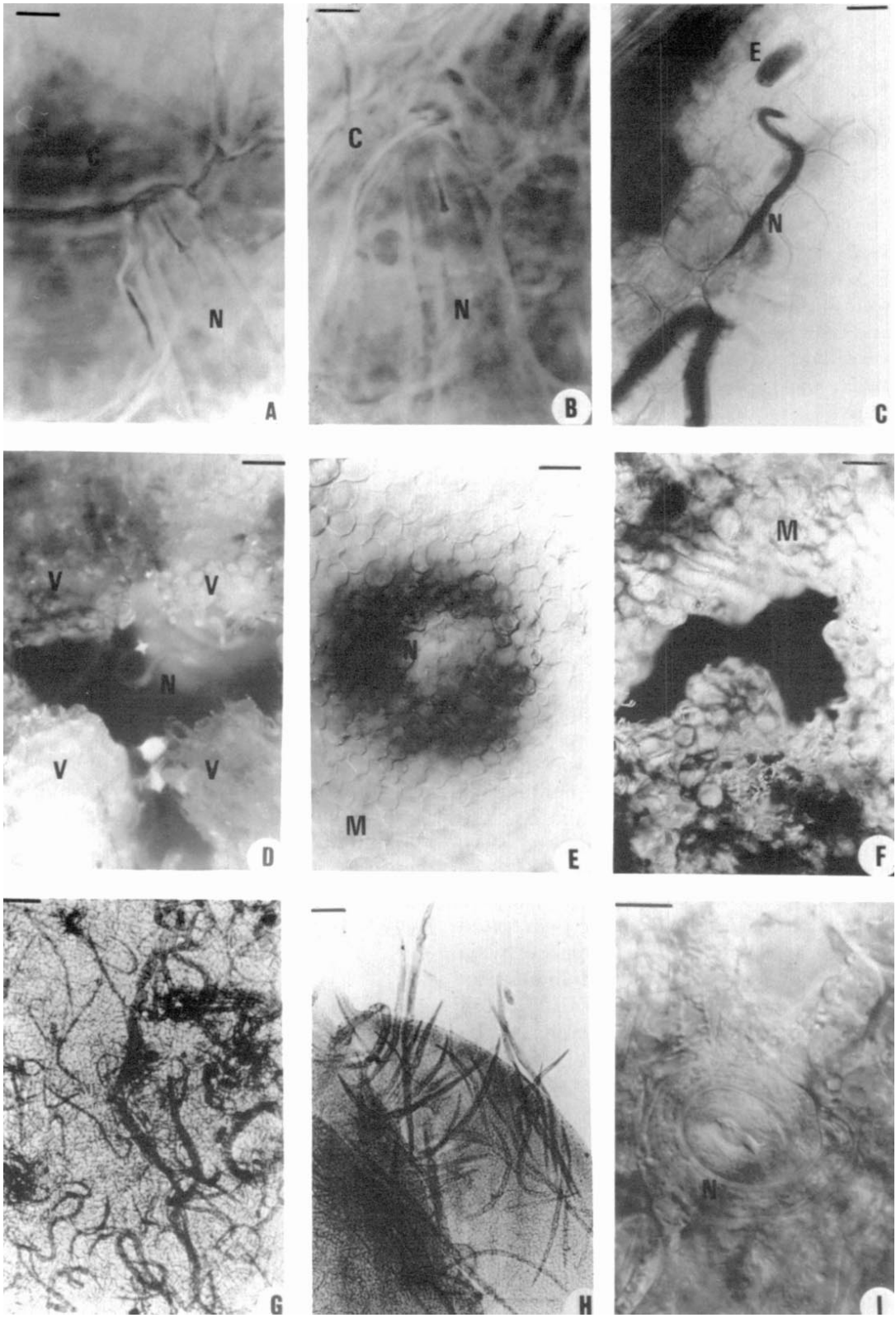


FIG. 1. Histopathology of peanut naturally infected with *Ditylenchus destructor*. C = cell, E = egg, M = mesocarp, N = nematode, V = vascular bundles. A, B) Longitudinal sections showing nematode feeding on parenchyma cells of a peg. B) Collapsed cell after removal of cell contents. C) Longitudinal section showing egg and nematodes in parenchyma tissue which extends from the peg into the exocarp of the pod. D) Transverse section through a peg near attachment to the pod showing a nematode in the opening created by the separation of the vascular bundles. E) Transverse section through the mesocarp at the base of a young

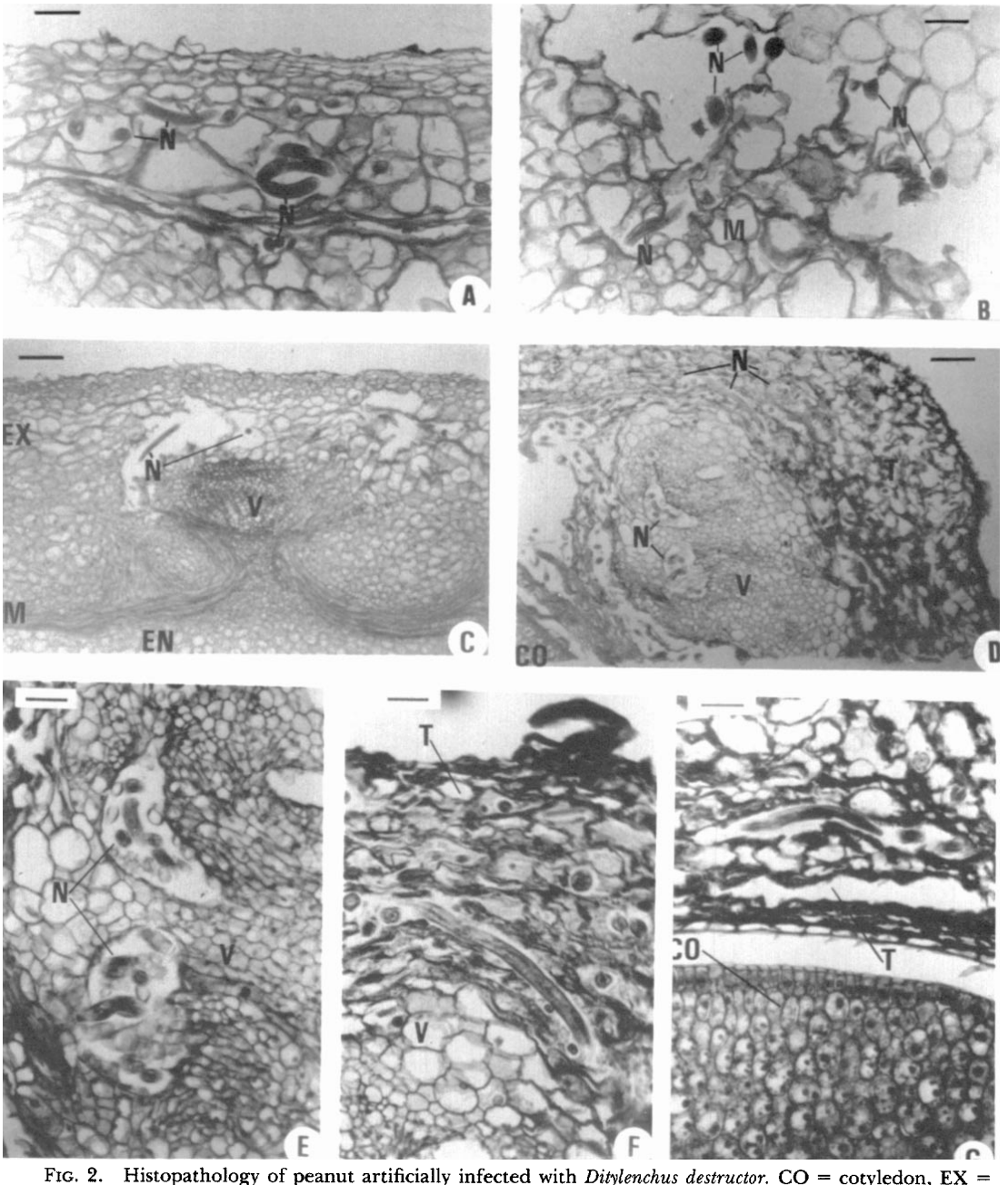


FIG. 2. Histopathology of peanut artificially infected with *Ditylenchus destructor*. CO = cotyledon, EX = exocarp, EN = endocarp, M = mesocarp, N = nematode, T = testa, V = vascular bundles. A) Transverse section showing nematodes in parenchyma tissue of a peg. B) Transverse section through the mesocarp at the base of a young pod showing discoloration of the sclerenchyma tissue and openings which developed following the presence of nematodes. C) Transverse sections through a peanut hull showing nematodes in the parenchyma tissue of the exocarp near the vascular bundles. D–F) Transverse sections through an immature peanut seed showing nematodes in the parenchyma tissue of the testa (E) and vascular bundles (F). G) Transverse section through a mature seed showing nematodes in the parenchyma tissue of the testa. Bars = 50 μm in A, B, E–G; 125 μm in C, D.

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pod showing discoloration of the sclerenchyma tissue which developed following the presence of nematodes. F) Transverse section through the mesocarp at the base of a young pod showing an opening created by nematodes. G) Enlargement of eggs and nematodes in testa of mature seed. H) Longitudinal section showing eggs and nematodes in embryo. I) Enlargement of nematode in coiled position on the surface of a cotyledon of a mature seed. Bars = 5 μm in A, B; 30 μm in C, E, I; 50 μm in D; 60 μm in F; 100 μm in G, H.

observed in the embryo or the central stele (2–4).

We do not know how *D. destructor* caused openings in the mesocarp or why it aggregated in large numbers near the vascular bundles in the exocarp. The discoloration of the mesocarp tissue at the base and top of the pod surrounding the feeding site of *D. destructor* may indicate that some enzymatic activity is involved, but mechanical damage cannot be excluded. The exocarp tissues adjacent to the vascular bundles may facilitate migration of the nematodes, but it is also possible that these tissues constituted an attractive feeding site.

## Literature Cited

1. Barbosa, P. 1974. Manual of basic techniques in insect histology. Amherst, MA: Autumn Publishers.

2. Bos, W. S. 1977. *Aphelenchoides arachidis* n. sp. (Nematoda: Aphelenchoidea), an endoparasite of the testa of groundnuts in Nigeria. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 84:95–99.

3. Bridge, J., W. S. Bos, L. J. Page, and D. McDonald. 1977. The biology and possible importance of *Aphelenchoides arachidis*, a seed-borne endoparasitic nematode of groundnuts from northern Nigeria. Nematologica 23:253–259.

4. Bridge, J., and D. J. Hunt. 1985. *Aphelenchoides arachidis*. C.I.H. descriptions of plant-parasitic nematodes, set 8, no. 116. London: Commonwealth Agricultural Bureau.

5. De Waele, D., B. L. Jones, C. Bolton, and E. van den Berg. 1989. *Ditylenchus destructor* in hulls and seeds of peanut. Journal of Nematology 21:10–15.

6. De Waele, E. 1987. A study of the interaction between *Pratylenchus* spp. (Nematoda) and *Zea mays* L. M.Sc. thesis, Rand Afrikaans University, Johannesburg.

7. Good, J. M., L. W. Boyle, and R. O. Hammons. 1958. Studies of *Pratylenchus brachyurus* on peanuts. Phytopathology 48:530–535.

8. Jones, B. L., and D. De Waele. 1988. First report of *Ditylenchus destructor* in pods and seeds of peanut. Plant Disease 72:453.

9. Van der Walt, P. C. W., and D. De Waele. 1989. Mass culture of the potato rot nematode *Ditylenchus destructor* on groundnut callus tissue. Phytophylactica 21:79–80.