

Istituto di Nematologia Agraria del C.N.R. — 70126 Bari, Italy

HISTOLOGICAL ALTERATIONS INDUCED BY *ROTYLENCHULUS RENIFORMIS* ON *COFFEA ARABICA* ROOTS¹

by
N. VOVLAS and F. LAMBERTI

Summary. Investigations on the histopathology of *Rotylenchulus reniformis* Linford *et* Oliveira, on coffee (*Coffea arabica* L.) roots revealed that the permanent feeding site of adult female is initiated in an endodermal cell. Syncytia induced in coffee feeder roots consist of a curved sheet of pericyclic cells which are conspicuously hypertrophied, with densely staining cytoplasm, extended 6-10 cells on either side of the feeding place, both circumferentially and longitudinally, involving 150- 200 modified cells. A small feeding peg surrounds the nematode stylet where it penetrates the thickened cell wall of the initial cell of syncytium. The stylet tip seems to be continuous with a small feeding tube which is included in the cell contents of the nematode feeding cell of the syncytium.

The reniform nematode *Rotylenchulus reniformis* Linford *et* Oliveira was first observed on roots of cowpea (*Vigna unguiculata* L.) Walp., in the Hawaiian islands. It is known to parasitize approximately 160 other plant species and is a severe pathogen of considerable economic importance for several crops in tropical and subtropical regions (Mac Gowan, 1977). The reniform nematode has been observed on coffee in India, Brazil, Puerto Rico and Sao Tomè (Gonzaga and Lordello, 1986; Vovlas and Lamberti, 1985). This article reports on the permanent feeding site of adult females and illustrates the histological effects caused by the nematode on *Coffea arabica* L. roots, collected during a nematode survey in coffee plantations in Sao Tomè.

Materials and methods

Nematode infected plant material was fixed in FAA (formalin- acetic acid-ethyl alcohol), dehydrated through a tetr-butyl alcohol series, and embedded in parawax (melting point 56-58°C). Serial sections (10-12 µm) were stained with safranin and fast green (Johansen, 1940) and selected sections photographed using a light microscope. The common terminology e.g. syncytium, initial cell, feeding cell, feeding peg, feeding tube, used for *R. borealis* and *R. reniformis* in other hosts (Cohn, 1973; Rebois *et al.*,

1975; Rebois, 1980; Jones and Dropkin, 1975; Vovlas and Inserra, 1983) have also been used in our observations.

Results and discussion

Rotylenchulus reniformis adult females with the posterior of the body protruding the root surface were found to be randomly distributed on coffee roots without any preferred location on the root (Fig. 1A). They were partially embedded in damaged cortical cells perpendicularly to the stele establishing a permanent feeding site in a stelar syncytium. The cell walls of the cortical cells through which the nematode passed were slightly thickened and stained more deeply with safranin (Figs. 1C, D; 2A). Syncytia induced by *R. reniformis* in coffee roots, consist of a curved sheet of pericyclic cells which are conspicuously hypertrophied and have densely staining cytoplasm, extending 6-10 cells on either side of the feeding cell, both circumferentially and longitudinally (Figs 2A, D; 3A, D).

Pericyclic cells near to the nematode head are usually 3-8 times the size of normal pericyclic cells (Fig. 2B). Expansion, in fact, occurs most in the initial cells of the syncytia and the degree of expansion of modified cells is reduced as the distance from these cells increases until there is no expansion (Figs 2C; 3A, B). The extent of cell modification caused by one nematode forming one 'syncytial unit' has been estimated to be 100-200 modified cells. The sheet of modified cells, occasionally in overlapped layers (Fig. 2C), has been observed sometimes to contact the vascular elements and occupy 1/3 of the stelar area, with predictable disorganization of the root structure. Closer examination of the feeding zone showed that the nematode

¹ This study was partially funded by the European Economic Community (EEC) Contract n. TS 2.0158. P. for cooperative studies on Nematodes of Coffee in S. Tomé and Príncipe with screening for resistance and host-parasite relationships).

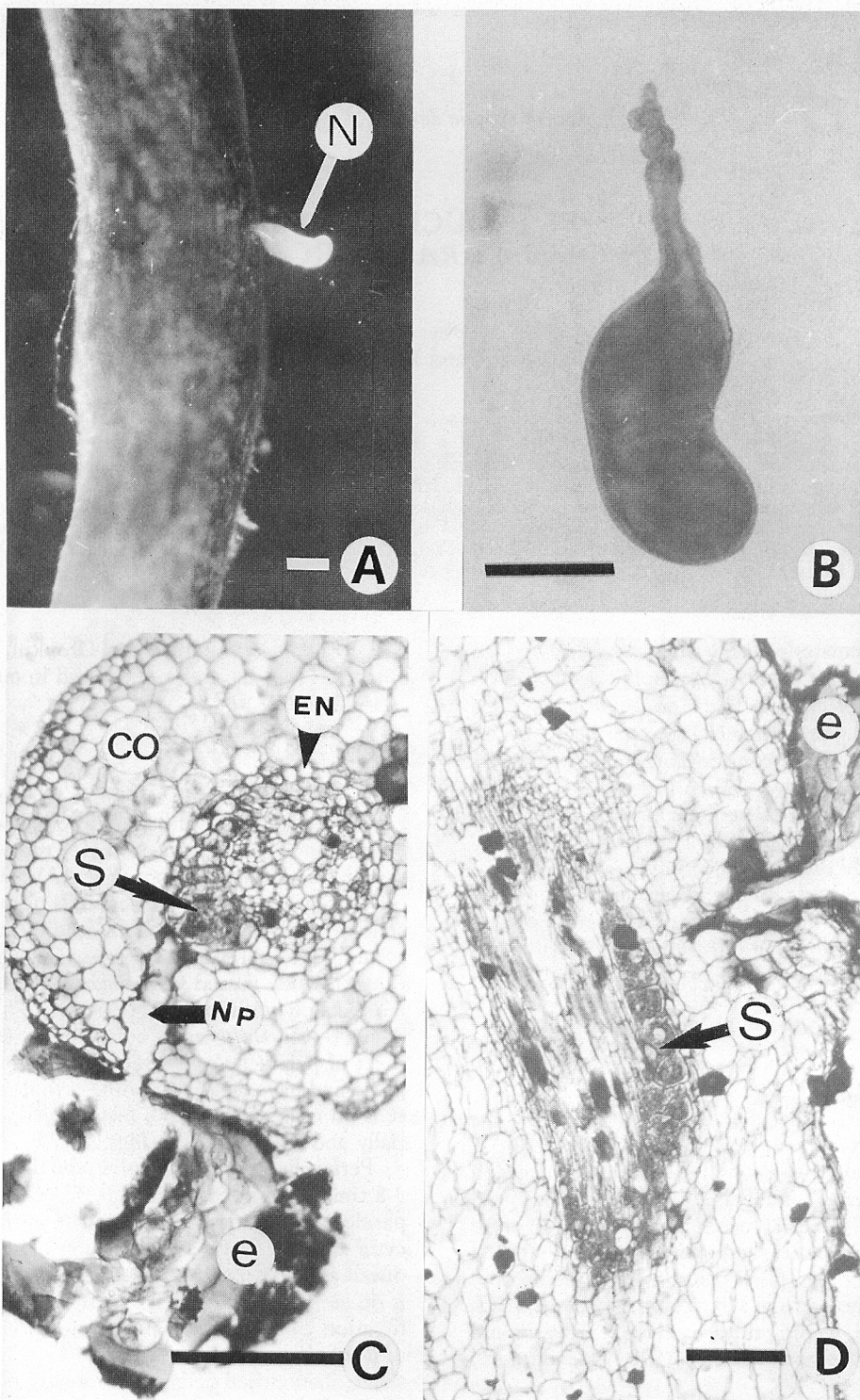


Fig. 1. A) Adult female (N) of *Rotylenchulus reniformis* inserted in a coffee root. B) Entire female body extracted from the root. C,D) Cross and longitudinal sections of infested roots showing nematode penetration (NP) through cortical tissues (CO) and syncytium (S) in the stele. (Other abbrev.: EN = endodermis; E = eggs; T = thickened cell walls) (Scale bars = 150 μ m).

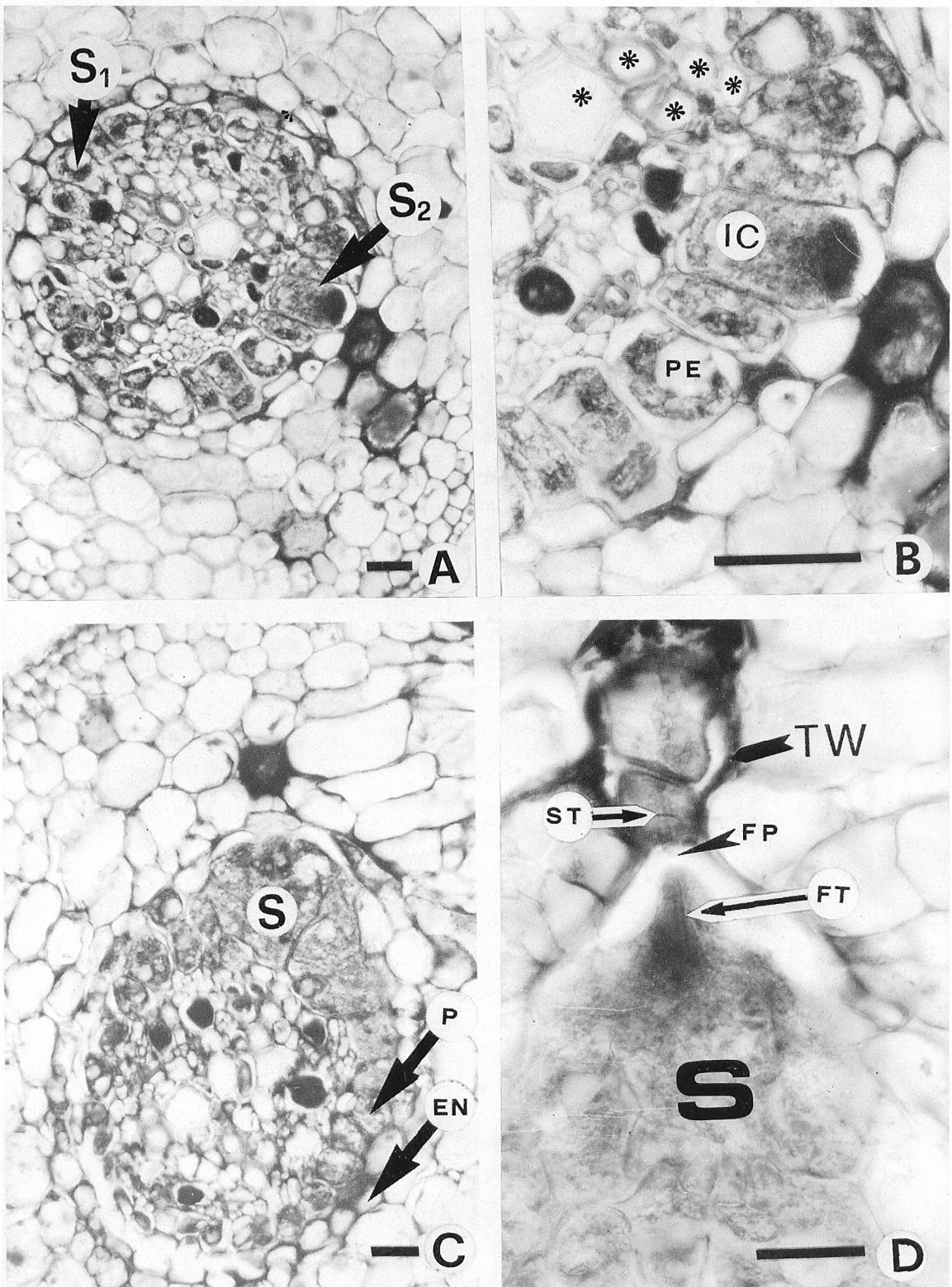


Fig. 2. Anatomical changes of coffee roots induced by *R. reniformis*: A) Cross section showing the stelar area with two, S1 and S2, sheets of modified pericyclic cells stimulated by two different females which feed on two diametrically opposite sites. B) Enlargement of the feeding zone of syncytium, S2, of the Fig. A. Note the expansion of the initial cell (IC) of the syncytium (Abbrev.: PE = pericycle; * = xylem elements). C) Cross section showing the stelar area with a multilayer syncytium (S) (Abbrev.: P = pericycle; E = endodermis). D) Enlargement of the feeding zone of Fig. C (Abbrev.: S = syncytium; ST = stylet of the nematode; FP = feeding peg; FT = feeding tube; TW = thickened wall) (Scale bars = 25 μ m).

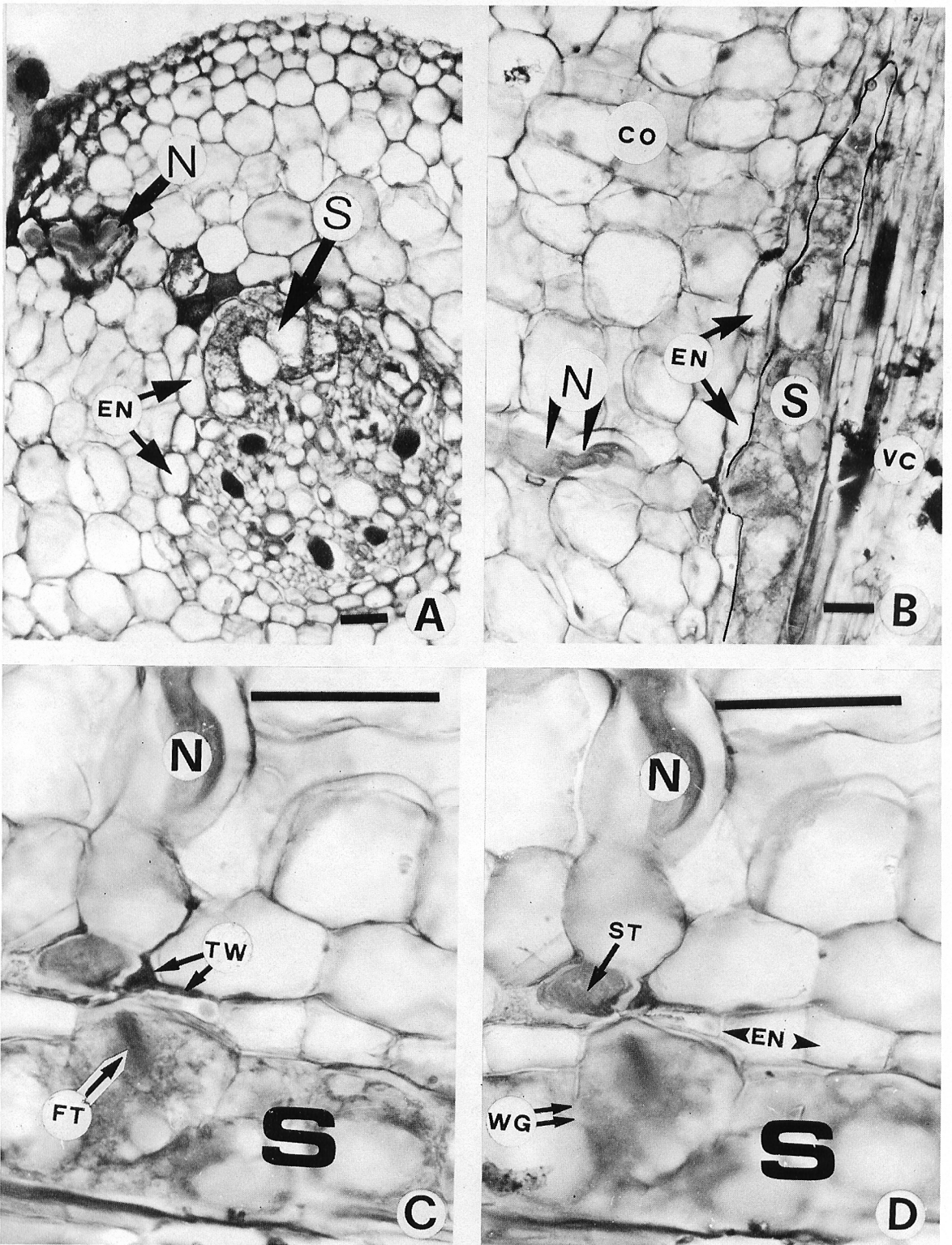


Fig. 3. Cross (A) and longitudinal (B-D) sections of coffee roots infected by *R. reniformis*: A) Intercellular penetration of cortical cells by a female (N) and well developed syncytium which has altered the symmetry of the stelar area. B) Longitudinal section showing a large syncytium (S) extending in opposite directions (about 6-7 cells) from the feeding point of the nematode (N). C and D) Enlargement of the feeding point of the section of Fig. B showing syncytia (S) at different foci. Note: the nematode stylet (ST) and the thickened cell wall (TW) of the endodermal cell in contact with the nematode (N) lips. (Additional abbreviations used: CO = cortex; EN = endodermis; VC = vascular elements; FT = feeding tube; WG = wall gap between syncytial cells) (Scale bars = 25 μ m).

stylet was inserted into one endodermal cell, the feeding cell (Fig. 3B). The cytoplasm of the feeding cell and other syncytial cells was very dense and granular (Figs 3A-D). The cell wall of the endodermal cells, where the nematode stylet was inserted, was thicker than in normal endodermal cells and deeply stained by safranin (Fig. 3C). Where the stylet penetrated the wall of the feeding cell a small peg-like structure was formed, 'feeding peg', containing the stylet and projecting about 6-8 μ m into the cytoplasm (Fig. 2D) of the cell. In front of the stylet tip there was a continuous small feeding tube which was *not coiled* (Figs 2D; 3C, D).

This study showed that in coffee also *R. reniformis* is a stelar parasite and feeds in pericyclic cells inducing profound anatomical changes.

The histological modifications induced by *R. reniformis* in coffee roots as described above, are very similar to those reported for this species in roots of cantaloupe soybean, cotton and sunflower (Heald, 1975; Rebois *et al.*, 1975; Robinson and Orr, 1982; Cohn, 1973), for *R. borealis* in corn (Vovlas and Inserra, 1983) and for *Meloidoderita* species on *Mentha* sp. (Cohn and Mordechai, 1982).

Literature cited

COHN E., 1973 - Histology of the feeding of *Rotylenchulus reniformis*. *Nematologica.*, 19: 455-458.

- COHN E. and MORDECHAI M., 1982 - Biology and host-parasite relations of a species of *Meloidoderita* (Nematoda: Cricone-matoidea). *Revue Nématol.*, 5: 247-256.
- HEALD C.M., 1975 - Pathogenicity and histopathology of *Rotylenchulus reniformis* infesting cantaloupe. *J. Nematol.*, 7: 149-152.
- JOHANSEN D. A., 1940 - Plant Microtechnique. New York, Mc Graw Hill Co., 523 pp.
- JONES M.G.K. and DROPKIN V.H., 1975 - Cellular alternations induced in soybean roots by three endoparasitic nematodes. *Phy-siol. plant pathol.*, 5: 119-124.
- LORDELLO L.G.E., 1986 - Plant parasitic nematodes that attack coffee. In: Plant Parasitic Nematodes of Bananas, Citrus, Coffee, Grapes and Tobacco. Union Carbide Agr. Products Company Inc., 33-41 pp.
- MAC GOWAN J.B., 1977 - The Reniform nematode. *Nem. Circular* 32. Oct. 1977. Fla. Dept of Agric. & Consumer Serv. D.P.I., 2 pp.
- ROBINSON A.F. and ORR C.C., 1980 - Histopathology of *Rotylenchulus reniformis* on sunflower. *J. Nematol.*, 12: 84-88.
- REBOIS R.V., 1980 - Ultrastructure of a feeding peg and tube associated with *Rotylenchulus reniformis* in cotton. *Nematologica*, 26: 396-405.
- REBOIS R.V., MADDEN P.A. and ELDRIDGE B.J., 1975 - Some ultrastructural changes induced in resistant and susceptible soybean roots following infection of *Rotylenchulus reniformis*. *J. Nematol.*, 7: 122-129.
- VOVLAS N. and INSERRA R.N., 1983 - Biological relationship of *Rotylenchulus borealis* on several plant cultivars. *J. Nematol.*, 14: 373-377.
- VOVLAS N. and LAMBERTI F., 1985 - Some Tylenchids from Coffee plantations of Sao Tomè. *Absrt. XVII meeting OTAN*, July 28- Aug. 2, 1985, St. Augustine, Trinidad, 38-39 pp.