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Istituto di Nematologia Agraria,
70126 Bari, Italy

ROOT CELL RESPONSE IN RICE ATTACKED BY *HEMICYCLIOPHORA TYPICA*

by
T. Bleve Zacheo, F. Lamberti and M. Chinappen

The feeding activity of the sheath nematodes, *Hemicycliophora arenaria, H. similis* and *H. nudata* on the roots of several plants has been described (Colbran, 1963; Zuckerman, 1961). The preferred feeding site is the root tip, which is transformed into a gall (Van Gundy and Rackham, 1961; McElroy and Van Gundy, 1968; Kisiel *et al.*, 1971). Large root swellings induced by *H. arenaria* on rough lemon showed an abnormal increase in cell division in the immediate vicinity of the stylet; a mass of small cells with deeply stained and enlarged nuclei represented the feeding site, which was surrounded by normal cells (Van Gundy and Rackham, 1961). Observations on cell responses to *H. arenaria* on root tips of tomato, grown in agar, showed that the feeding site was predominantly in undifferentiated endodermal and pericyclic tissues. The nurse cells increased in size as the contents of adjacent cells were removed. Depleted cells were then crushed and replaced by the continuous formation of lateral meristems contiguous with the main active meristem within the swelling root tips. These cells then became a part of the bulk of the enlarging gall and supplied nutrients to the nematode (Mc Elroy and Van Gundy, 1968).

In the studies referred to observations on feeding and cellular response were made by light microscope.

In the present study the interaction between *Hemicycliophora typica* (de Man) (syn. *H. membranifer* Micoletzky), which is widespread in Mauritius (Lamberti *et al.*, 1987), and rice roots was observed at the ultrastructural level by electron microscopy.

Materials and Methods

A suspension of 30 individuals of *H. typica*, handpicked from soil obtained from Mauritius was pipetted onto the rhizosphere of each rice seedling, cv. Lac, 23 (red) grown in sterilized sand in 5 cm diameter clay pots. The plants were maintained in growth chambers at 22°C and at 2,5 and 7 days, after nematode inoculation samples of roots were removed and washed. The root tips, with nematodes attached, were fixed in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2 for 4h, rinsed in the same buffer and post-fixed in 2% osmium tetroxide for 4h at 4°C, then stained in 0.5% uranyl acetate, dehydrated in an ascending series to absolute ethanol and embedded in Spurr's medium (1969).

Sections, 2 μm thick, were cut with an LKB ultratome III, stained with toluidine blue and observed under the light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400T transmission electron microscope at 80kV.

Results

The nematodes were attracted to lateral roots and lateral initials rather than the main tap root. Growing roots of rice seedlings were usually attacked at the root tips and the feeding sites ranged from the root cap to the zone of elongation where many nematodes were found aggregated at a single site (Fig. 1a, b, c). As a consequence of this feeding activity the rootlet growth slowed and finally stopped. Only a slight swelling developed and this never developed into a gall. The swelling of the root tip when attacked by several nematodes was similar but, as might be expected, occurred more rapidly than with a single nematode.

Sections about 2 μm thick through the parasitized emerging roots, revealed that the nematode had deeply inserted its stylet, so that the few cell layers were almost completely penetrated. This resulted in a marked distortion of the meristematic tissues and heavy differentiation of the cortical cells behind the feeding site (Fig. 1d).

There were distinct cellular modifications in the emerging roots, between the root apex and zone of elongation following the attack of a single nematode or several individuals (Fig. 2). There was always a clearcut demarcation between the necrotic and modified cells. The necrotic cells could be a result of both mechanical and chemical stimuli. The ruptured rhizodermis cell wall indicated where the nematode had probably inserted

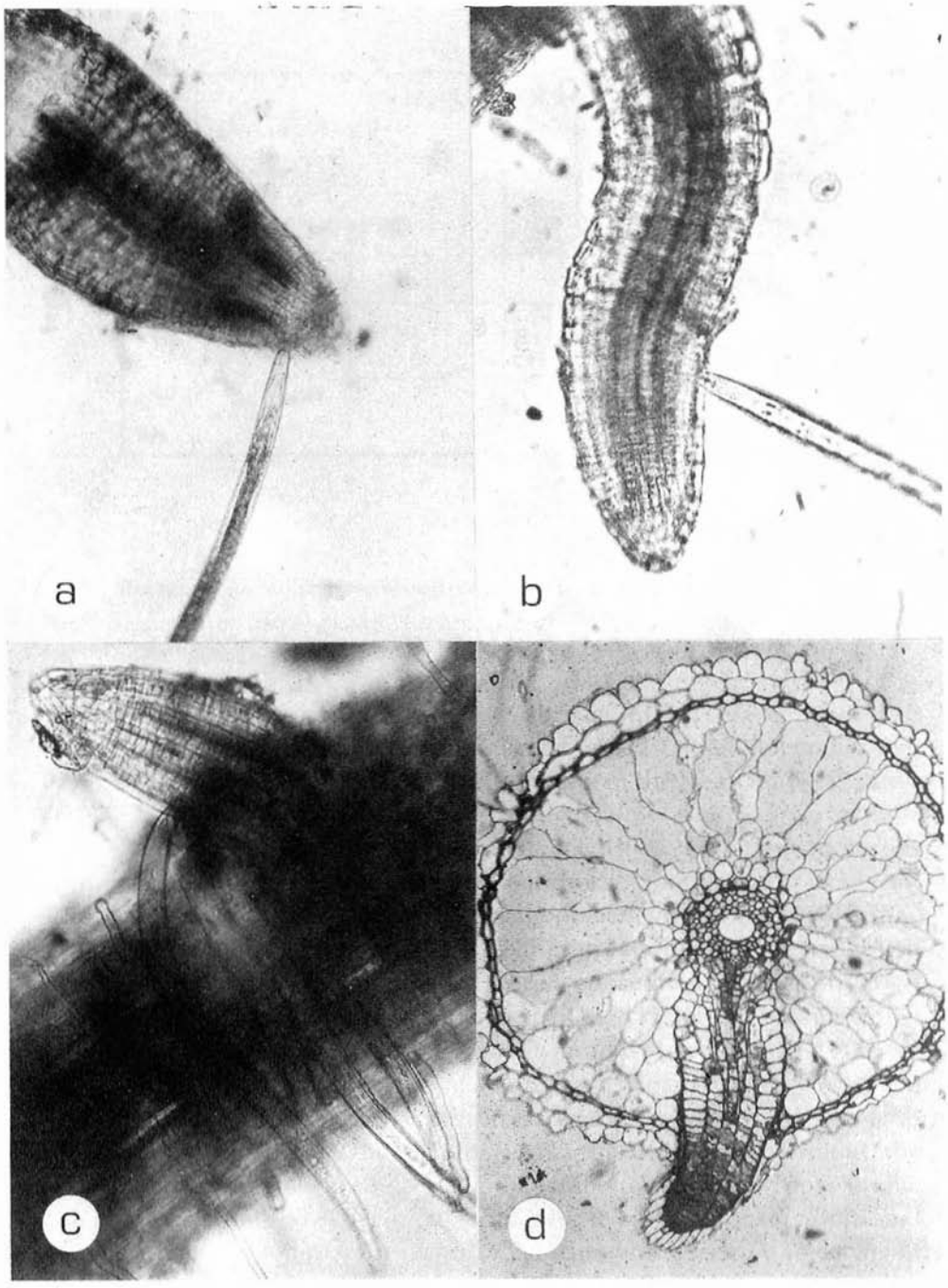

Fig. 1 - Feeding of *Hemicycliophora typica* on emerging root tips, on the initial cells (1a, 760×), on the zone of elongation (1b, 820×). Several nematodes feeding on the same root tip (1c, 730×). Necrotic empty cells in the meristematic area of the root tip and abnormal differentiation in the zone of elongation (1d, 710×).

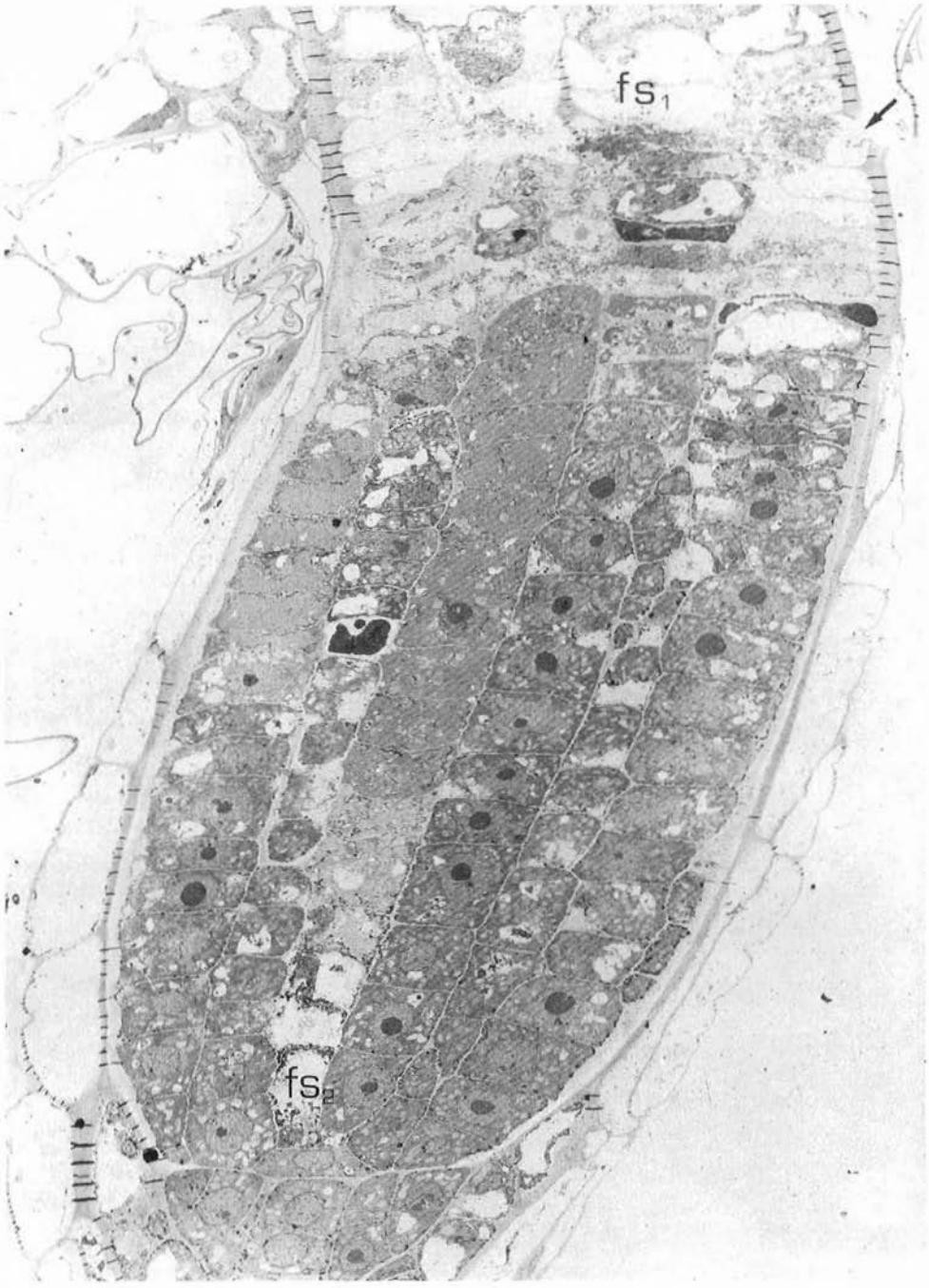

Fig. 2 - Micrograph of a longitudinal section through an emerging root tip. The rhizodermis (arrow) is broken, probably because of the insertion of the nematode odontostyle. The feeding site (fs_1) occupies the radius of the root; almost all the cells are empty. Three or four layers around the feeding site show evidence of necrosis. The growing root tip has also been attacked near the root cap (fs_2). The meristematic cells still appear to be metabolically active, as in normal meristematic tissue (1540×).

its stylet in the selected feeding site. The whole feeding site extended radially along all of the root as indicated by the almost empty cells whose contents have presumably been ingested by the nematode. These cells were bordered by cells, filled with partially degraded cytoplasm (Fig. 2). Observations on a root tip, two days after nematode infestation, showed that the feeding site was localized near the root cap. It consisted of a row of necrotic cells, in the middle of the meristematic tissue, fused as a result of a progressive cell wall degradation. Neither cytoplasmic organelles nor nuclei were seen. The cells bordering the feeding site showed ingrowths on their walls, in the form of finger-like deposition of secondary walls. The cytoplasm was typical of meristematic cells i.e. rich in ribosomes and mitochondria, apart from enlarged profiles of endoplasmic reticulum which frequently surrounded the cell walls and nuclei. The latter were relatively large and more or less spherical as in unaffected root tips (Fig. 3).

Cytological changes in root tips, five days after nematode inoculation, showed a flux of cytoplasm towards the feeding site because of the continuous demand of the nematode for nutrients. Multinucleate cells were formed by fusion of the initially stimulated cells with those in the adjacent layer. Continuity of cytoplasm and ease of movement of the nuclei from cell to cell was facilitated by openings in adjacent cell walls. This led to a syncytium-like structure, which longitudinally encompassed tiers of cells. The openings in the cell walls appeared to have been caused by chemical dissolution and progressed from cell to cell (Fig. 4). One week after nematode inoculation, the whole meristematic tissue had become a large syncytium, connected to the feeding site. All the nuclei were in a lacuna of amorphous material and membranes, representing the remains of the disintegrated cytoplasm. The stubs of the cell walls, projecting into the lacuna, marked the position of the original cells (Fig. 5).

Discussion

The cellular modifications induced by the ectoparasite tylenchid nematode *H. typica* on rice roots differs extensively from those induced by *H. arenaria* on several hosts. Van Gundy and Rackham (1961) state that root tips infested by *H. arenaria* were transformed into galls, resulting from hyperplastic cells with enlarged nuclei, in the immediate vicinity of the nematode stylet. Removal of the nematodes allowed the return to apparently normal root growth. The cellular adaptations induced by *H. typica* partially resemble those induced by cyst nematodes; in that both stimulate the development of the syncytium.

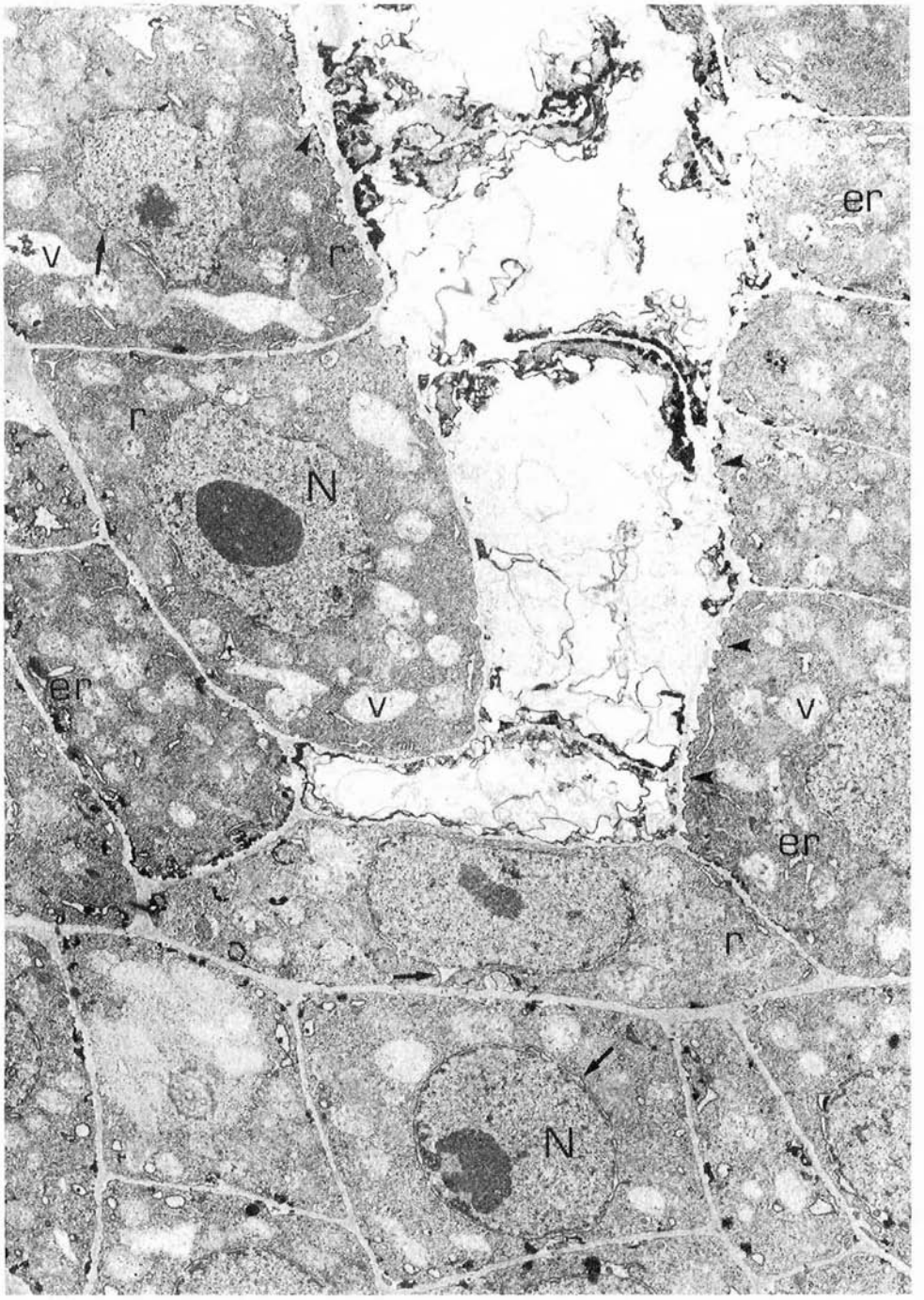

Fig. 3 - Longitudinal section through a root tip, 2 days after nematode inoculation. The feeding site is localized in the meristematic cells close to the root cap. It consists of a row of cells in which the cytoplasm is completely deranged and the cell walls partially dissolved. The cells adjacent to the feeding site have the characteristic features of meristematic tissue with large nucleus (N), ribosomes (r) and small vacuoles (v) in the cytoplasm. Rough endoplasmic reticulum (er) has enlarged profiles as have the nuclear membranes (arrow). Cell walls show finger-like appositions (head arrow) as an initial response to limit the damage (6550×).

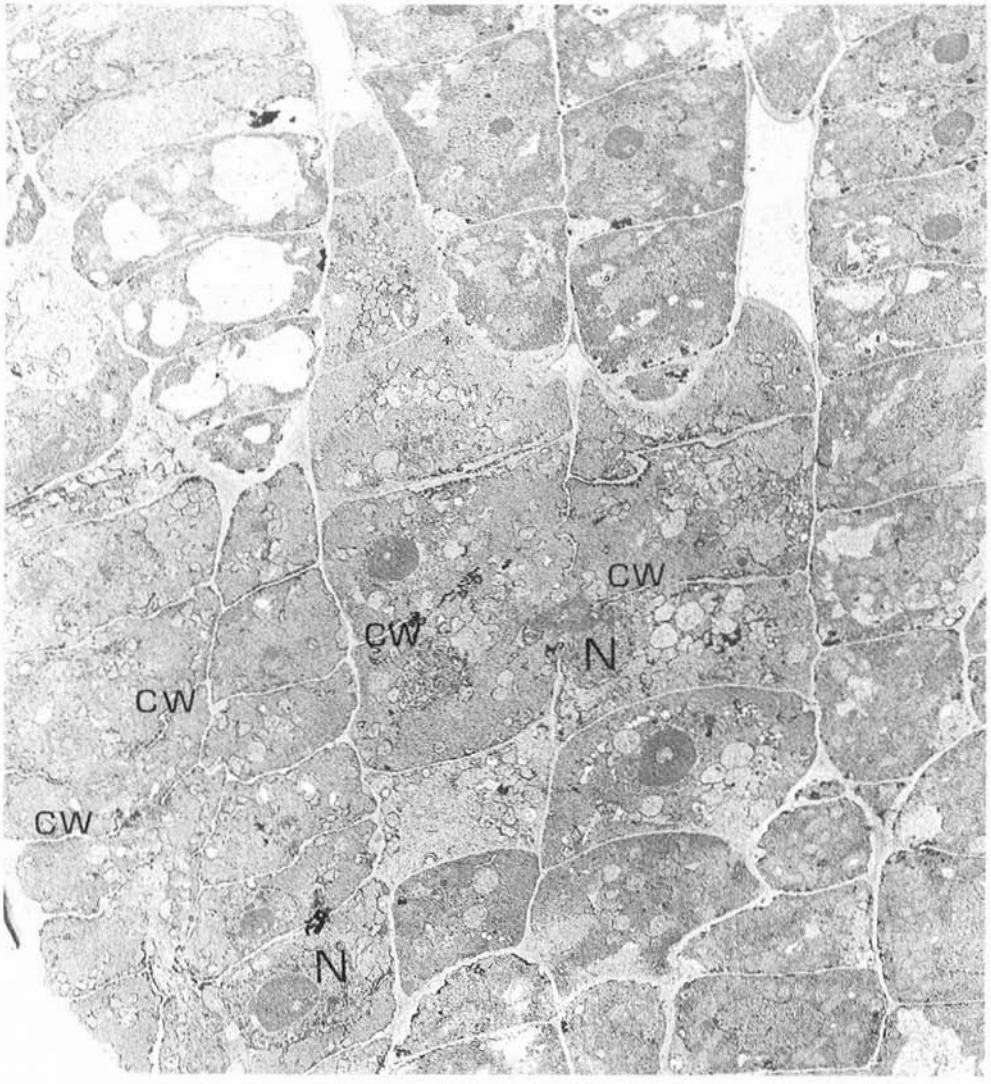

Fig. 4 - Longitudinal section through a root tip 5 days after nematode inoculation. The meristematic cells, furthest from the feeding site, show extensive wall breakdown (cw). Some cytoplasmic organelles are still evident. Nuclei are amoeboid in form and have migrated through the spaces in the cell walls. Cell wall thickenings are present all around the syncytial cells (2550×).

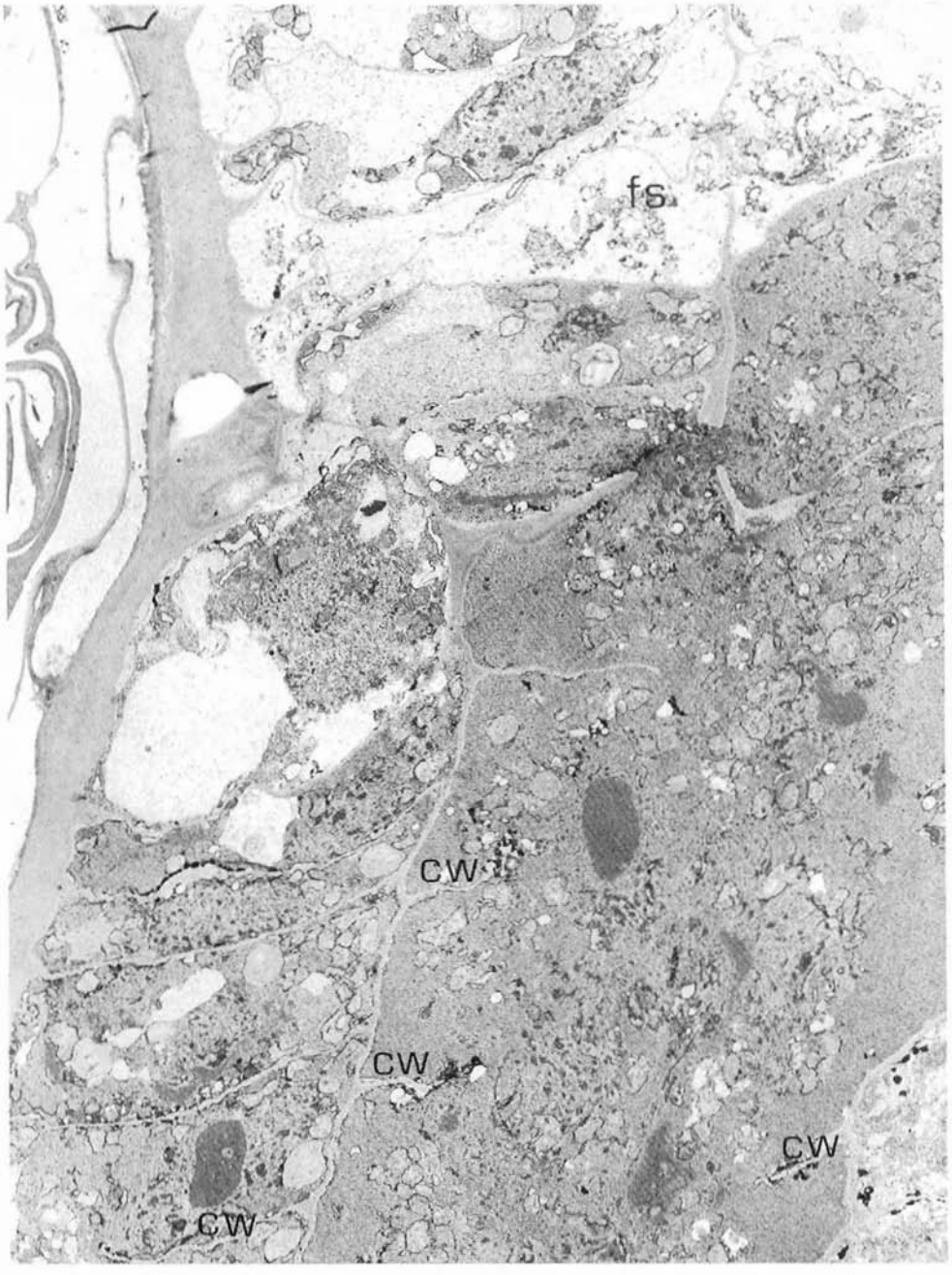

Fig. 5 - Longitudinal section through a root tip 7 days after nematode inoculation. The process of cell wall dissolution, as evident from the wall stubs, indicates the increasing number of cells involved in the formation of the lacuna. The direction of the wall stubs indicates the flux of cytoplasm towards the feeding site. The cytoplasm in the lacuna has deteriorated and the nuclei are scattered (5460×).

The term syncytium, when referring to *H. typica* infection, pertains to a multinucleate condition which results from a partial dissolution of cell walls and a coalescence of cytoplasmic components. Cell wall lysis and/or breakdown which occurs between adjoining cells is similar to the response induced by many endoparasitic nematodes. Bird *et al.* (1975) have speculated that cell wall dissolution during the formation of syncytia is a result of the secretion of polysaccharide degrading enzymes by the nematode. Because of the delicate balance between host and parasite it seems more plausible that plant enzymes are involved in wall digestion and that nematode secretions exaggerate a normal plant cell process (Jones, 1981). The syncytia induced by *H. typica* and by *Heterodera* spp., are similar in that, in both cases they extend further longitudinally than radially, probably because of the distribution of the plasmodesmata in the cell walls. The sites of digestion are probably pit fields, where enzymes may be released at the plasmodesmata. The characteristic structure of the syncytium is formed as a response to specific substances introduced by the nematodes. The different mechanisms all lead to the creation of large volumes of cytoplasm which are tapped by the nematodes.

Syncytia induced by endoparasites are sites of intense metabolic activity. They are specialized structures which provide nutrients for the nematodes. Thus they act as nutrient sinks, while at the same time maintaining functional integrity (Jones, 1981).

Hemicycliophora typica as an ectoparasitic nematode has no fixed feeding site. The cytoplasm in the induced syncytia consists of an amorphous mass without structures or organelles. Therefore the «syncytia» detected in rice roots may be compared in their function to the «lysigenous cavities» induced by the ectoparasitic *Longidorus apulus* (Bleve Zacheo *et al.*, 1979) rather than to those induced by endoparasitic sedentary nematodes. The sheath nematode seems to inject through its stylet specific substances into the selected feeding site to facilitate the almost complete ingestion of cell contents from the root tissues; subsequent root tip deterioration causes the nematode to search for a new feeding site.

SUMMARY

The sheath nematode *Hemicycliophora typica* fed exclusively on emerging root tips of rice seedlings. Response to nematode feeding was the transformation of root tip cells to form a syncytium. Electron microscopy observations indicated that syncytia were formed by perforations in the cell walls. Abnormal perforations were apparent 2 days after nematode

inoculation. The site of the perforations increased as nematode feeding continued and at 7 days, all of the root tip was transformed into a large syncytium, connected to the site where the nematode seemed to have inserted its stylet. The formation of the syncytium involved deterioration of the cytoplasm; the nuclei were free within the amorphous background. Similarities between the syncytia induced by *H. typica* and endoparasitic nematodes are discussed.

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