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Ultrastructure of feeding tubes formed in giant-cells induced in plants by the root-knot nematode *Meloidogyne incognita*

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Summary. The plant pathogenic nematode *Meloidogyne incognita* forms conspicuous tubular structures referred to as feeding tubes in special food cells, called giant-cells, induced and maintained in susceptible host roots by feeding nematodes. Feeding tubes are formed by nematode secretions injected into giant-cells via a stylet and apparently function to facilitate withdrawal of soluble assimilates by the parasite. In giant-cells in roots of the four host species examined in this study, feeding tube morphology was identical. Tubes were straight to slightly curved structures just less than 1 μm wide and up to slightly more than 70 μm long. At the ultrastructural level, each tube consisted of a 190–290 nm thick, electron-dense, crystalline wall surrounding an electron-transparent lumen with a diameter of 340–510 nm. The distal end of the tube was sealed with wall material. Older tubes were found free in the host cytoplasm while the proximal ends of young tubes were attached to the host cell wall via short wall ingrowths through which the nematode's stylet was inserted. An elaborate membrane system was associated with the feeding tubes and was most extensive around newly formed tubes. Contiguous to the feeding tube wall, this membrane system consisted of strands of smooth endoplasmic reticulum while rough endoplasmic reticulum predominated toward the outer margin of the membrane system. Vacuoles and mitochondria were excluded from a zone of cytoplasm surrounding feeding tubes. This zone of exclusion, as well as the membrane system noted above, tended to be less pronounced or absent around older tubes no longer being used by the nematode.

Keywords: Crystalline wall; Feeding tube; Giant-cell; Host–parasite interaction; Membrane system; Nematode secretions; Plant-parasitic nematode; Ultrastructure.

Introduction

Root-knot nematodes belonging to the genus *Meloidogyne* have evolved highly specialized feeding relationships with their hosts. These obligate parasites, which parasitize more than 2,000 plant species (Sasser 1980), are dependent on modified plant tissue for the nourishment needed for their growth, development, and reproduction. These nematodes enter roots as vermiform second-stage juveniles and, after feeding commences, swell and become immobile. Further development of the parasite is dependent upon nourishment provided by a few host cells adjacent to the nematode's head. These cells are modified by nematode esophageal gland secretions to form specialized food cells, called giant-cells, which become the permanent feeding sites for the parasite (Hussey 1989). These highly specialized cellular adaptations function as nutrient sinks in which soluble assimilates are continuously ingested by the nematode and replenished by the plant (Hussey 1985). Giant-cells are characterized by many enlarged, lobed nuclei with prominent nucleoli, increased numbers of cell organelles, and protoplasts with high rates of metabolism (Huang 1985). The enlarged nuclei of giant-cells contain 14–16 times more DNA than root-tip nuclei in pea and tomato (Wiggers et al. 1990). The increased metabolic activity of giant-cells stimulates mobilization of photosynthates from shoots to roots and, in particular, to the giant-cells were they are removed and utilized by feeding adult female nematodes (Bird and Loveys 1975, McClure 1977). Elaborate wall ingrowths form in giant-cells giving these cells the phenotype of transfer cells (Jones 1981, Pate and Gunning 1972).

Secretions injected into the cytoplasm of the giant-cells through a stylet during feeding by second-stage juveniles and adult females of *Meloidogyne* species form tubular structures that have been called feeding tubes (Rumpenhorst 1984). While feeding tubes were first

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observed in 1911 by Nemec (1911), not until 1970 were they observed in electron micrographs by Paulson and Webster (1970), who described them as protein inclusion bodies. Razak and Evans (1976) first used the terms "feeding tube" in a light microscopy study of feeding sites induced in cowpea roots by the reniform nematode, *Rotylenchulus reniformis*. In subsequent ultrastructural studies, a feeding tube was shown being formed from secretions being extruded from the aperture of the stylet of *R. reniformis* (Rebois 1980). A new tube is formed each time a nematode removes its stylet and reinserts it into a food cell to resume feeding (Rumpenhorst 1984). Feeding tubes are a common structure in all specialized feeding sites induced by sedentary endoparasitic nematodes examined thus far. They are also produced by some nematodes that feed either as ectoparasites (Jones 1978) as well as by migratory endoparasites (Schuerger and McClure 1983). While the exact function of the feeding tube has yet to be determined, the observation that feeding tubes can be associated with the aperture of the nematode's stylet has led to speculation that these structures are required for efficient withdrawal of soluble assimilates by the parasitic nematode. Razak and Evans (1976) proposed that feeding tubes might function as cytoplasmic filters that prevent cell organelles from obstructing the aperture of the stylet. On the other hand, Wyss et al. (1984) considered feeding tubes to serve as a means for facilitating the flow of soluble nutrients toward the stylet aperture. They also reported that the cytoplasm around feeding tubes formed by *Heterodera schachtii* appeared modified. The present study was undertaken because detailed information on the ultrastructure of feeding tubes formed by *Meloidogyne* species is lacking. *Meloidogyne* species are distributed worldwide and parasitize many plant species which are of significant economic value.

Materials and methods

Galled root tissue infected with *Meloidogyne incognita* (Kofoid & White) Chitwood (Hussey and Barker 1973) was collected from greenhouse-grown tomato, *Lycopersicon esculentum* Mill. cv. Rutgers, garden pea, *Pisum sativum* (L.) cv. Wando, and common bean, *Phaseolus vulgaris* cv. NB 86. Additional *M. incognita*-infected tissue was obtained from monoxenic cultures of *Impatiens balsamifera* L. cv. Tom Thumb and Rutgers tomato root explants (Huettel and Rebois 1985). Galls 28 to 35 days-old containing a single nematode were collected and processed for electron microscopy.

Three fixatives and fixation protocols were used in processing infected root tissue from the different host species: (1) 4% paraformaldehyde in 0.05 M phosphate buffer (pH 7.2) for 4 h at room temperature followed by 3% glutaraldehyde in the same buffer at 4 °C overnight, (2) 2% acrolein and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 6 h, and (3) 2% paraformaldehyde and 2.5% glutaraldehyde in 0.01 M phosphate buffer (pH 7.2) for 4–5 h. These are referenced in the figure legends as fixatives 1, 2, or 3, respectively. In all fixation procedures the posterior ends of nematodes were punctured with a fine needle after the specimens had been in fixative for 30 min. This procedure was necessary to reduce shrinkage of nematodes during fixation and embedding procedures. Following fixation, galls were transferred to vials containing the phosphate buffer and maintained overnight at 4 °C. After three changes of buffer, specimens were post-fixed for 2 h at 4 °C in similarly buffered 1% OsO_4. Specimens were then washed in distilled water, bulk-stained overnight in aqueous uranyl acetate, washed in distilled water, dehydrated in an ethanol series to acetone, and infiltrated with Spurr's resin. Specimens were embedded using 60 × 15 mm Lux Contur Permanox disposable tissue culture dish as an embedding mold (Mims et al. 1988). Following polymerization of the plastic, specimens were excised and glued onto specimen stubs so as to yield longitudinal sections of giant-cells. Serial ultrathin (70–80 nm) sections were cut with a diamond knife, collected on Formvar-coated copper slot grids, stained with aqueous uranyl acetate and lead citrate, and examined using transmission electron microscopy.

Light microscopy was used to observe feeding tubes in frozen sections of giant-cells of infected tomato roots. In this case, galls from infected roots first were fixed overnight at 4 °C in phosphate buffered 4% paraformaldehyde. Prior to equilibrating the tissue with a cryoprotectant, cortical tissue was carefully dissected from galls. Samples were quick frozen in liquid nitrogen in BEEM capsules and stored at − 80 °C until they could be sectioned. Sections 8 µm thick were cut from frozen tissue using a Reichert-Jung 2800 E Frigocut cryostat, mounted on subbed glass slides, and viewed with Nomarski optics.

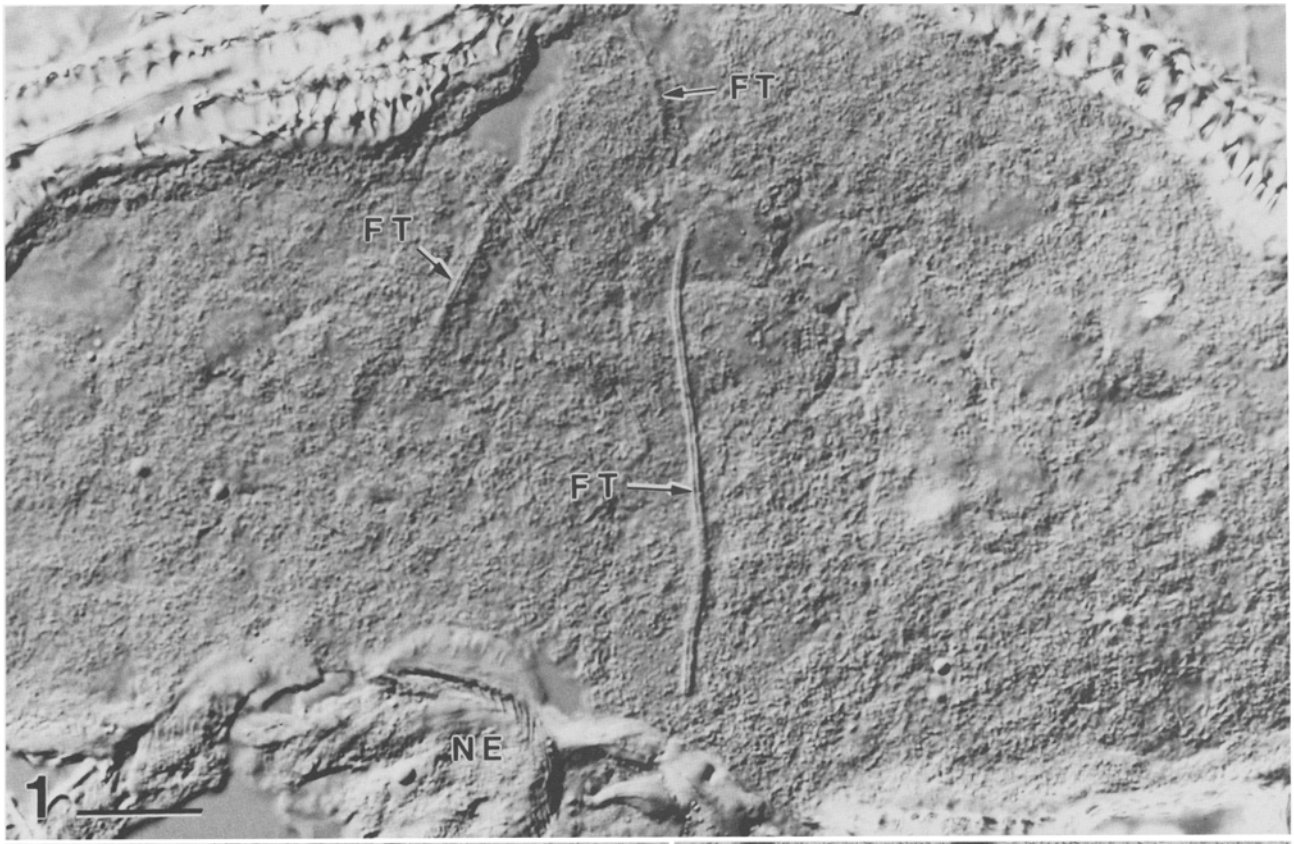
Results

Feeding tubes were observed in the cytoplasm of giant-cells induced by *M. incognita* in roots of all hosts examined. In frozen sections of one giant-cell in a tomato

Fig. 1. Nomarski light micrograph of a cryosection of a giant-cell from a root of a tomato plant infected by *Meloidogyne incognita*. Several feeding tubes (*FT*) are visible in the cytoplasm. A section of the head of the adult female nematode (*NE*) that was feeding from the giant-cell is visible. Bar: 20 µm

Fig. 2. Portion of three giant-cells (*GC*) in an infected bean root radiating outward from the lip region of an adult female nematode (*NE*). Wounds (▼) are visible in walls of GC were the nematode's stylet had been inserted. The cell walls near the nematode's lips were much thinner than elsewhere around the cells. A portion of a thickened cell wall (*TCW*) is visible. A stylet knob (*SK*) is visible in the nematode, although the remainder of the stylet was out of the plane of section. Fix 3. Bar: 5 µm

Fig. 3. Detailed view of the cell wall (*CW*) of a giant-cell in a tomato root in the region where the nematode's stylet had been inserted (▼). The proximal end of a newly formed feeding tube (*FT*) is visible. The site where the tip of the stylet was positioned next to the feeding tube is indicated by an arrow. A portion of the nematode (*NE*) is visible. Fix 3. Bar: 1 µm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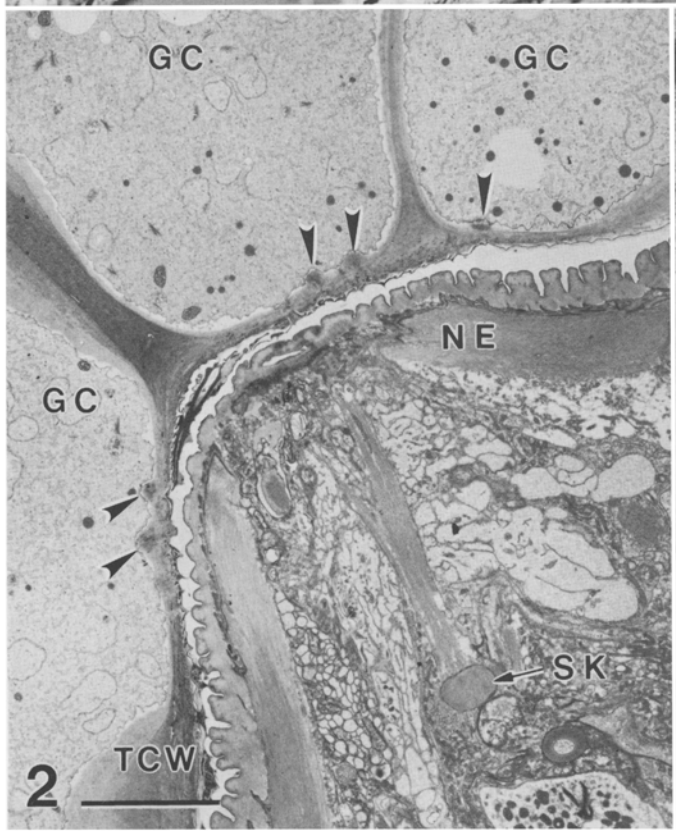
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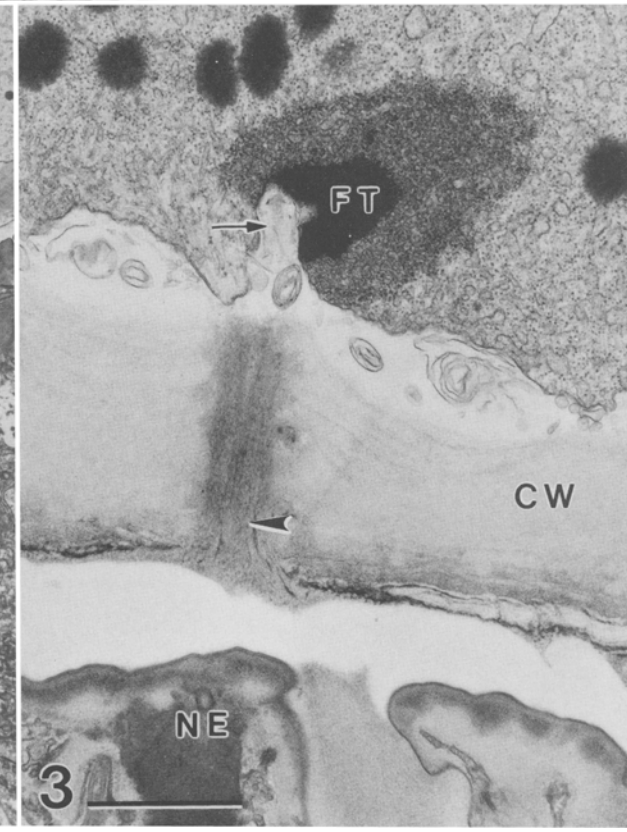
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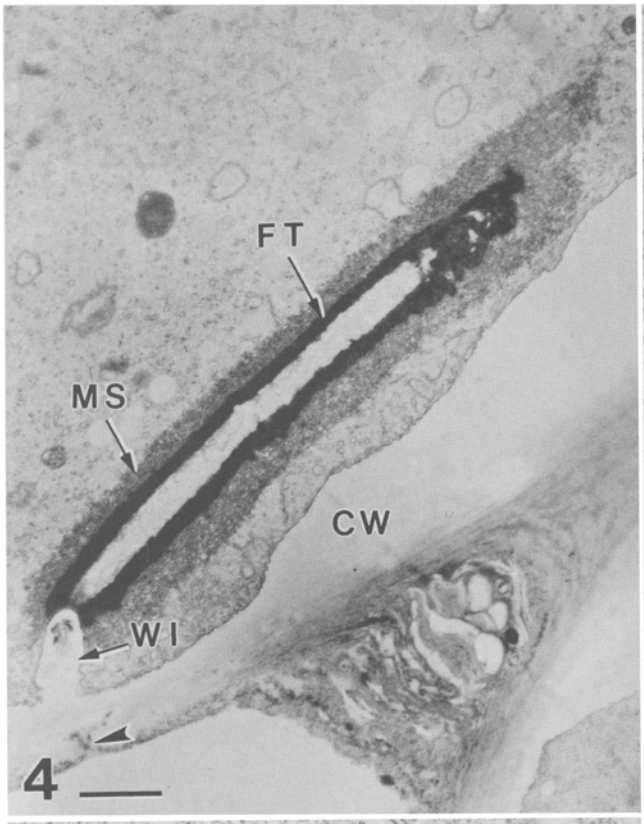
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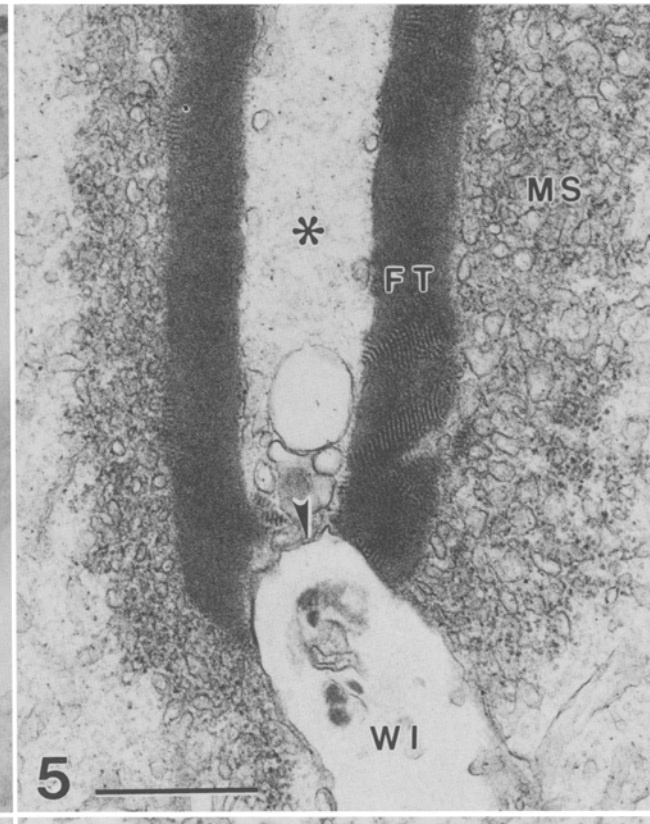
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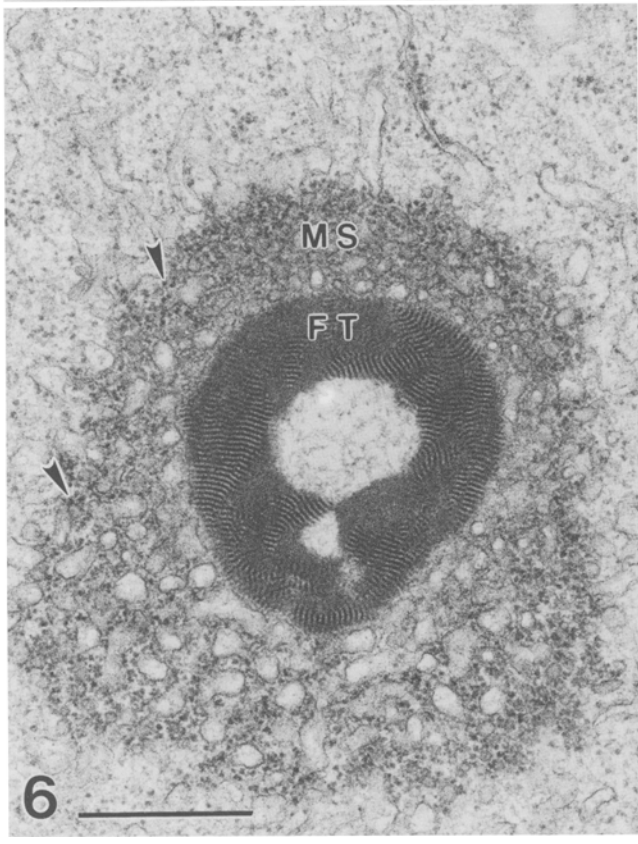
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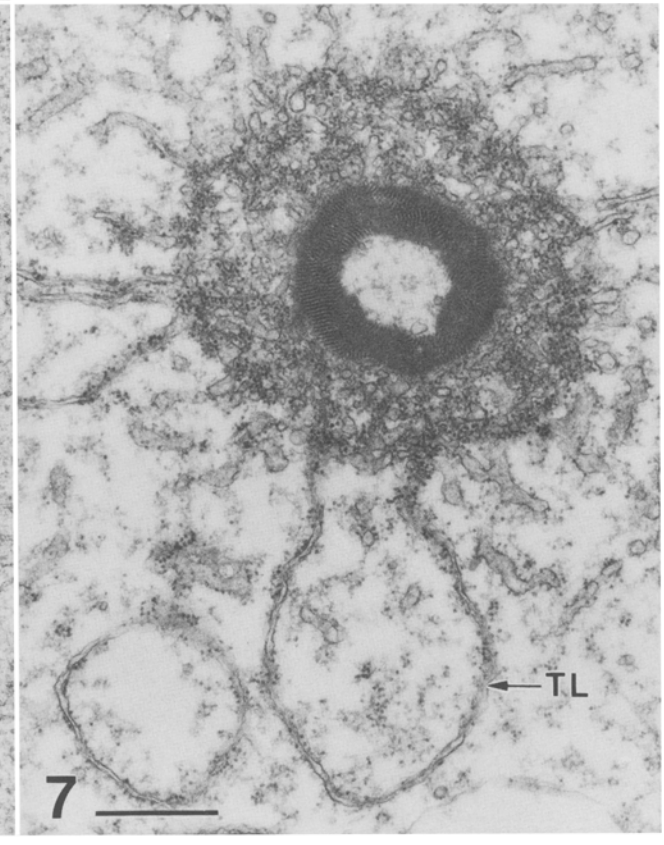
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root, one tube was observed to be 73 μm long (Fig. 1). Several feeding tubes were normally present in each giant-cell.

Removal of nutrients by the adult female nematode from each of the 4–5 giant-cells induced was facilitated by radial arrangement of the giant-cells around the lip region of the parasite (Fig. 2). This type of cell arrangement permitted the sedentary adult female nematode to feed on different giant-cells with only limited movement of its head. The normally extraordinarily thickened walls (10–15 μm) of giant-cells were only 0.8–2.3 μm wide opposite the nematode's lips (Fig. 2). The different fixatives were used to improve ultrastructural preservation of giant-cells and to attempt to rapidly kill adult female nematodes that were either in the process of injecting secretory components that form feeding tubes in a giant-cell or actually feeding from a giant-cell. The use of paraformaldehyde or acrolein in combination with glutaraldehyde provided better preservation of endomembrane systems in giant-cells than did glutaraldehyde alone. However, a nematode with its stylet inserted in a giant-cell was never observed in this study.

Numerous wounds where stylets had penetrated were discernible in giant-cell walls (Figs. 2 and 3). Furthermore, analysis of serial sections of giant-cells from several galls, revealed feeding tubes in contact with giant-cell walls at the penetration sites of the stylets (Fig. 3). Although lips of the female nematodes were always in close proximity to the wall punctures in these sections, their stylets were retracted within their oral apertures. We interpreted these observations to indicate that these stylet punctures in the walls of the giant-cells were the most recent ones made by the adjacent nematodes and that the stylets probably were retracted from the giant-cell wall during the fixation process. In several giant-cells, feeding tubes projected from short wall ingrowths through which the nematode's stylet was undoubtedly inserted (Fig. 4). In all hosts examined in this study, feeding tubes ranged from 820 to 990 nm in diameter. Each tube consisted of an electron-dense crystalline wall 190–290 nm thick and an electron-transparent lumen ranging in diameter from 340 to 510 nm in different tubes (Figs. 5 and 6). In any one tube, the lumen diameter was relative uniform throughout its length. All observed distal ends of feeding tubes were sealed (Fig. 4). In higher magnification views of the juncture of a tube with the giant-cell wall, the plasma membrane apparently was not disrupted between the wall and lumen of the feeding tube (Fig. 5).

An elaborate membrane system developed around newly formed tubes (Figs. 4–7). The membrane system immediately contiguous to the wall of the feeding tube was similar in appearance to smooth endoplasmic reticulum while ribosomes were bound to tubular elements at the periphery of the system. The compact membrane system around newly formed tubes extended approximately 450–500 nm outward from the tube into the surrounding cytoplasm. In giant-cells induced in bean roots, conspicuous tubular loops studded with ribosomes extended from the membrane system (Fig. 7).

Mitochondria and small vacuoles were excluded from the zone of cytoplasm surrounding feeding tubes that possessed the elaborate membrane system described above (Fig. 8). Tubular elements of endoplasmic reticulum and numerous dictyosomes were characteristically present in the exclusion zone surrounding the dense membrane system associated with most tubes. The integrity of the membrane system varied among different feeding tubes present in most giant-cells. Some tubes, which we judged to be newly formed, were enveloped by very dense membrane systems, while other, apparently older tubes, lacked a membrane system (Fig. 9). In some instances, membrane systems associated with some tubes appeared to be in various stages of deterioration.

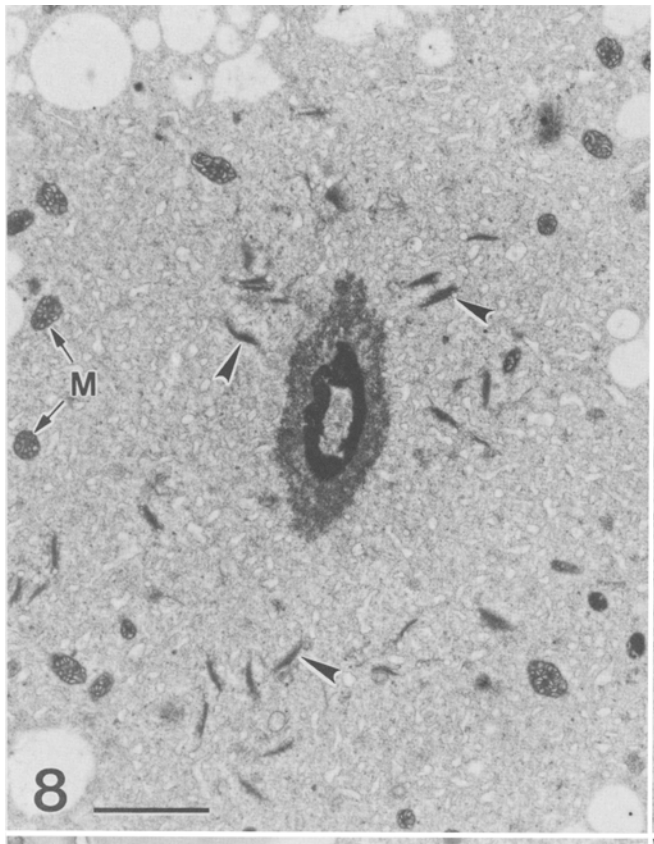
In one giant-cell observed in a pea root, secretions

Fig. 4. Longitudinal section through a feeding tube (*FT*) with its proximal end attached to a wall ingrowth (*WI*) of a tomato giant-cell. The ingrowth formed at the point where the nematode's stylet was inserted (▼) into the cell wall (*CW*). The distal end of the tube is sealed by crystalline wall material. The FT is enveloped in a compact membrane system (*MS*). Fix 1. Bar: 1 μm

Fig. 5. Detailed view of the juncture of a feeding tube (*FT*) with a giant-cell wall ingrowth (*WI*). The host cell plasma membrane (▼) between the wall and lumen (*) of the FT appears intact. A dense membrane system (*MS*) is present around the crystalline wall of the feeding tube. Fix 1. Bar: 0.5 μm

Fig. 6. Cross-section of a feeding tube (*FT*) in a giant-cell from a balsam root. The electron-dense crystalline wall of the FT is surrounded by an elaborate membrane system (*MS*). Ribosomes are attached to peripheral tubular elements (▼) of the membrane system. Fix 1. Bar: 0.5 μm

Fig. 7. Cross-section of a feeding tube in a giant-cell in bean. Tubular loops (*TL*) of endoplasmic reticulum studded with ribosomes extend outward from the membrane system surrounding the tube. Fix 3. Bar: 0.5 μm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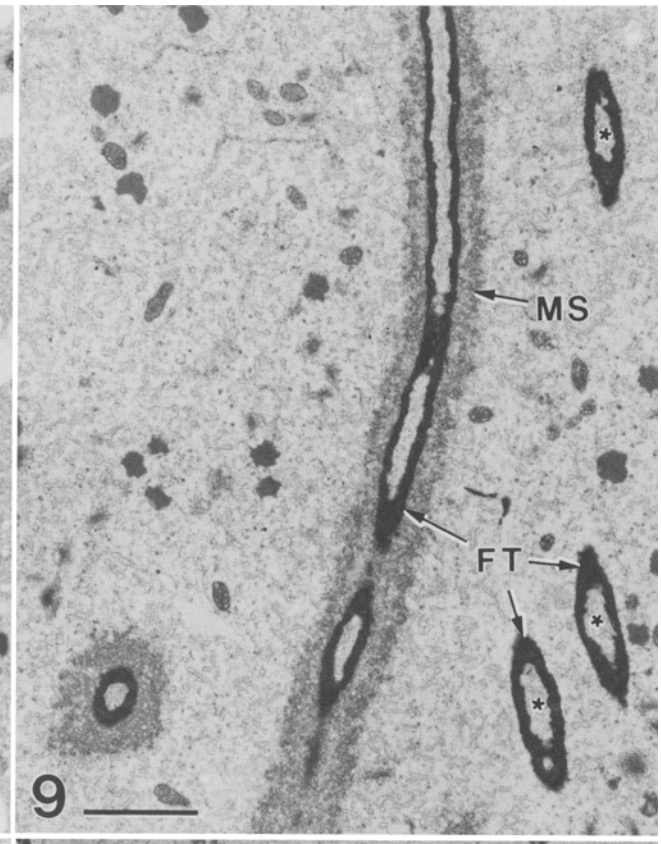
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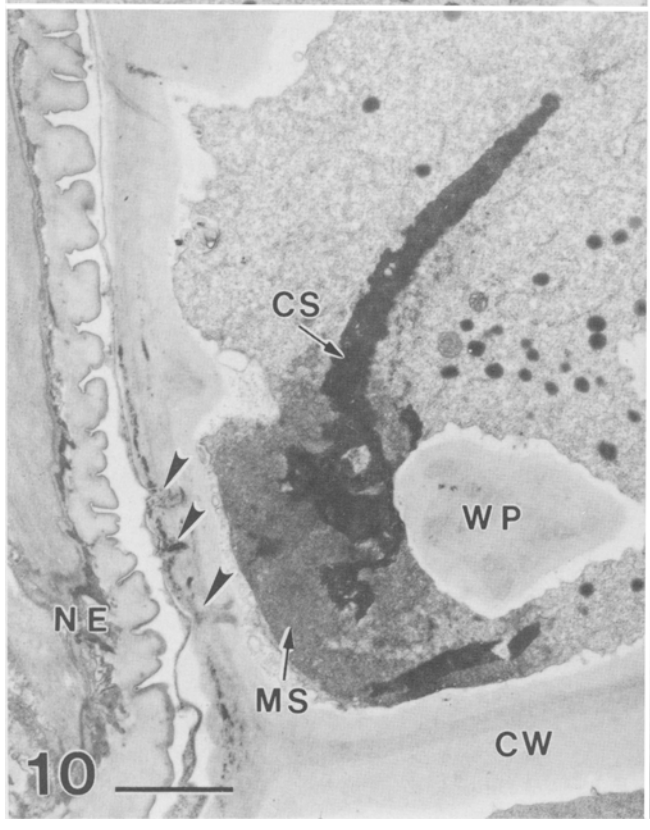
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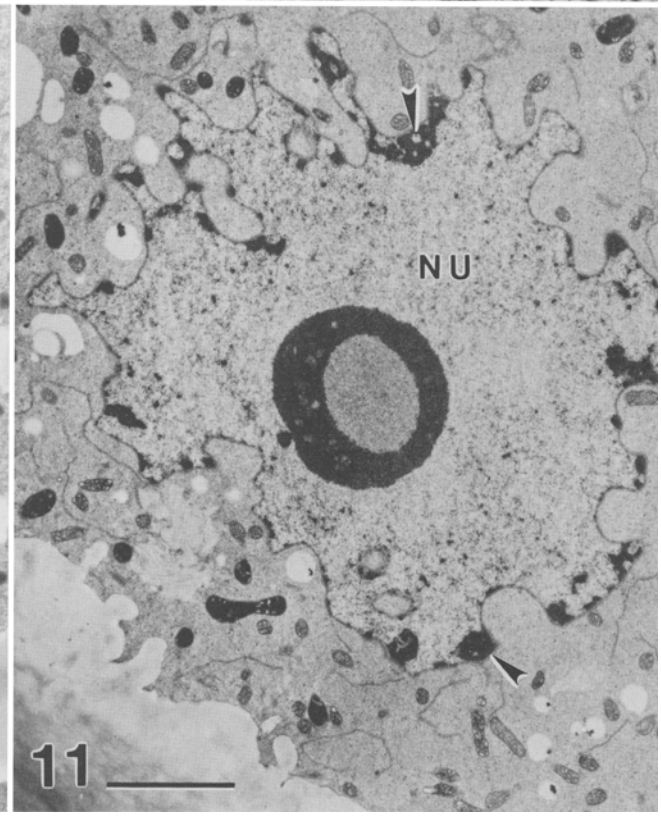
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injected into the cell by an adult female nematode were trapped in a corner of the cell behind a wall projection and were prevented from forming an intact feeding tube (Fig. 10). Three punctures visible in the wall of this giant-cell indicated that the nematode repeatedly inserted its stylet into the cell while attempting to form a tube. These repeated injections of secretions resulted in an accumulation of a mass of crystalline tube components in the cytoplasm in the corner of the giant-cell. Even though a feeding tube had not formed, a dense membrane system developed around the aggregated secretions.

An example of an enlarged nucleus typical of those present in giant-cells is shown in Fig. 11. The extraordinarily lobed nuclei contained enlarged nucleoli and dense peripheral clumps of condensed chromatin. It was not uncommon to observe as many as 10–15 nuclear profiles in a single section of a giant cell.

Discussion

Feeding tubes formed in giant-cells from secretions of *Meloidogyne* species differ from feeding tubes formed by other plant-parasitic nematodes. The wall of a feeding tube formed by a species of *Meloidogyne* was crystalline and considerably thicker than those of feeding tubes formed by *Heterodera glycines* (Endo 1987) and *H. schachtii* (Wyss et al. 1984). Furthermore, the highly organized and dense membrane system that was formed by the giant-cell and which enveloped feeding tubes produced by *M. incognita* has not been reported in association with feeding tubes produced by other nematodes. To date, only swirls of endoplasmic reticulum have been observed around feeding tubes formed in feeding sites of other host–nematode interactions (Rebois 1980, Rumpenhorst 1984).

The crystalline feeding tube wall formed by *M. incognita* is similar in morphology to a crystalline secretory component observed in the end-sac of an open dorsal esophageal gland valve in an adult female of the same species (Hussey and Mims 1990). Feeding tubes have been observed to form from secretory components synthesized in the dorsal esophageal gland of *H. schachtii* (Wyss and Zunke 1986). Although one secretory component synthesized in the dorsal gland of *M. incognita* is a glycoprotein (Hussey et al. 1990), the composition of the wall of the feeding tube is unknown. Polymerization of the secretory components into tubular structures apparently is induced as the secretion is emitted from the nematode's stylet and contacts giant-cell cytoplasm. Stylet secretions produced when adult female nematodes are dissected from galled roots and incubated in aqueous solutions in vitro do not form tubular structures (Hussey et al. 1990).

The function of the compact membrane system enveloping the feeding tubes remains unknown. This membrane system seemed to be interconnected with the endomembrane system of the multinucleated giant-cells. Ultrastructural cytochemistry possibly could provide information about the relationship of this membrane system with the endomembrane system of the giant-cell (Harris 1986). In plant cells, functions of the endoplasmic reticulum include intracellular communication and transport and membrane and protein synthesis (Chrispeels 1980). The stimulus for the formation of the membrane system appears to be associated with the crystalline secretions as they are extruded from the stylet aperture. Support for this conclusion comes from the observation that aborted tube formation still resulted in a dense membrane system forming around aggregated secretions. The organization of the membrane system around feeding tubes and the outward projection of its tubular elements usually created a cytoplasmic zone free of most large cell organelles. Similar organelle free zones in food cells have been observed around feeding tubes produced by other plant parasitic nematodes (Rumpenhorst 1984, Wyss et al. 1984). The close association of the complex membrane system with newly formed feeding tubes indicates that it might function in synthesizing and/or transporting soluble assimilates in the metabolically active giant-

Fig. 8. Portion of a giant-cell in a balsam root. Note the zone in the cytoplasm around a feeding tube which is free of vacuoles and most mitochondria (*M*). Numerous dictyosomes (▼) are present around the membrane system associated with the feeding tube. Fix 1. Bar: 2 μm

Fig. 9. Section of a tomato root giant-cell containing several fragments of feeding tubes (*FT*) both with and without (*) the membrane system (*MS*). Fix 3. Bar: 2 μm

Fig. 10. Electron-dense crystalline secretions (*CS*) from an adult female nematode (*NE*) which aggregated behind a wall projection (*WP*) in a corner of a pea root giant-cell. A dense membrane system (*MS*) formed around a portion of the CS. Wounds (▼) where the nematode's stylet had been inserted are visible in the wall (*CW*) of the giant-cell. Fix 3. Bar: 2 μm

Fig. 11. Detailed view of a lobed nucleus (*NU*) with a prominent nucleolus in a tomato root giant-cell. Condensed chromatin (▼) is visible at the periphery of the nucleus. Fix 2. Bar: 3 μm

cells to the feeding tube for withdrawal by the parasite. The feeding behaviour of *H. schachtii* in living root tissue has been studied using high resolution video-enhanced contrast light microscopy (Wyss and Zunke 1986). This nematode feeds in cycles that include a secretion phase when a feeding tube is formed in the food cell, a food ingestion phase, and resting phase when the stylet is withdrawn from the food cell. After a few minutes the stylet tip is reinserted into the food cell to begin another feeding cycle. A new feeding tube is formed each time the stylet tip is inserted into the food cell. The presence of numerous feeding tubes in the cytoplasm and stylet punctures in the walls of each giant-cell induced by *M. incognita* in a root gall suggests that this nematode also feeds in cycles. Crystalline walls of feeding tubes no longer used by feeding nematodes appear to persist for long periods of time.

An electron-dense feeding plug forms around the stylet when it is inserted into the wall of food cells during feeding by *Heterodera* species (Endo 1978, Wyss et al. 1984). No structure similar to a feeding plug was observed at sites where stylets of *M. incognita* penetrated walls of giant-cells. Since the stylet of *M. incognita* is smaller in diameter than the stylet of *Heterodera* species, the small puncture produced by *Meloidogyne* species may not require a plug to seal the giant-cell wall during feeding or when the stylet is withdrawn.

The manner in which feeding tubes facilitate removal of soluble assimilates from giant-cells by *Meloidogyne* species is unclear. Although the distal end of the feeding tube was sealed, it was not possible to determine at the proximal end of the tube whether the plasma membrane remained intact between the stylet aperture and the lumen of the tube as observed with other nematodes (Rebois 1980, Wyss et al. 1984). Rumpenhorst (1984) proposed that feeding tubes function as ducts through which special cell products are directed toward the cytosol around the aperture of the nematode's inserted stylet. In the present study, the frequent observation of the feeding tube lumen abutting giant-cell wall ingrowths with stylet punctures possibly indicates a direct relationship between the stylet and feeding tube. However, the slow action of chemical fixatives precluded observations of nematodes with extended stylets and the manner in which the aperture of the stylet was associated with the lumen of the feeding tube during feeding by adult female nematodes inside galled roots. The utilization of rapid fixation techniques, e.g., high pressure freezing (Muller and Moor 1984), might help overcome this problem.

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