The ultrastructure of cereal and leguminous root tips parasitized by *Longidorus belloi*

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SUMMARY

Sections of galls induced by *Longidorus belloi* on the root tips of wheat, barley, rye grass, lentil and vetch revealed a column of necrotic cells, singly or in small groups within the root apex. These cells, with features of a hypersensitive reaction had obviously been penetrated by the nematode odontostyle and represented the feeding site. Sometimes breakdown of the cell wall was observed, probably due to perforation by the odontostyle. Wheat root tips responded to nematode attack by a hypertrophic reaction of the cells around the feeding site. Their nuclei had amoeboid profiles and contained several nucleoli with small " vacuoles ", an indication of an increased metabolic activity. The response of barley root tips was similar but more severe, because the feeding site was transformed into a lysigenous cavity. This led to the destruction of the root tip in shorter time than in wheat. In rye grass, lentil and vetch root hyperplasia was detectable with cells dividing in a highly ordered manner. The cell walls, particularly in rye grass, were very wavy. Mature protoxylem and protophloem elements occurred in the procambium, as a response to nematode feeding. A common response in the cells not directly affected by the nematode was the formation of paramural bodies, probably involved in the wall apposition by tentatively repairing cellular damage.

RÉSUMÉ

Ultrastructure des racines de céréales et de légumineuses parasitées par Longidorus belloi

Des séries de coupes effectuées dans des galles provoquées par *Longidorus belloi* sur des racines de blé, d'orge, d'ivraie, de lentille et de vesce révèlent la présence de files de cellules nécrotiques, isolées ou groupées, à l'extrémité de la racine. Ces cellules, pénétrées par le stylet du nématode et constituant son site de prise de nourriture, montrent des réactions d'hypersensibilité. Une rupture de la paroi cellulaire, due probablement à l'action du stylet, a été parfois observée. La réaction des racines de blé se traduit par une hypertrophie des cellules situées autour du site de nutrition du nématode. Le noyau de ces cellules devient amiboïde et présente plusieurs nucléoles ayant de petites vacuoles; ces dernières représentent des organisateurs nucléaires, indiquant une forte augmentation de l'activité de synthèse des cellules. La même réaction, mais plus accentuée, est observée dans les racines d'orge : le site de nutrition est en effet lysé, transformé en une cavité dépourvue de structure cytoplasmique. La racine d'orge est donc détruite plus rapidement que celle de blé. Les cellules des racines d'ivraie, de lentille et de vesce se divisent régulièrement, provoquant une hyperplasie. La paroi des cellules, particulièrement chez l'ivraie, a un aspect fortement ondulé; en réaction à l'infestation par le nématode, des éléments des assises du protoxylème et du protophloème sont observés dans le procambium. Une réaction générale des cellules non directement atteintes par le nématode se traduit par la présence de corps paramuraux; selon toute probabilité, ceux-ci ont fonction de colmater la paroi cellulaire, contribuant à une tentative de réparation des dommages causés à la cellule.

The root tip is an important infection court for many pathogens, including nematodes. In particular Longidoridae, with the exception of few *Xiphinema* spp., feed exclusively at root tips. They insert their odontostyle five to six cells deep into the meristematic tissue and feed for a relatively short period before moving to other feeding sites (Bleve-Zacheo *et al.*, 1977). As a consequence most cells are emptied of their contents, the tissues collapse and the roots are invaded by soil bacteria and fungi (Dropkin, 1979). According to Perry and Evert (1983) the root tip is the most vulnerable portion of the root for vascular infection.

Like with other *Longidorus* spp., *L. belloi* Andres & Arias, 1988 feeding induces prominent terminal swellings on the roots; meristematic activity of the root tip is suppressed and a gall is formed by hyperplasia or hypertrophy of the cortical and/or procambial cells (Cohn, 1975; Bleve-Zacheo *et al.*, 1977; Griffiths & Robertson, 1984; Andres, Arias & Bleve-Zacheo, 1988). Andres and Arias (1989) evaluated hosts for *L. belloi* by determining the rate of total nematode population increase in the field. Studies under controlled conditions have shown that this species feeds and induces more galls on some plant species which are better hosts than others (Andres, Arias & Bleve-Zacheo, 1988).

Griffiths and Trudgill (1983) have found that there was little difference in the number of galls formed by *L. elongatus* on strawberry (good host) and turnip (poor host) indicating that differences in host status are probably associated with differences in gall quality. Wyss (1977, 1978) has suggested that the formation of galls is essential for the successful reproduction of *Xiphinema index*.

A preliminary study of the histological changes occurring in galls of cereals and leguminous plants, induced by *Longidorus belloi*, indicated that root tip swellings were due to hyperplasia or hypertrophy of the tissue (Andres & Arias, 1988*a*). The work described here examines, for the first time, the cytological changes induced by the feeding of *L. belloi* on different hosts.

Materials and methods

Seedlings of wheat, barley, rye grass, vetch and lentil were transplanted singly into 5 cm diameter clay pots containing 10 ml sterilized sand. Each pot was inoculated with five adult female *L. belloi*. The pots were kept in a growth chamber at 24°. Three days after nematode inoculation seedlings were randomly selected for histological observations of swelling root tips. Affected root tips were cut off, fixed in 3 % glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.2 for four hours, then rinsed in the same buffer and post-fixed in 2 % osmium tetroxide for four hours at 4°, followed by staining in 0.5 % aqueous uranyl acetate, dehydration in an ascending series to absolute ethanol and embedding in Spurr's medium. Semi-thin and ultrathin sections were cut with an LKB ultratome III, stained with uranyl acetate and lead citrate and examined in a Philips 400 T transmission electron microscope operated at 80 kV.

Results

The normal cells of the cortical ground meristem of cereal roots are uninucleate and contain large populations of ribosomes and small vacuoles. In addition, plastids, mitochondria, dictyosomes and rough endoplasmic reticulum are fairly abundant. The nucleus, when it is in interphase, occupies the larger part of the cell. Part of the chromatin in the nucleus is in a condensed form (heterochromatin) and the dispersed chromatin varies in density, especially near the nucleolus. The nucleolus has the three typical components, the fibrillar component, the nucleolar organizer surrounded by the granular component (Fig. 1).

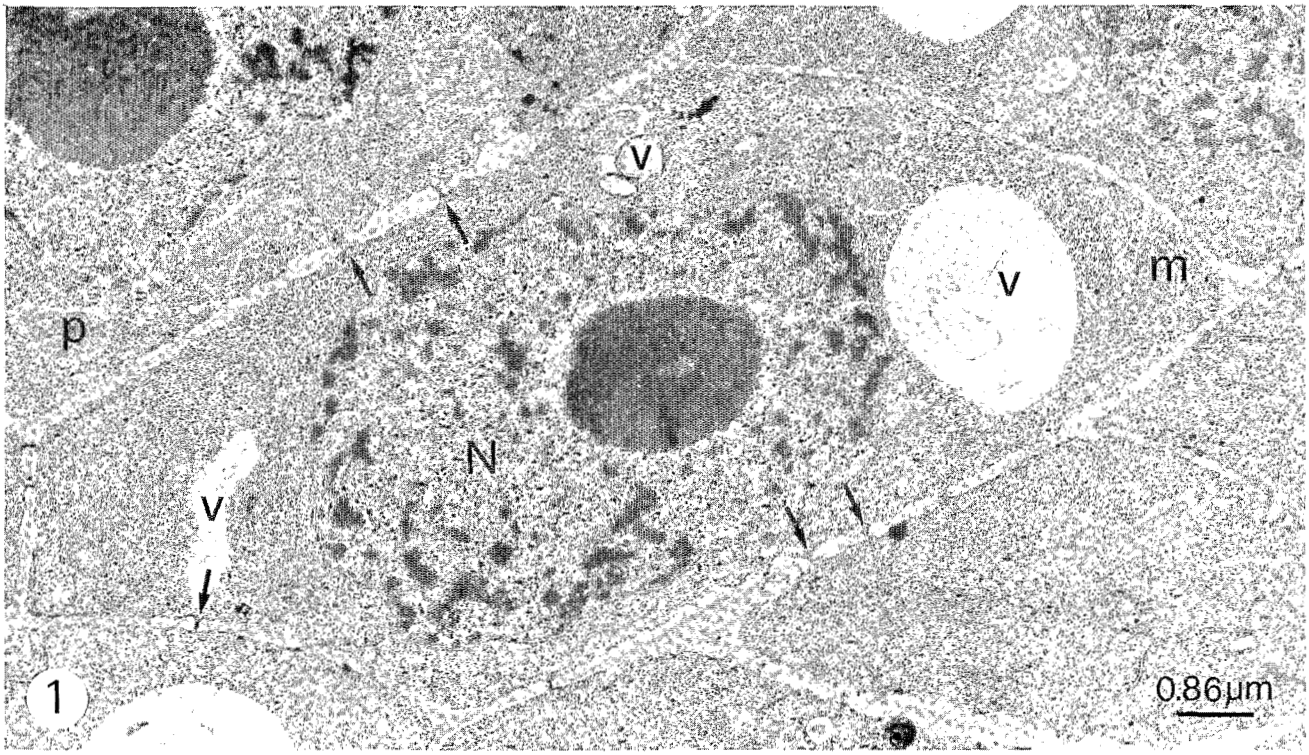

Fig. 1. Longitudinal section through an unattacked wheat root tip, showing a meristematic cell. The cytoplasm is very dense with many ribosomes and organelles and small vacuoles. The nucleus, in interphase, has a large nucleolus; heterochromatin is in condensed form and mostly lines the double nuclear envelope. Note the frequent plasmodesmata along the cell wall (arrow).

Abbreviations used in the figures : N = nucleus, nu = nucleolus, m = mitochondrion, v = vacuole, p = proplastid, cw = cell wall, cy = cytoplasm, nc = necrotic cell, rh = rhizodermis, d = dictyosomes, mi = microtubules, ER = endoplasmic reticulum, ve = vessels, fs = feeding site.

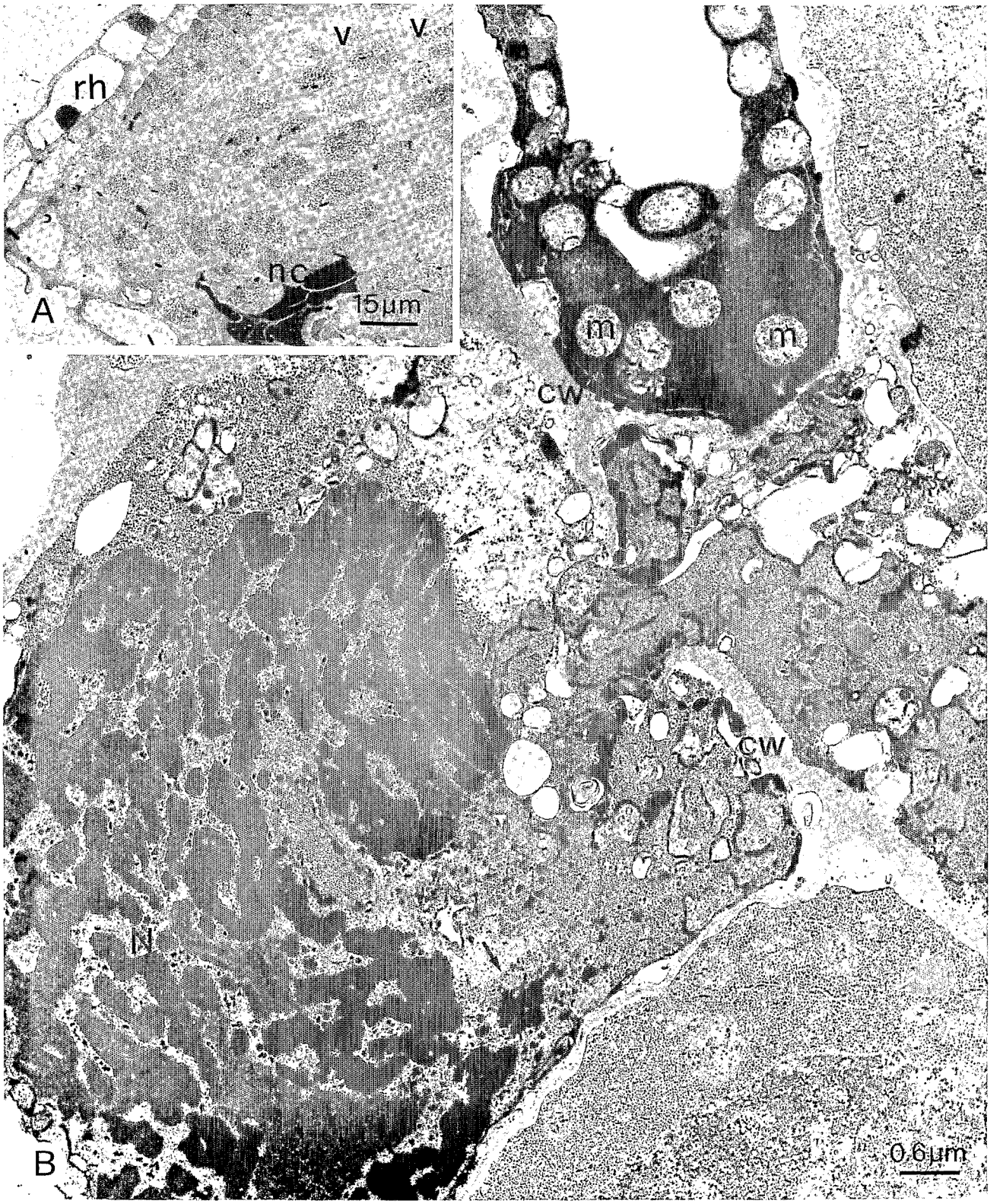

Fig. 2. A - Longitudinal section through a wheat root tip three days after inoculation with *L. belloi*. A column of necrotic cells starting from the rhizodermis indicates the insertion and pathway of the nematode odontostyle. Cells on the opposite side have enlarged vacuoles because of the precocious maturity of the tissues. B - Necrotic cells of wheat root tip with cell wall breakdown, probably representing the site of insertion of the nematode odontostyle and subsequent feeding. The protoplast is completely deranged and nuclear content is in condensed form and diffused, after the breakdown of the nuclear envelope (arrow). Plasmolysis is evident along the cell wall. Note the flow of the cell content through the broken wall.

For abbreviations see Fig. 1.

Three days after *L. belloi* inoculation, cells in the galled root tip had begun to lose their meristematic appearance and many of them showed a marked increase in vacuolisation, just behind the directly parasitized cells (Figs 2 A, 4 A). The column of the cells in wheat roots, most probably penetrated by the nematode odontostyle, became necrotic and the cell content consisted of dark material (Fig. 2 A). Fig. 2 B shows the characteristic feature of necrotic cells, still partially filled with degraded contents. The nucleus was highly disorganized, with chromatin condensed in large layers, it lacked a detectable nucleolus and the double membrane envelope appeared to be broken. The cytoplasm lost its original structure, now being composed of amorphous ground material, with organelles transformed into myelin-like figures; only mitochondria, in very poor condition, were recognizable. A break in the cell wall (Fig. 2 B), was probably due to perforation by the nematode stylet. Previously holes were reported in the cytoplasm of the cells that had been perforated by the stylet of Longidoridae (Bleve-Zacheo & Zacheo, 1983) but never the breakdown of the cell wall. Through the broken wall the cytoplasm flowed from one cell to the other (Fig. 2 B). Cells around the feeding site, represented by the necrotic cells, were greatly enlarged. Nuclei, in interphase, had a nearly amoeboid profile and contained more than one nucleolus. The cytoplasm was still well preserved with small vacuoles scattered in it (Fig. 3).

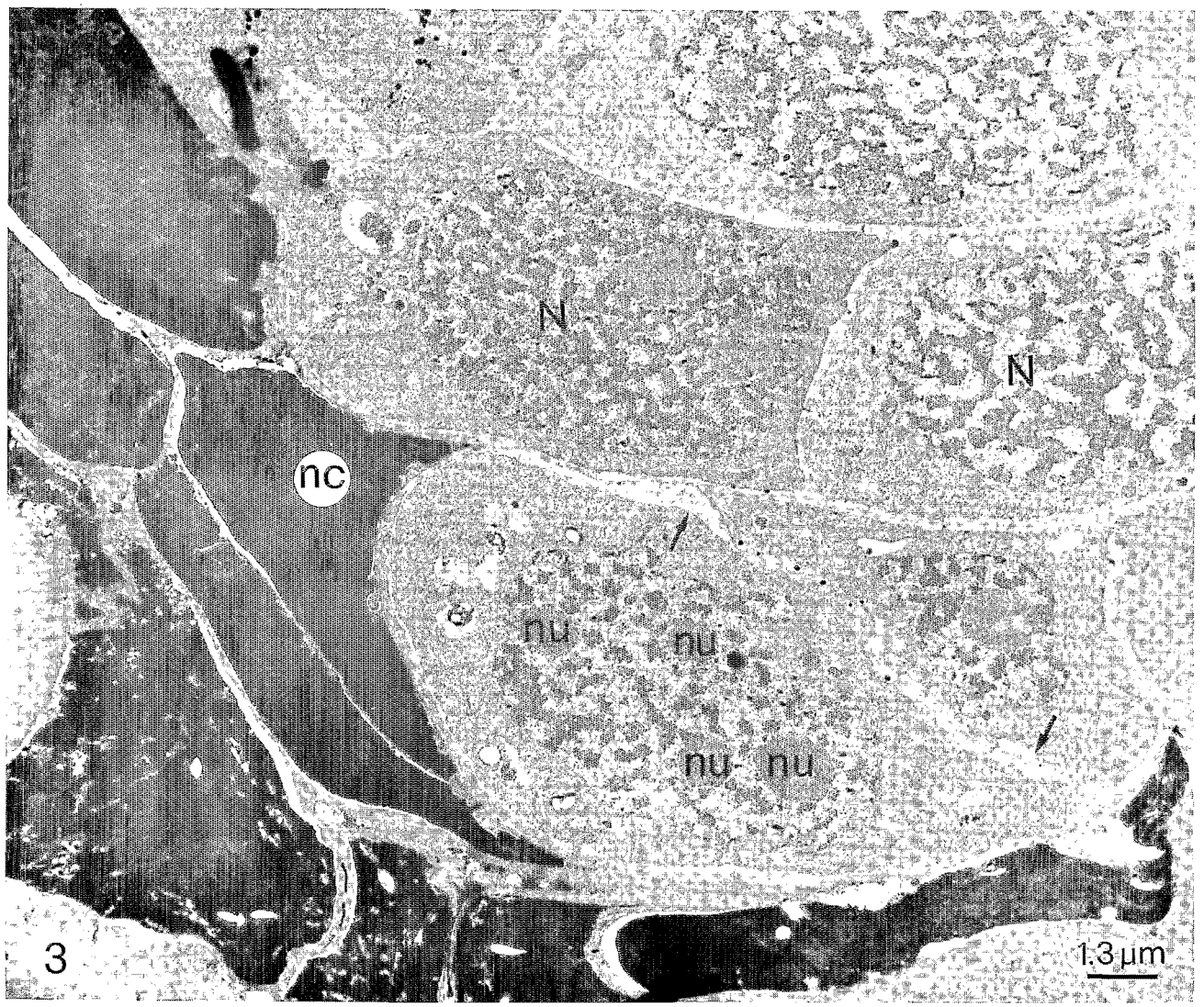

Fig. 3. Cells of wheat root tip adjacent to the feeding site, represented by dark necrotic cells, are hypertrophied. The protoplasts with very dense cytoplasm are subjected to false plasmolysis (arrow). The nucleus, with an amoeboid profile, is hypertrophied, rich in heterochromatin and contains several nucleoli.

For abbreviations see Fig. 1.

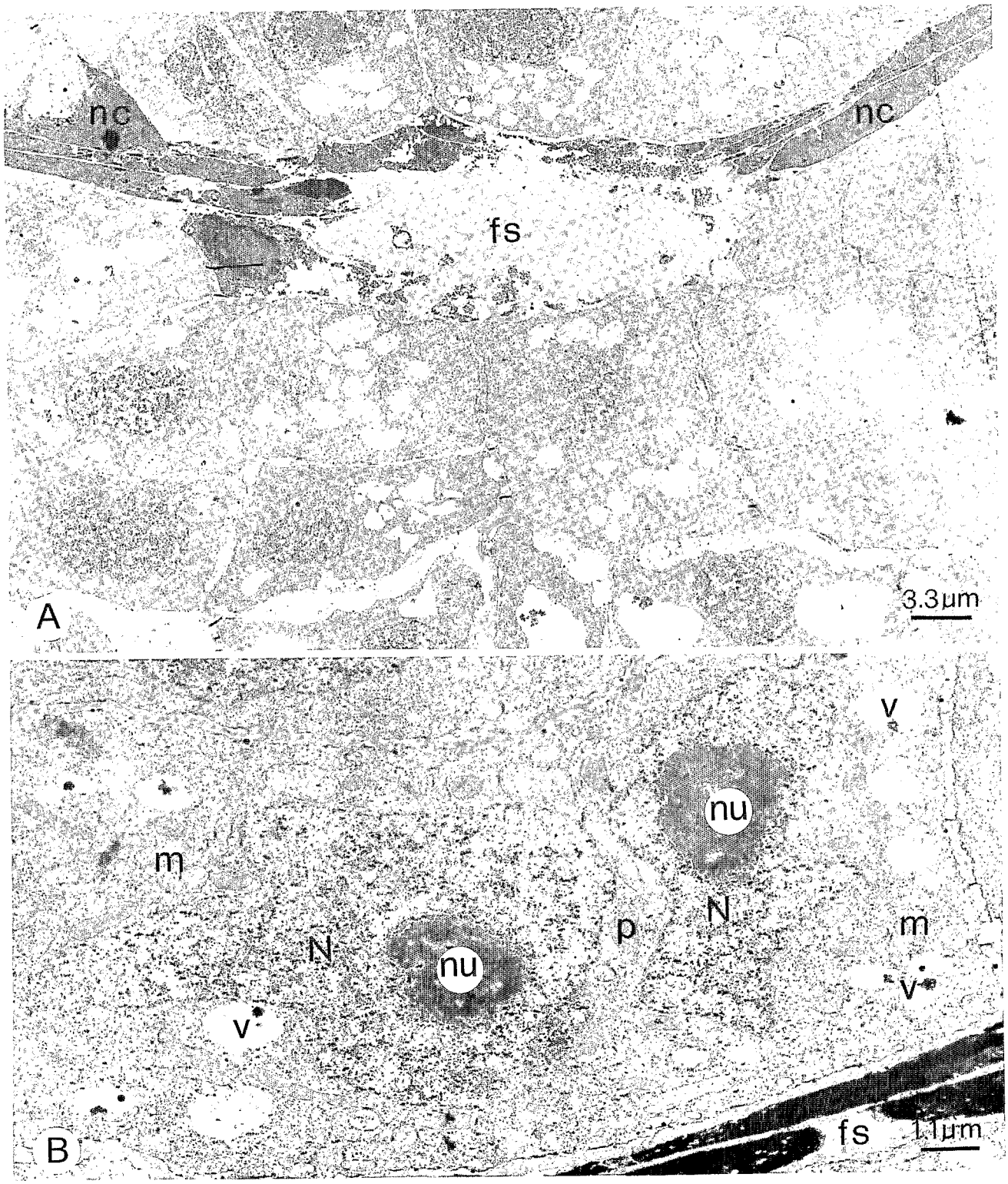

Fig. 4. A - Longitudinal section through a barley root tip, three days after nematode inoculation. The feeding site of the nematode is an empty cavity delineated by necrotic cells. The remaining tissue has been severely affected by the feeding action. B - Detail of a cell in barley root tip next to the feeding site. The cytoplasm is rich in organelles and dark spots in the vacuoles indicate protein storage. The two nuclei that appear to be present are in reality only one with a highly amoeboid profile. The two visible nucleoli are proliferated as in wheat.

For abbreviations see Fig. 1.

The feeding of *L. belloi* on barley root tips resulted in more widespread histological changes than in wheat roots; four axial layers of cells appeared to be involved. The presence of necrotic cells (dark and collapsed cells) indicated the pathway of the odontostyle which ended in an empty cavity that formed the feeding site (Fig. 4 A). Neighbouring cells were typical of the meristem apart from changes in the nuclei; slight plasmolysis was also present in some cortical cell layers (Fig. 4 A).

Fig. 4 B shows details of a cell in juxtaposition to the necrotic area. The cell appeared to be binucleate, but this was not the case as the plane of the section was here through an enlarged and highly invaginated nucleus. Its nucleoli were hypertrophied and had large " vacuoles " and chromatin was very diffused (Fig. 4 B).

In rye grass root tips fed upon by *L. belloi* there was an abundance of cells throughout the section. The cells were subjected to hyperplasia that resulted in many small cells with sinuate walls (Fig. 5 A). Vetch and lentil root tips that had been subjected to nematode feeding lost their tapered form and the apical meristem had matured (Figs 5 B, 6). All the cells of the cortical ground meristem were highly vacuolated with intercellular spaces; mature protoxylem and protophloem elements occurred in the procambium (Fig. 6). The primary endodermis was severely affected by nematode feeding and cells were distorted and necrotic (Fig. 6). The phenomenon of the hyperplasia involved the procambial cells and this more evident in lentil roots (Fig. 5 B).

A common response of the cells not directly injured by the nematode and found in all the hosts tested was the presence of paramural bodies in many of the cells (Fig. 7 A-D) often relatively far from the nematode feeding site. These structures appeared to be invaginations of the plasmalemma containing small vesicles or tubules and membranes. They were not associated with any localized modification of the adjacent cell wall (Fig. 7 A-D) but they appeared to be associated with dictyosomes in some cells (Fig. 7 A, C). The paramural bodies were less well defined (Fig. 7 A, C) in cells far from the feeding site and more extensive in those adjacent to the feeding area (Fig. 7 D). Frequently they were found associated with microtubules that appeared to be linked to each other (Fig. 7 D). Sometimes the plasmalemma was in digitate form; this was a consequence of the fusion of vesicles produced in the cytoplasm (Fig. 7 E). In these cells vesiculation of the endoplasmic reticulum and dictyosomes and fuzzy-coated vesicles were present with transitional elements, both indicating that they were involved in the synthesis of some products transferred in vesicles to the plasmalemma (Fig. 7 C).

Discussion

The analysis of cytological changes showed that all the cells directly fed upon by the nematode became empty and necrotic most probably due to ondontostyle penetration and the removal of cell contents. Interestingly the remaining cells of the root tip also showed changes in their metabolism due to the parasitism. In wheat, the cells became hypertrophied, increased in size and in the nuclei there was a relative increase in the number of nucleoli. It is common for a diploid organism to have one large or two small nucleoli.

The number of nucleoli per nucleus is a reflection of the number of organizers present, having the organizers the capacity to form nucleoli. The total volume of nucleolar material is higher in cells in an early stage of differentiation and drops to a minimum at the end of their differentiation when they are quiescient.

The presence of several nucleoli with about the same surface area as the single nucleolus in cells of uninfested roots indicates that the activity of the cells is highly increased in roots subjected to nematode feeding. Some reports show that there is an increased total nucleolar volume as a consequence of an increase in cellular RNA (Jordan, Timmis & Trewavas, 1980; Griffiths, Robertson & Trudgill, 1982). Spherical inclusions of low density are also present in the nucleoli : the nucleolar " vacuoles ". They are characteristic of active nucleoli and have been assigned the role of RNA transport (Rose, Setterfield & Fowke, 1972).

In rye grass infested by *L. elongatus*, Griffiths and Robertson (1984) reported modification such as concentration of RNA and protein indicating host metabolism enhancement.

The feeding effect of *L. belloi* on barley roots resembles that induced by *L. apulus* on celery roots, with the formation of a lysigenous cavity and an ingrowth of the cell walls in the layers of cells adjacent to the feeding site.

The morphology of the enlarged nuclei with irregular, lobed outlines and with hypertrophied nucleoli indicates metabolically active cells such as those induced in fig roots by *Xiphinema index* (Wyss, Lehman & Jank-Ladwig, 1980). However in infested barley roots the damage is more severe and quickly leads to the destruction of the whole root tip.

The different responses by the host plant depends on the specific interaction with the parasite in the early stages of infection. Many stimulatory events seem to be associated with *L. belloi* parasitism; for example an increase in the volume of host cytoplasm, ribosomes, accumulation of proteins in plastids and hypertrophy of the nuclei. Stimulatory changes to nuclei have also been observed during fungal invasion (Hadwiger & Adams, 1978). Pseudoplasmolysis, retraction of the plasmalemma and an increase in vacuolar content is in agreement with the evidence for the lysosomal character of the root cells (Matile, 1975). In view of the similarity of cellular responses to different form of injury — nematode pathogenesis, senescence and herbicides (Anderson & Thomson, 1973) — it is difficult to ascertain whether

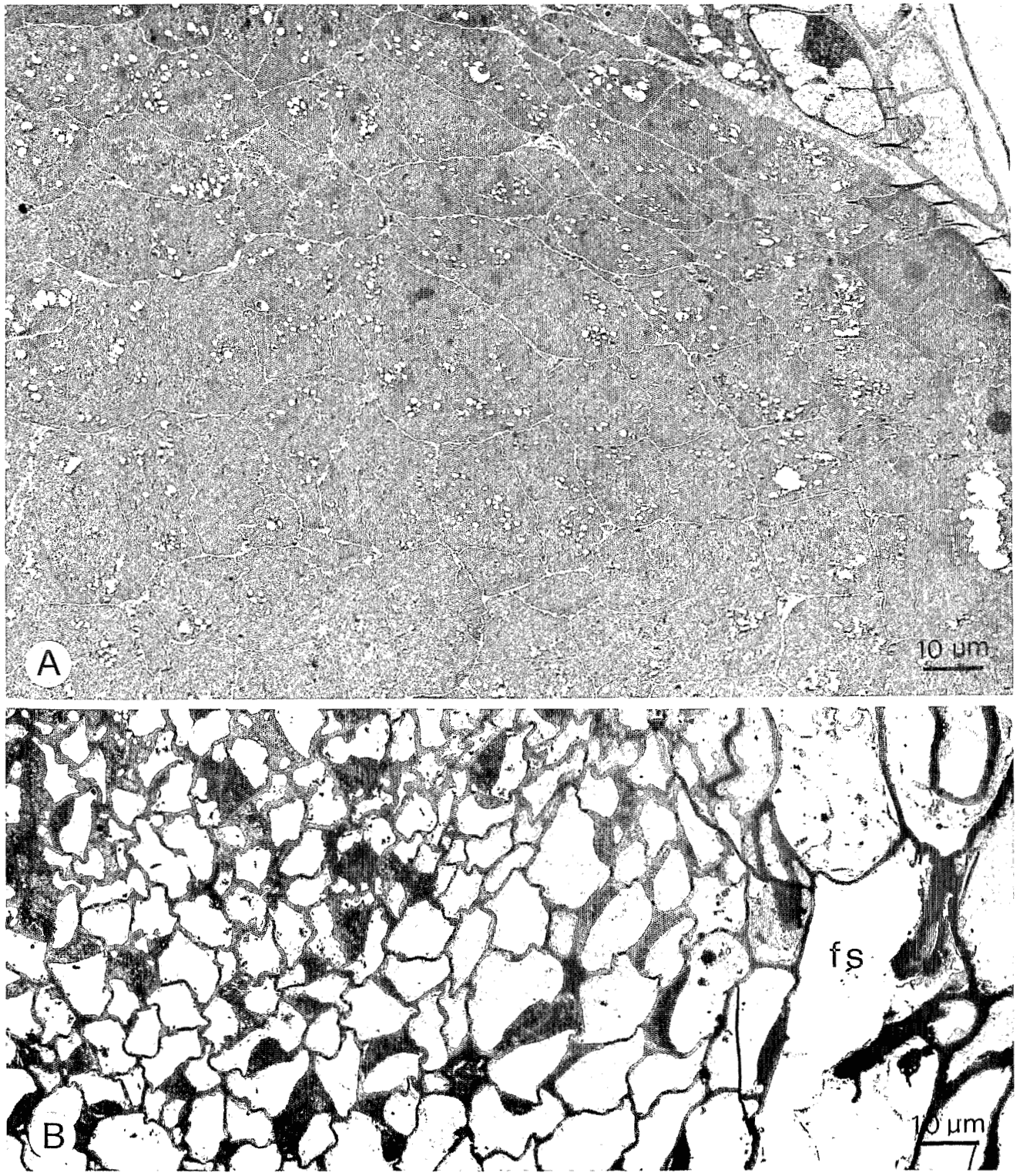

Fig. 5. A - Longitudinal section through an infested rye grass root tip. The excessive number of cells indicate that the meristematic tissue is subjected to a hyperplastic response. The cell walls are wavy and small vacuoles are scattered in the cytoplasm. B - Transverse section of infested vetch root tip. The feeding site of the nematode is localized in the cortical cells and the remaining tissues show hyperplastic response, indicated by many small daughter cells, with irregular wall profiles.

For abbreviations see Fig. 1.

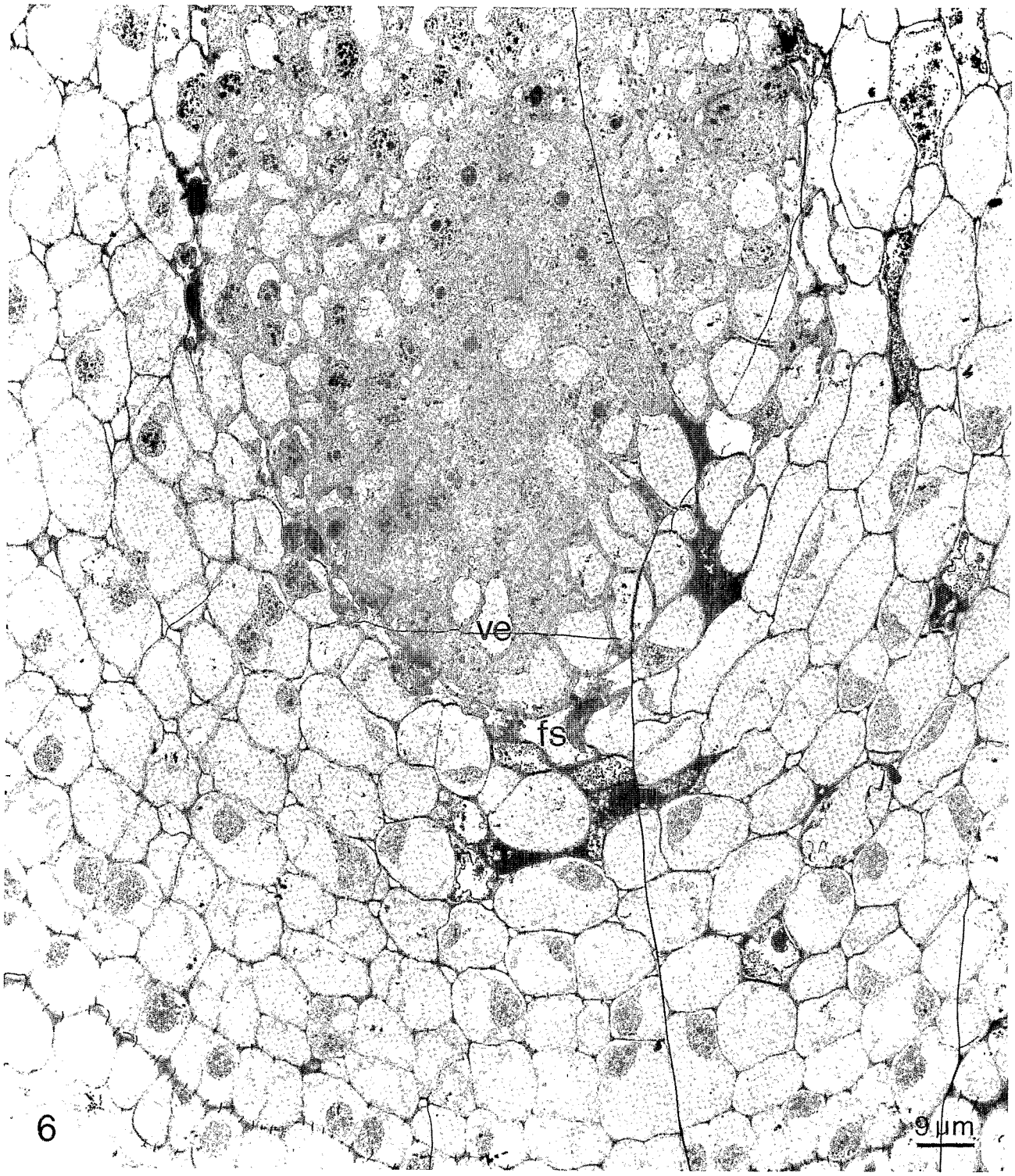

Fig. 6. Transverse section of lentil root tip three days after nematode inoculation. The feeding site is localized between the primary endodermis and four cortical layers. Cells, that have been fed on, are necrotic and sometimes empty after the removal of the cytoplasm during feeding. All the cortical tissue is completely differentiated. The hyperplastic response of the cells is evident in the procambium; protophloem and protoxylem elements are present.

For abbreviations see Fig. 1.

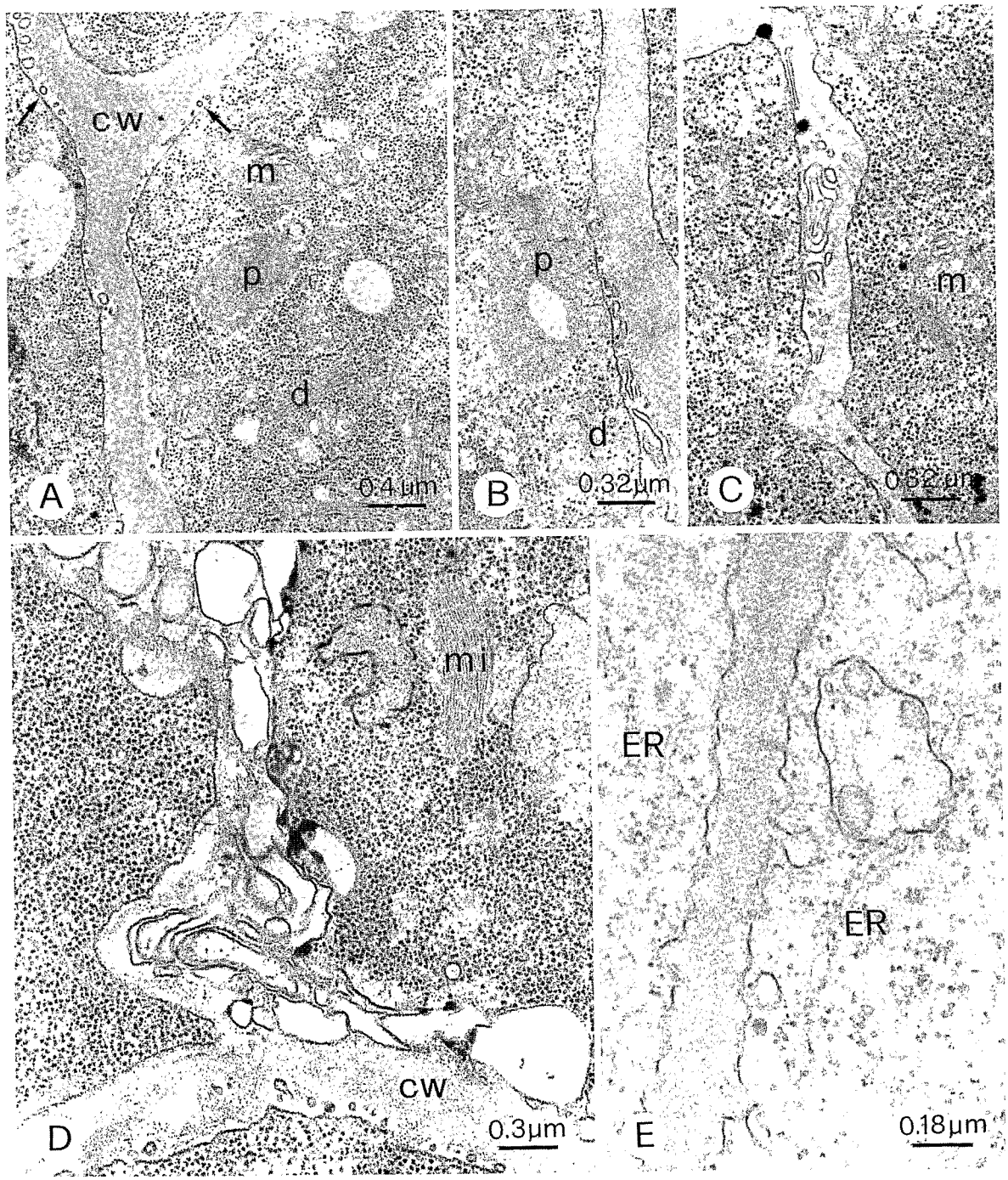

Fig. 7. Micrographs of paramural bodies formed in root cells of wheat, barley and lentil parasitized by *L. belloi* (longitudinal sections) : A) Vesicles (arrow) compressed between plasmalemma and cell wall in wheat cells. Note the presence of active Golgi bodies; B) Enlarged vesicles and tubules (wheat cells). Transitional vesicles and others with dense core are released by dictyosomes, close to the plasmalemma; C) Multivesicular bodies in barley cells; D) Disconnection of plasmalemma due to development of vesicles in barley cells. Note assembled microtubules in the cytoplasm; E) Extensive multivesicular bodies in lentil cells. The plasmalemma assumed digitate form because of the fusion of big vesicles. Endoplasmic reticulum is enlarged and dictyosomes actively synthetizing.

For abbreviations see Fig. 1.

such effects are a direct result of nematode feeding or of secondary compounds, generated during infection.

One of the earliest and most regular responses to *L. belloi* feeding is the rapid aggregation of host cytoplasm from which secretory vesicles contribute to the formation of wall apposition in the paramural space beneath the directly injured cells. The presence of paramural bodies in celery roots parasitized by *L. apulus* was considered to be an indicator of a mechanism for regulating nematode damage (Bleve-Zacheo *et al.*, 1979). In the root cells of cereal and leguminous hosts attacked by *L. belloi* the paramural bodies were scattered at random and were not associated with modified regions of the cell wall, implying that they were a manifestation of a generalized alteration of the plasmalemma. Nevertheless, the presence of transitory elements of dictyosomes and clustered microtubules, in the region of cell wall thickening, indicates the initiation of a cellular response to repair the damage. The microtubules appear to anticipate and control cell wall thickening by directing a flow of organelles (Golgi vesicles) or metabolites required for fibril formation (Dustin, 1984) towards the cell membrane. Association between microtubules and dictyosome vesicles is frequent in *Zea mays* and perhaps relates to cell wall formation (Galatis, 1982).

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