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ULTRASTRUCTURAL RESPONSE OF CELERY ROOT CELLS TO *LONGIDORUS APULUS*

by
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Several authors have reported the presence of numerous lomasomes (paramural bodies) and the deposition of callose during the initial phase of infection by viruses (Kim and Fulton, 1973; Conti *et al.*, 1974; Martelli, 1980), fungi (Ehrlich *et al.*, 1968; Heath and Heath, 1971; Bracker and Littlefield, 1973; Politis and Wheeler, 1973), bacteria (Sequeira *et al.*, 1977; Politis and Goodman, 1978), and nematodes (Huang and Maggenti, 1969; Bleve-Zacheo *et al.*, 1979; Bleve-Zacheo *et al.*, 1980) in plant cells. It has been suggested that callose plugs in the plasmodesmata might be the main factor in the localization of viruses inhibiting their diffusion (Allison and Shalla, 1974; Favali *et al.*, 1978). Large areas of callose have also been observed in response to fungal infections and they appeared to surround completely the haustoria as they grew (Ehrlich and Ehrlich, 1971; Littlefield and Bracker, 1972; Bracker and Littlefield, 1973; Politis and Wheeler, 1973; Sargent *et al.*, 1973). Callose has also been detected in clover root cells at the sites of infection by *Rhizobium* sp. (Kumarasinghe and Nutman, 1977), and in strawberry tissues attacked by *Ditylenchus dipsaci* (Bleve-Zacheo *et al.*, 1980).

There is little information about these types of response in plant tissues attacked by nematodes, especially ectoparasitic nematodes. This paper describes the reactions of celery root cells injured by *Longidorus apulus*.

Materials and Methods

Celery seedlings were grown into 5 cm diameter plastic pots containing sterilized quartz sand. The pots were placed in a growth chamber (22ºC, 65% RH, 3,000 lux) and one root of each plant was separated into a 2 cm diameter plastic pot and inoculated with one female of *L. apulus*. Three days after inoculation the roots were removed and observed with a stereomicroscope. Terminal portions (about 3 mm) were excised from damaged root apices, where the feeding sites of nematodes were localized in meristematic and differentiating areas. The apices were fixed for 2 h in 3% glutaraldehyde at 4º C, rinsed overnight in 0.1 M phosphate buffer at pH 7.2 and postfixed for 2 h in 1% osmium tetroxide. They were then dehydrated in a graded ethanol-propylene oxide series and embedded in Araldite.

Ultrathin and 2 μm thick sections were made for electron microscopy and for histochemical detection of callose, respectively. For the detection of callose the sections were immersed for 2 h in NaOH methanol solution which was changed repeatedly to eliminate the resin and stained with an aqueous solution of 0.05% aniline blue, in 0.067 M Na_3PO_4 phosphate buffer (Fulcher *et al.*, 1976; Peterson *et al.*, 1978). An examination for autofluorescence was made on sections treated as previously described, but without aniline blue treatment. The sections were examined with a Leitz Dialux 20 EB microscope with fluorescent light and exciter filter KP 490 and barrier filter TK 510/K 515. Ultrathin sections, made on a LKB ultratome III, were stained for 1 h in uranyl acetate and for 10 min in lead citrate (Reynolds, 1963) and examined at 80 Kv in a JEM 100 B electron microscope.

Results

Modifications caused by *L. apulus* in the cells of the root apex were evident from the initiation of the nematode attack. In four or five cells, usually those penetrated by the odontostyle as previously described (Bleve-Zacheo *et al.*, 1977), the cytoplasm was in an advanced state of degeneration, three days after inoculation with the nematode (Fig. 1). Cells adjacent to this area did not show much modification. They had numerous vacuoles containing granular ma-

terial and sequestered cytoplasm, similar to those defined by Marty (1978) as digestive vacuoles. The plastids were of normal appearance but richer in their starch content, as previously observed by other workers who considered this to be the initial symptom of pathogen attack (Kosuge, 1978).

The damage caused by nematode feeding was localized behind the fourth layer of cells from the rhizodermis. The cytoplasm of the cells beneath this layer was extensively degraded and, as previously reported (Bleve-Zacheo et al., 1979), the rapid death of the cells gave rise to a lysigenous cavity (Fig. 2).

In those cells directly affected by the feeding of the nematode numerous protrusions of the walls were observed: this could be interpreted as the beginning of the differentiation of the multinucleate transfer cells, as usually induced by the feeding of the endoparasitic nematodes (Fig. 3). However, L. apulus failed to induce complete differentiation to form transfer cells because of the severe damage to the cytoplasm.

The cells surrounding the lysigenous cavity but not actually attacked by the nematode, showed an intense growth of the cell wall-plasma membrane complex and the formation of wide areas callose-like adjacent to it. Numerous lomasomes were present in these cells.

The morphology of the lomasomes varied. In some instances they were simple invaginations of the plasmalemma in which vesicular bodies were recognizable; these were of different shape, size and content, and were embedded in a matrix either similar or clearly in contrast with that of the cytoplasm (Fig. 4). Other vesicles consisted of a complex system of membranes, very similar to the myelinic configurations (Fig. 5) and considerably extending the plasmalemma.

The amplification of the plasmalemma does not appear to be linked exclusively to the lomasomes, but also the endoplasmic retic-

Figs. 1-3 - 1, Electron micrograph of celery root-tip: cells (arrow) injured by the odontostyle of the nematode, Longidorus apulus; the adjacent cells show plastids richer in starch than the healthy ones, and numerous vacuoles; lytic vacuoles with sequestered cytoplasm (inset x 7,000), (x 7,600). 2, Feeding site of the nematode (lysigenous cavity) with degraded cellular structure. Three layers of paren-chymatous cells and the rhizodermis are recognazable (x 3,800). 3, Protrusions on cell walls bordering the lysigenous cavity, possibly associated with differ-entiation as in trasfer cells (x 23,600).
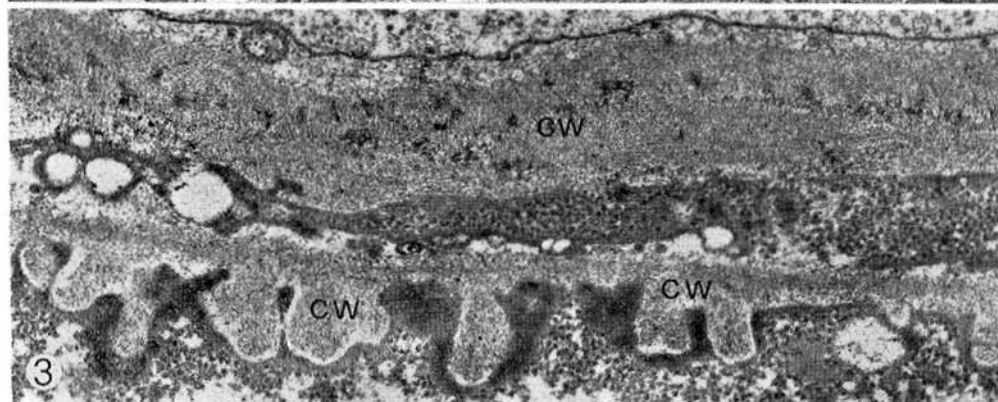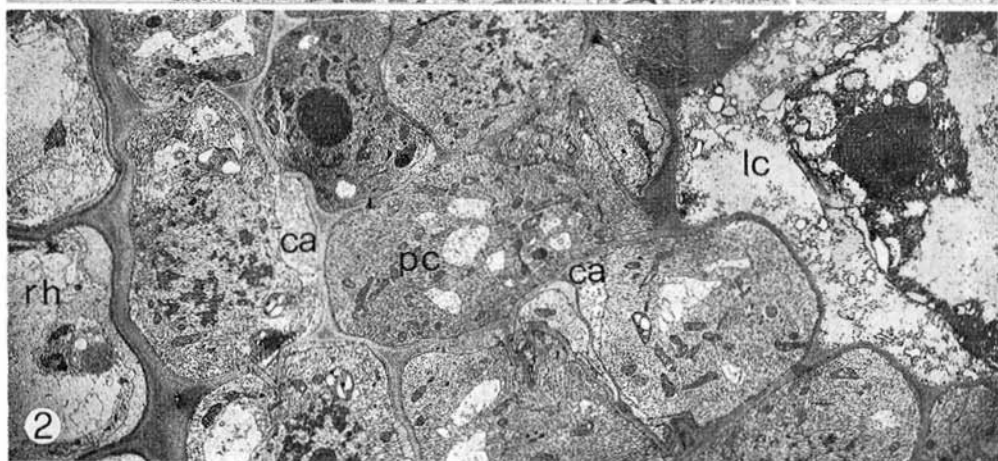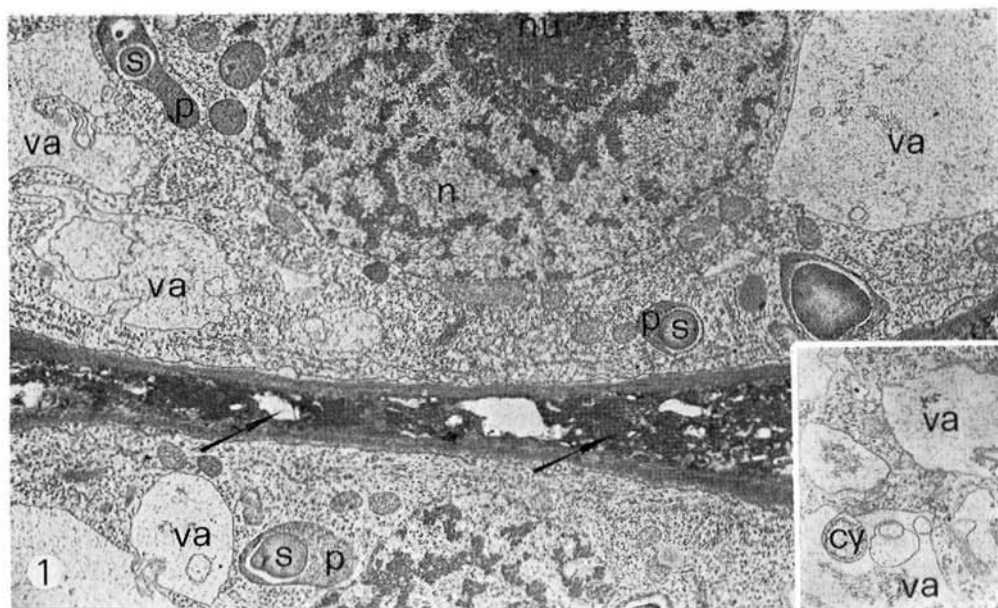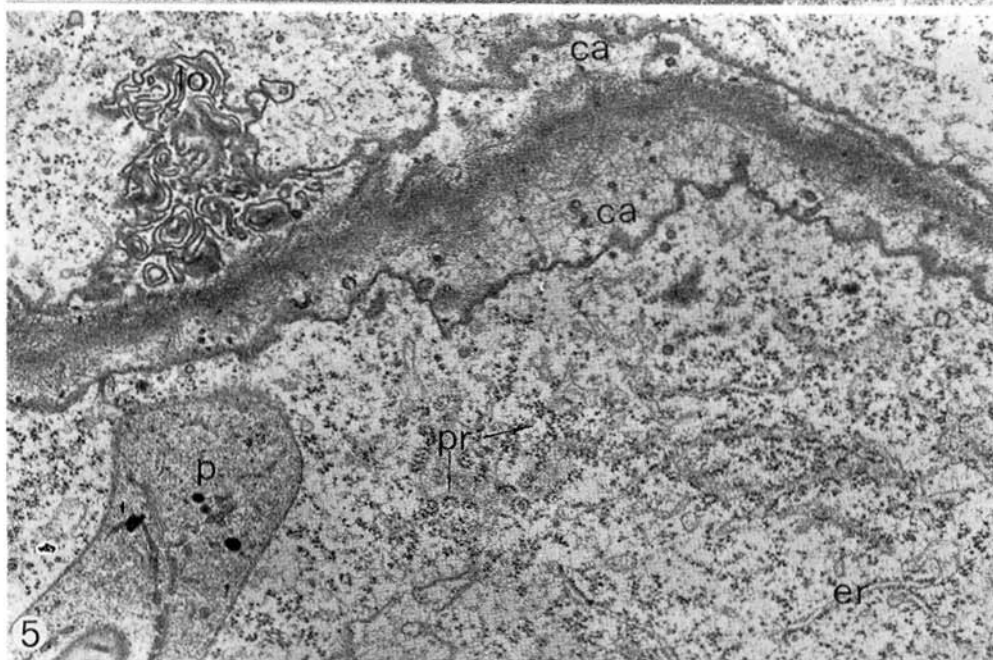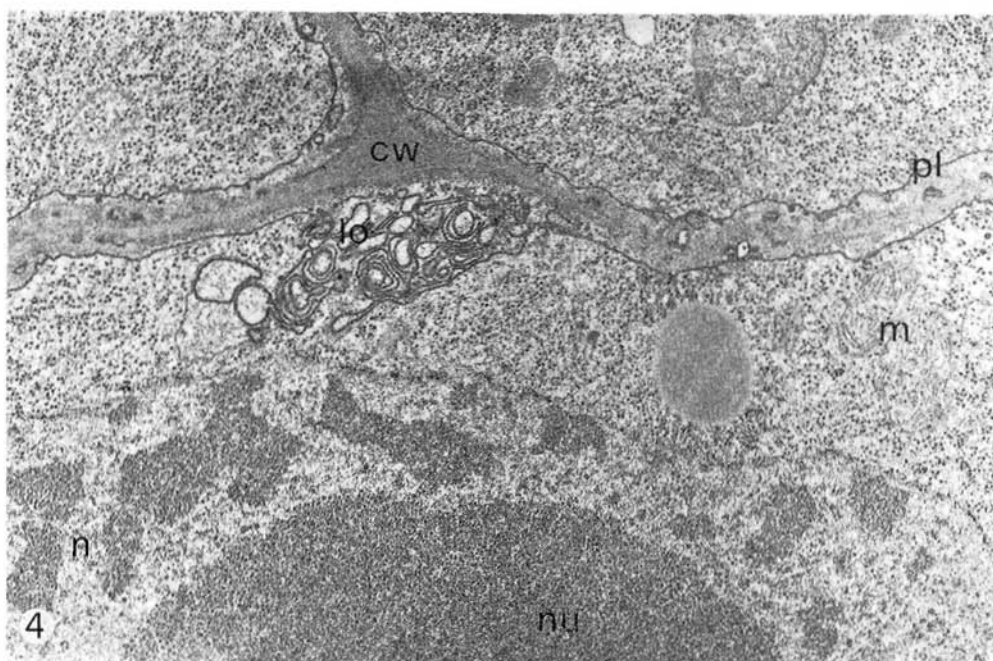
Figs. 4-5 - 4, Cell adjacent to the lysigenous cavity with translucent multivesicular body. The vesicles differ in shape and size, and show a double membrane (x 34,300). 5, Lomasome with numerous vesicles and electron dense material like myelinic body (x 29,800).
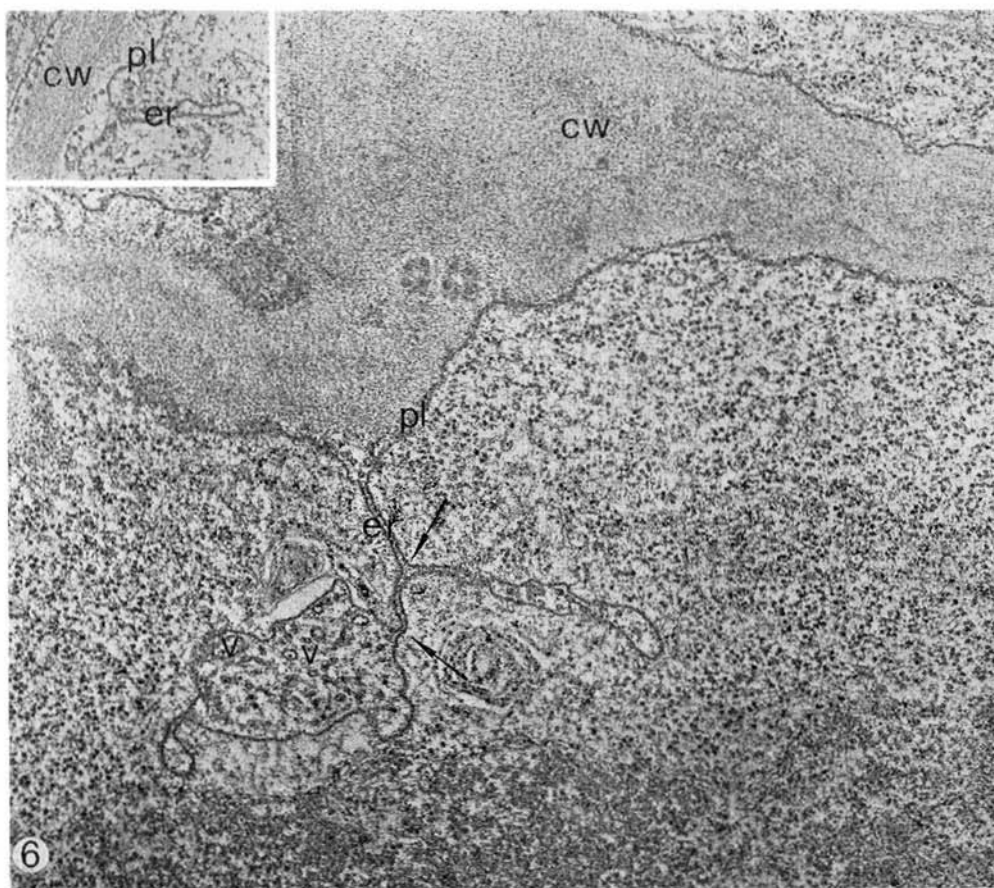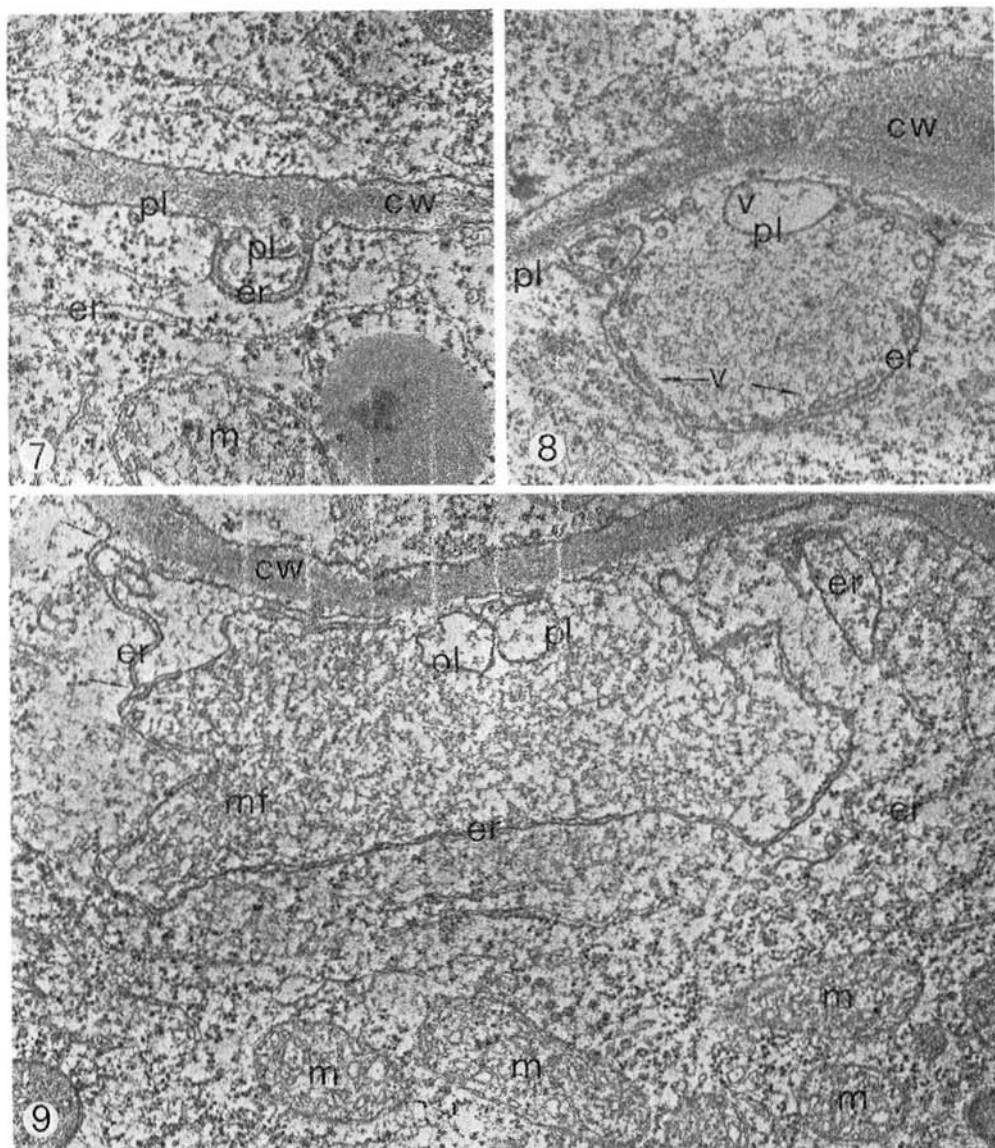
Fig. 6 - Endoplasmic reticulum fused to the plasmalemma: fusion of endoplasmic reticulum membranes (inset x 38,000); fusion of E.R. membranes (arrow) extending the plasmalemma. Material rich in vesicles is present between the endoplasmic reticulum membrane (x 40,000).

ulum appears to be heavily involved in this process. Portions of endoplasmic reticulum were observed fused to the plasmalemma in indifferent ways.

Sometimes membranes of the endoplasmic reticulum appeared to be fused to the plasmalemma only at one point (Fig. 6, Inset). On these membranes new fragments of endoplasmic reticulum could be inserted from which both this structure and the plasmalemma grew (Fig. 6).

Figs. 7-9 - 7, Bridge of membranes of endoplasmic reticulum on the plasmalemma. (x 38,000). 8, The plasmalemma and the inner membrane of the endoplasmic reticulum give rise to some vesicles (x 36,800). 9, Cell below the feeding site (as Figs. 7, 8). Portions (arrow) of endoplasmic reticulum fused to the membrane delimiting the structure toward the cytoplasm. Inside electron lucent matrix and amorphous material is recognizable (x 26,600).
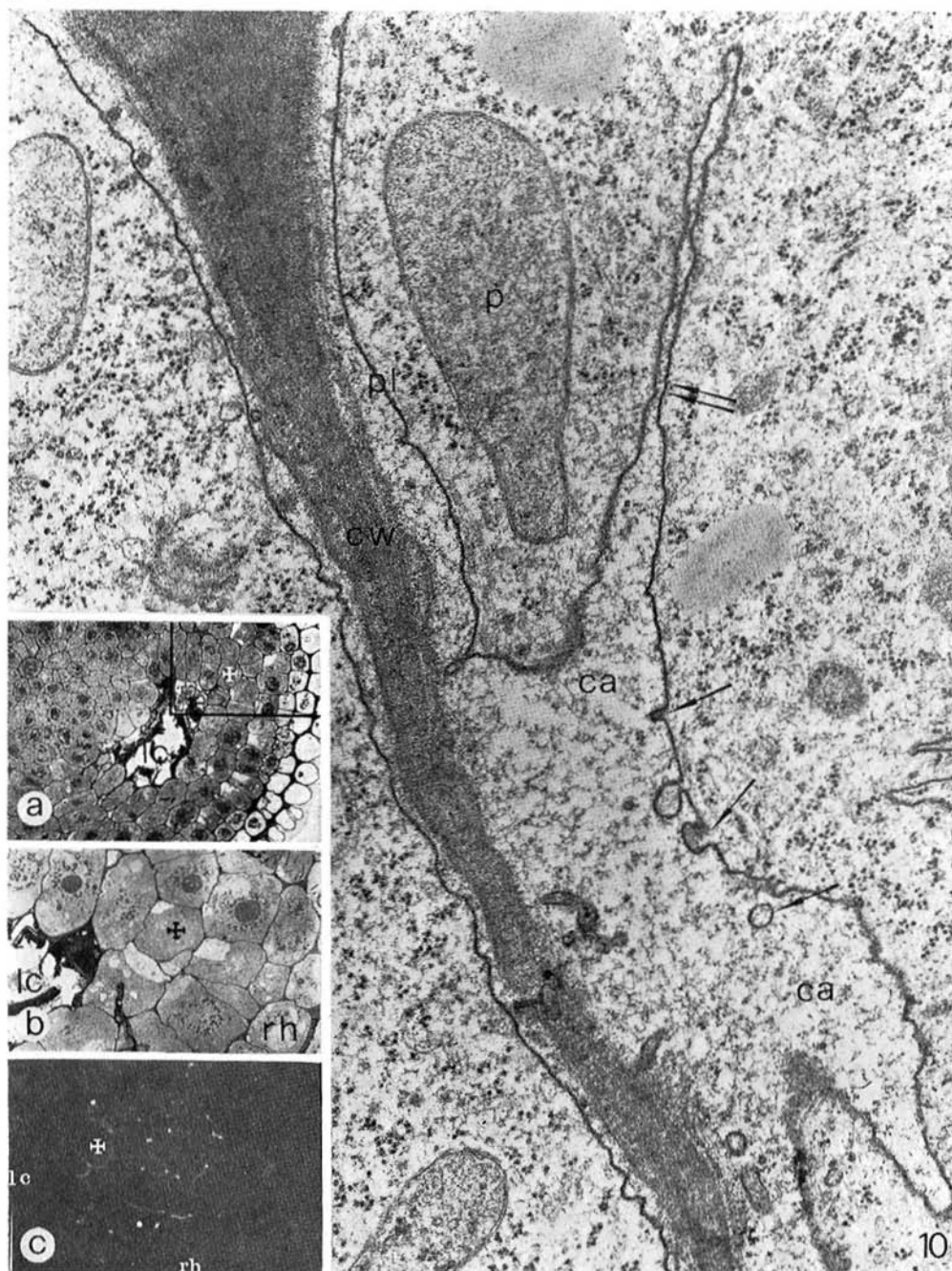
Fig. 10 - Wide area of callose-like material acculumation delimited by a membrane on which vesicles at different stages (arrows) are discernible. Extension of the area is developed from the continuous fusion of endoplasmic reticulum. A portion of endoplasmic reticulum (double arrows) can be identified (x 38,000). Inset shows: a) section showing lysigenous cavity at light microscope (x 110); b) detail of figure 10 a (square) observed at E. M. (x 850); c) the same cells as in 10 b, stained with aniline blue (x 200).

In other instances the endoplasmic reticulum appeared to be attached to the plasmalemma at two distinct points, forming a bridge; one of the two membranes of the endoplasmic reticulum was adjacent to the cytoplasm and the other formed a vesicle enclosing the preexisting plasmalemma (Fig. 7). The plasmalemma wa salso observed to form vesicles whose content was clearly different from the remaining part of the structure (Figs 8, 9), while the inner membranes of the endoplasmic reticulum were transformed into small vesicles (Fig. 8). The content of these structures appeared to be similar to that reported by other authors as callose (Ingram *et al.*, 1976; Favali *et al.*, 1978; Politis and Goodman, 1978). These structures, containing also fibrillar material expanded progressively to form large masses on the whole cell wall (Figs. 10, 11).

The callose-like material accumulated on the cell wall fluoresced by the examination of sections of the same cells treated with aniline blue, in a fluorescent microscope. By this means it was possible to follow the progressive increase of the fluorescent areas in the surrounding tissues starting from the cells furthest from the feeding site and progressing toward the lysigenous cavity. From these observations it was ascertained that the accumulation of material aniline blue sensitive extended from the feeding site as far as the cell layer beneath the rhizodermis, thus forming a ring surrounding the lysigenous cavity. Sections not stained with aniline blue, but examined in phosphate buffer, at the same pH and in the same ultraviolet wavelengths and barrier filters, did not show any fluorescence.

In cells accumulating callose-like material the Golgi apparatus showed intense activity and formed numerous vesicles, similar to those found outside the protoplast. Numerous polysomes were always observed close to the membranes of enlarged endoplasmic reticulum containing electron dense material (Fig. 11).

The plasmalemma was also seen to be involved in the deposition process. Figure 10 illustrates the different stages of evagination of the membranes, with primordial vesicles, newly formed or free in the deposition area.

All of these cellular structures may be involved in the synthesis of callose-like material as it is suggested for other pathogens (Ingram *et al.*, 1976; Martelli, 1980).

Discussion

The formation of the lysigenous cavity is the first result of the trophic action of *L. apulus*.

Some of the cells which form the lysigenous cavity initiate a process of differentiation such as seen in the formation of transfer cells in roots attacked by endoparasitic nematodes (Huang and Maggenti, 1969). However, damage caused by the ectoparasitic nematode such as *Longidorus* is more severe than that induced by an endoparasite, and the process is soon halted.

Numerous lomasomes appeared in the cells surrounding the lysigenous cavity soon after the start of the nematode attack on the root. Many authors (Heath and Heath, 1971; Politis and Wheeler, 1973; Sequeira *et al.*, 1977; Bleve-Zacheo *et al.*, 1979; Martelli, 1980) consider that lomasomes are closely associated with pathogen injury. However, lomasomes are normal constituents of a plant cell and it has been demonstrated that they are involved in cell expansion (Clowes and Juniper, 1968). Nevertheless the role of the lomasomes in the process of cell wall growth is difficult to interpret.

Lomasomes have been generally associated with accumulation of callose (Kim and Fulton, 1973, 1975; Cooper and Atkinson, 1975; Russo *et al.*, 1978a, b), although our observations indicate that this is not necessarily the case. Another sign of deposition of callose-like material seems to be the fusion of portions of the endoplasmic reticulum to the plasmalemma; these fusions increase as the size of the deposition areas increase. This observation is of particular significance as an alignment of the endoplasmic reticulum with the plasmalemma has often been reported, although a fusion was never observed (Morré and Mollenhauer, 1974; Ingram *et al.*, 1976).

Fig. 11 - Wide area of callose-like apposition on both sides of the cell wall. Amorphous matrix embedded with fibrillar material and numerous vesicles produced by evagination of the modified plasmalemma are visible (arrows). The cytoplasm is rich in light and electron dense vesicles which seem to migrate toward the cell wall (double arrows); numerous polysomes are present (x 35,800).

List of abbreviations used in the figures: ca = callose-like deposition; cw = cell wall; cy = cytoplasm; ER = endoplasmic reticulum; G = Golgi bodies; lc = lysigenous cavity; lo = lomasome; m = mitochondrion; mf = microfibrils; n = nucleus; nu = nucleolus; p = plastid; pc = parenchyma cells; pd = plasmodesma; pl = plasmalemma; pr = polysomes; rh = rhizodermis; s = starch grain; v = vesicles; va = vacuole.
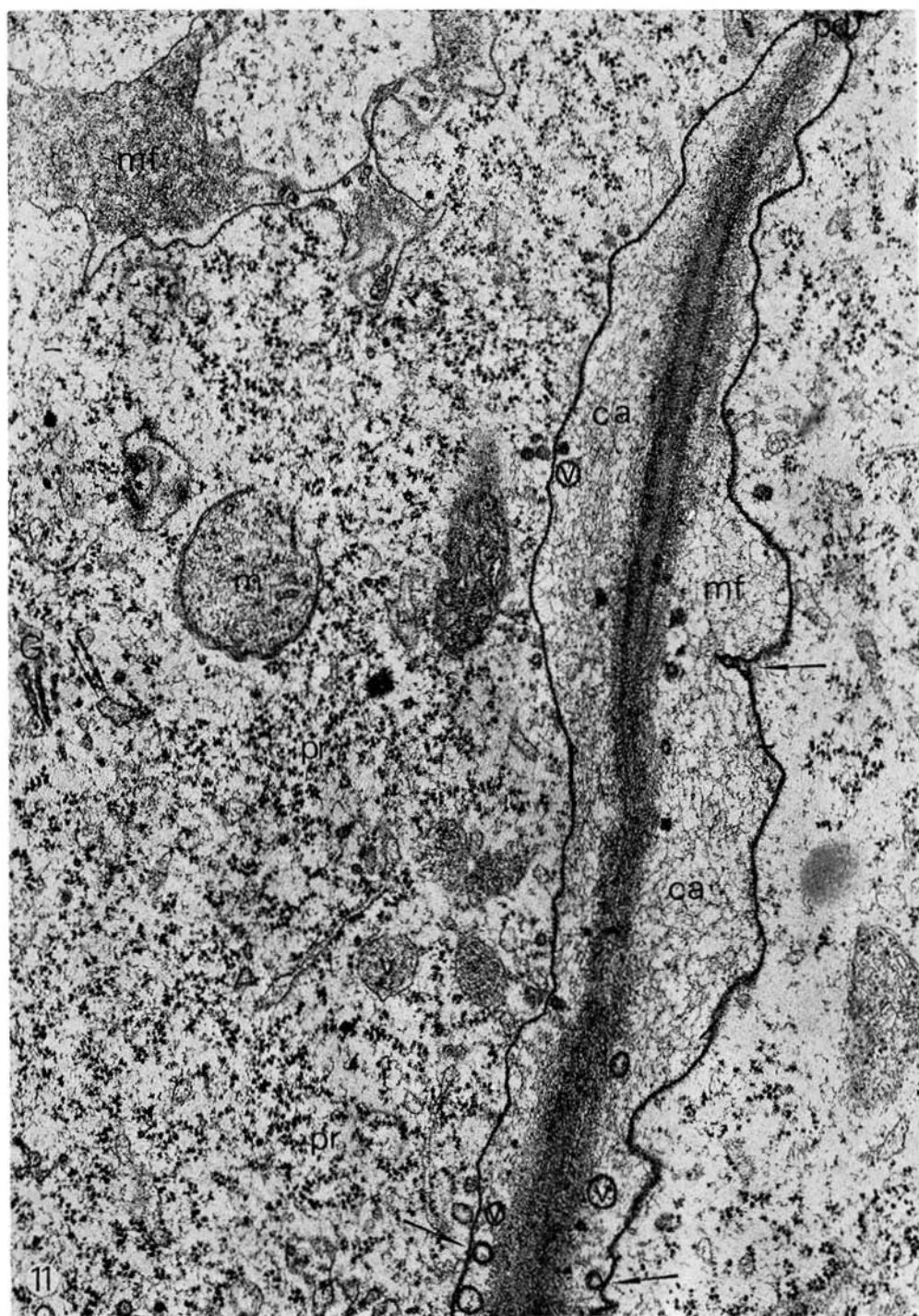
The Golgi apparatus, through two types of vesicles (Matile, 1975), seems to be involved in the process. Golgi-derived vesicles could contribute to the enlargement of the plasmalemma and the production of callose-like areas, building blocks and enzymes. These materials, packaged in vesicles could be then transported through the cytoplasm and transferred across the plasmalemma.

Anderson and Ray (1978), recently, have reported that glucan synthetase on the plasma membrane of pea cells may serve for formation of wound callose and this callose synthetase has the property of being labile to cell disruption. This may serve to ensure that upon injury, callose is formed by still viable cells and not by those injured beyond hope of rescue.

Accumulation of callose or callose-like in virus-infected tissues has been considered to be a barrier induced in the host cells to the diffusion of the virus (Wu and Dimitian, 1970; Allison and Shalla, 1974); and the more rapid is the synthesis of callose on the plasmo-desmata between infected and non-infected cells, the more effective would this barrier be (Wu et al., 1969). The same barrier function is attributed to callose in cells attacked by fungi (Ingram et al., 1976) and bacteria (Kumarasinghe and Nutman, 1977).

The results of this study indicate that following the attacks of ectoparasite nematodes a rapid accumulation of callose-like material occurs in cells surrounding the lysigenous cavity and that deposition areas are not restricted to the pit fields but involve the whole cell wall.

The formation of a barrier to counteract the action of the ecto-parasite nematode does not seem to provide any advantage to the host. It is more likely that callose-like accumulation in the cells immediately adjacent to those involved in the feeding process is a nonspecific reaction of the plant to wounding.

It seems logical to assume that the beginning of the callose-like material synthesis is induced in uninjured cells by an unknown biochemical message from cells directly attacked by the nematode. Even if it can be assumed that the production of callose is consequential to wounding there are, however, instances in which this does not occur. For example, pollen-stigma interaction causes an immediate callose production in an incompatible stigma, but callose production does not occur when the pollen is compatible with the stigma (Heslop-Harrison et al., 1975; Howlett et al., 1975). It has been demonstrated that the callose wall functions as a molecular filter, which permits the passage

of some molecules but excludes others (Heslop-Harrison and Mac-Kenzie, 1967; Southworth, 1971).

Thus, callose or callose-like material formation in plant tissue is a complex phenomenon and its physiological meaning is not, at present, well understood.

SUMMARY

The ultrastructure of celery roots parasitized by *Longidorus apulus* was studied. The nematode induced a lysigenous cavity and numerous protruded cell walls in the host cells involved in the trophic action. The parenchymatous cells surrounding the lysigenous cavity showed many modifications: pronounced formation of lomasomes, elements of endoplasmic reticulum connected with the plasmalemma, and conspicuous callose-like deposition outside the protoplast. Deposition areas, induced by the ectoparasitic nematode, were interpreted as wound response.

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