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CELL WALL BREAKDOWN AND CELLULAR RESPONSE
IN DEVELOPING GALLS INDUCED BY *LONGIDORUS APULUS*

by

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Some aspects of the histological and histochemical modifications induced by *Longidorus apulus* Lamberti *et* Bleve-Zacheo in the roots of celery (*Apium graveolens* L.) have been reported previously (Bleve-Zacheo *et al.*, 1977). Further studies, reported here, have extended the information on cell response to *L. apulus* feeding particularly with reference to morphological and ultrastructural changes.

MATERIALS AND METHODS

Celery seedlings, one month old, were transplanted into 100 ml plastic pots containing steam sterilized sand. One hundred female *L. apulus*, from a population reared on celery in the glasshouse, were added in a small volume of water to the root zone of the plants when transplanted. The pots were then placed in a controlled-temperature chamber at $22 \pm 1^\circ$ C. Seven and 15 days after inoculation the damaged root apices were excised, fixed for 2 h in 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2, washed overnight in buffer and postfixed for 2 h in 1% osmium tetroxide, at 4° C. Alternatively, the apices were fixed for 2 h in 2% potassium permanganate in phosphate buffer at pH 7. Both types of fixed roots were dehydrated in a graded ethanol-propylene oxide series and embedded in Araldite.

Sections 2 μ m thick were stained in an aqueous solution of toluidine blue for light microscopy. Ultrathin sections were stained

1 h in uranyl acetate and post-stained 10' in lead citrate (Reynolds, 1963) and examined at 60 kV in a JEM 100B electron microscope.

RESULTS

The feeding site of *L. apulus* on celery roots was limited to the apical meristematic area. The odontostyle was inserted perpendicular to the root axis and usually penetrated six to seven cell layers. Cells in the vicinity of the feeding site were swollen and the cytoplasm became granular and stained intensely with toluidine blue. The cells around the feeding site became prematurely hypertrophic with the nuclei appressed to the cell walls (Fig. 1, a and b). In the area of the root, opposite the site of odontostyle insertion, cell division was active but disordered, resulting in disorganization of the tissues. This abnormal tissue proliferation resulted in the formation of a gall. Some slight disorganization of the root tissue also occurred for a distance of a few millimetres immediately above the gall.

The hypertrophic cells formed near the point of odontostyle insertion ceased division; the cells had little cytoplasm and the walls became convoluted. On the opposite side of the root the cell walls appeared normal but the cytoplasm was dense with many organelles, abundant vacuoles and some were binucleate (Fig. 1, c).

The procambium may be modified in two ways. When the penetration of the odontostyle is limited to the cortical meristem, as observed in cross section, the procambial cells were polygonal and small near the site of insertion and larger on the opposite side; later, when cellular differentiation occurred, as a result of nematode feeding, the pericycle cells underwent a periclynal division and later became hypertrophic, causing compression of the endodermis (Fig. 1 d).

When the odontostyle of the nematode reached the procambial cells, these first became hypertrophic, then a process of lysis of the cell wall started which led to the formation of a syncytia like structure. Some of the cell walls were intact, but many were disintegrated and associated with other cultural material that has been lysed. This syncytium was multinucleate; it appeared to progressively absorb adjacent hypertrophic cells with vesiculous nuclei (Fig. 2, a, b, c).

A similar process has also been observed in the cortical meristem, where whole layers of cells, with dotted walls were present, from

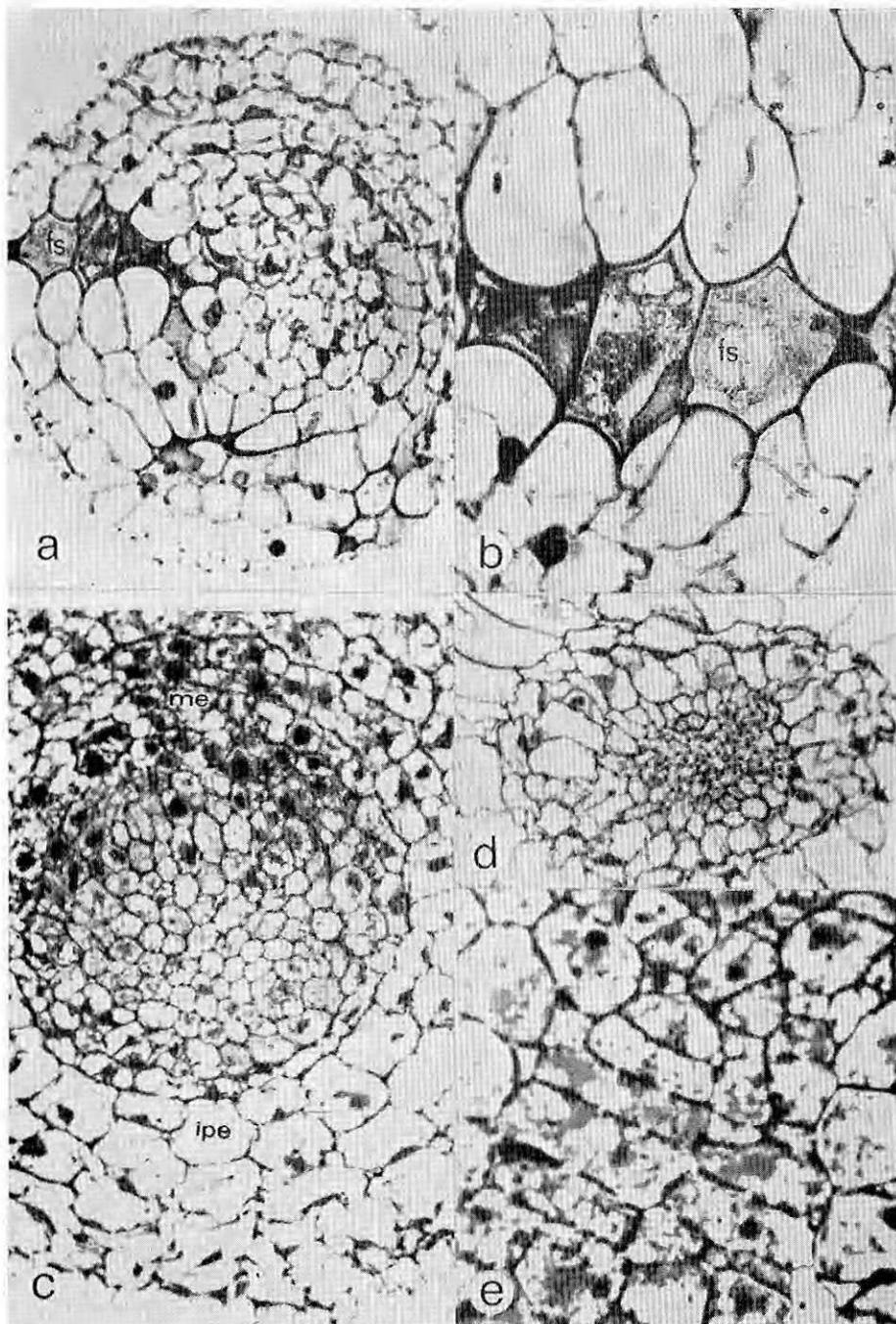


Fig. 1 - a: cross section of celery roots with cells damaged by the feeding action of *Longidorus apulus* (fs); b: feeding site (fs); c: feeding site of the nematode with hypertrophic cells (ipe) compared with normal meristematic cells (me); d: abnormal growth of the pericycle with consequent compression of the endodermis; e: cell wall breakdown.

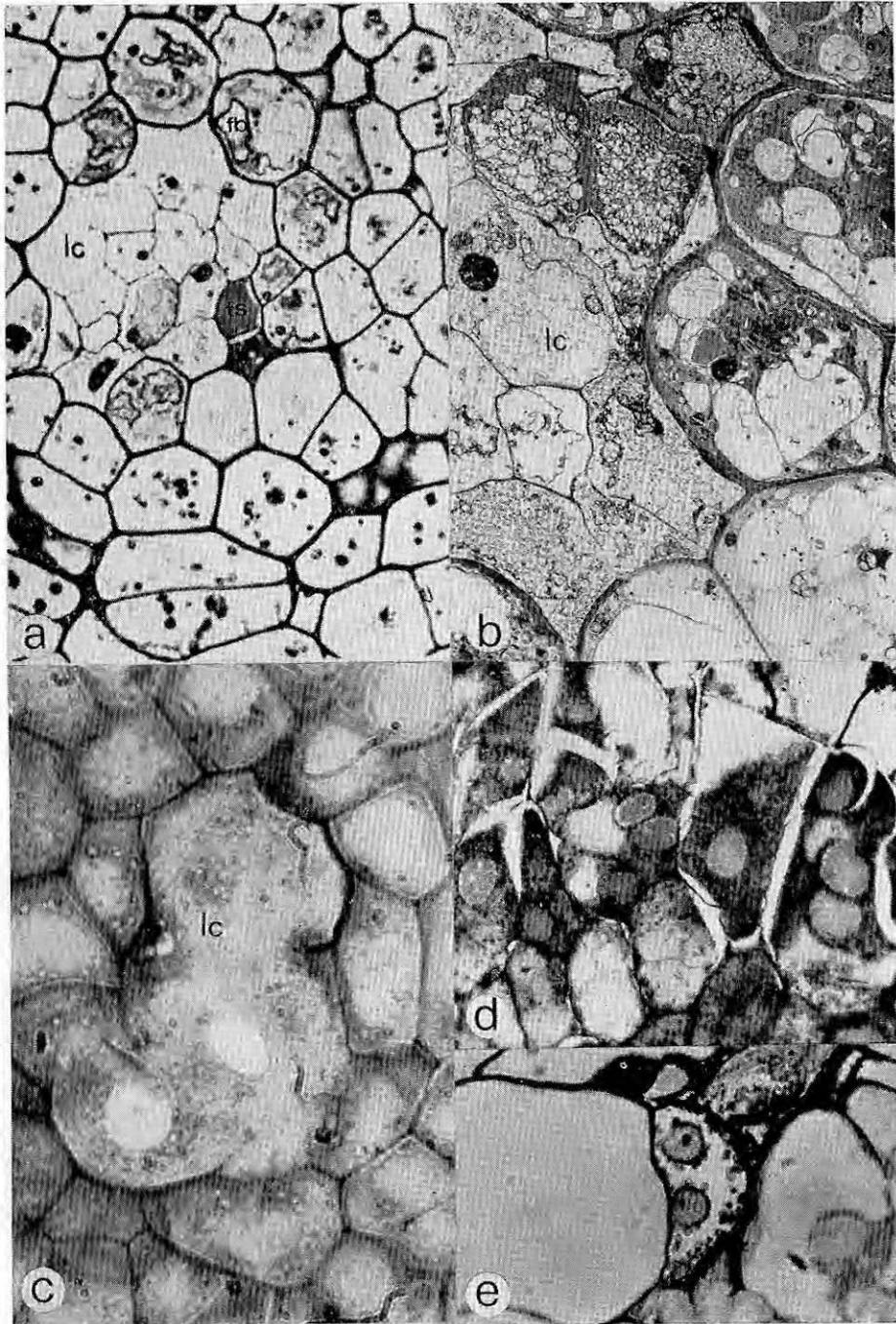


Fig. 2 - a, b (electron micrograph), and c: lysigen cavities (lc) showing partially dissolved cell walls and several nuclei; cells surrounding the cavities have thickened walls and dense cytoplasm with filling bodies (fb); d and e: hypertrophic multinucleate cells.

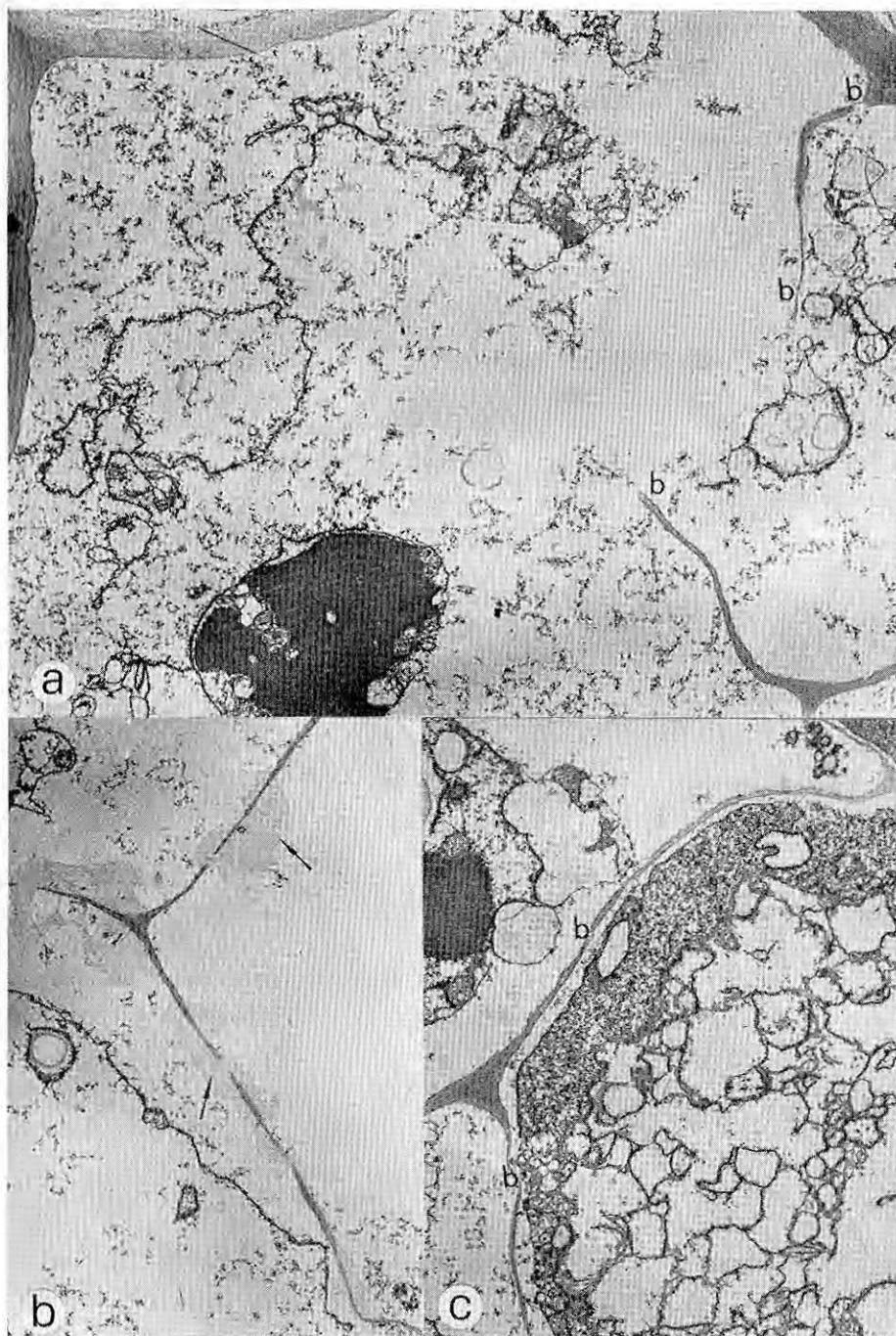


Fig. 3 - a, b, and c: electron micrographs showing breakdown (b) and dissolution of the cell walls (arrows) 6500 x.

the epidermis to the central cylinder (Fig. 1, e). The breakdown of the cell walls resulted in the formation of cavities along the cortical meristem (Fig. 2, d, e). Electron microscopy observations indicated that there were no preferential sites from which the dissolution of the cell wall started.

The cell walls delimiting the syncytium were always thickened with a rather irregular inner profile. Electron dense material was present near the sites of lysis and this presumably originated from the wall dissolution (Fig. 3, a, b, c).

In roots which were severely damaged after 15 days exposure to the nematode feeding hypertrophic and sometimes multinucleate cells of the cortical meristem were adjacent to the syncytia; 6 to 8 nuclei have been counted (Weischer and Wyss, 1976). The cytoplasm of these cells stained more intensively with toluidine blue than less damaged cells.

DISCUSSION

The structural modifications observed in root cells of celery attacked by the ectoparasitic *L. apulus* differ little from those described for endo- and semi-endoparasitic nematodes (Bird, 1972; Endo, 1975; Jones and Gunning, 1976; Rebois *et al.*, 1976). Although there is a structural similarity in response to nematode feeding, we consider that the physiological response is different. Feeding by endo- and semi endoparasitic nematodes causes an intense flux of metabolites through the modified cells, which can thus be considered as true transfer cells. The syncytia caused by the trophic action of *L. apulus*, however, appear to act more as lysigen cavities, which are an intermediate step to the complete destruction of the root apex.

ACKNOWLEDGMENTS

The cooperation of Mr. Emanuele Olivieri who has worked on this matter preparing his thesis is gratefully appreciated.

S U M M A R Y

Studies on the morphological and ultrastructural modifications caused by *Longidorus apulus* Lamberti et Bleve-Zacheo, in celery (*Apium graveolens* L.) root cells indicate that the nematode feeds upon the meristematic cells by inserting its odontostyle perpendicularly to the root axis. In response to feeding the root apex develops asymmetrically because of an increase of cell division in the area opposite the insertion of the odontostyle; on the side where the odontostyle penetrates cell walls dissolve and syncytia are formed.

R I A S S U N T O

Alterazioni cellulari indotte da Longidorus apulus in galle di radici di Sedano.

Studi sulle alterazioni cellulari in galle, indotte da *Longidorus apulus* Lamberti et Bleve-Zacheo in radici di Sedano (*Apium graveolens* L.), indicano che il nematode si nutre su cellule meristematiche introducendo l'odontostile perpendicolarmente all'asse della radice. In risposta, l'apice radicale si sviluppa asimmetricamente poiché, nell'area opposta a quella d'inserzione dell'odontostile, si ha una divisione cellulare. Nel punto di penetrazione dell'odontostile le pareti cellulari si dissolvono e si formano delle cellule sinciziali.

L I T E R A T U R E C I T E D

- BIRD A. F., 1972 - Cell wall breakdown during the formation of syncytia induced in plants by root-knot nematodes. *Int. J. Parasit.*, 2: 431-432.
- BLEVE-ZACHEO T., ZACHEO G. e LAMBERTI F., 1977 - Reazioni istologiche ed istochimiche indotte da *Longidorus apulus* in radici di Sedano e Cicoria. *Nematol. medit.*, 5: 85-92.
- ENDO B. Y., 1975 - Pathogenesis of nematode-infected plants. *An. Rev. Phytopath.*, 13: 213-238.
- JONES M. G. K. and GUNNING B. E. S., 1976 - Transfer cells and nematode induced giant cells in *Helianthemum*. *Protoplasma*, 87: 275-279.
- REBOIS R. V., MADDEN Ph. A. and ELDRIDGE B. J., 1975 - Some ultrastructure changes induced in resistant and susceptible soybean roots following infection by *Rotylenchulus reniformis*. *J. Nematol.*, 7: 122-139.
- REYNOLDS E. S., 1963 - The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.*, 17: 208-212.
- WEISCHER B. and WYSS U., 1976 - Feeding behaviour of *Xiphinema* on grape. *Nematologica*, 22: 319-325.

Accepted for publication on 18 October 1977.