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Istituto di Nematologia Agraria, C.N.R. - 70126 Bari, Italy

GALL INDUCTION ON TOMATO ROOT TIPS BY *PARALONGIDORUS BUCHAE*: ULTRASTRUCTURAL ASPECTS

by
T. BLEVE-ZACHEO, G. ZACHEO, F. LAMBERTI and M. CHINAPPEN (1)

The general pattern of development of galls induced on host root-tips by *Longidorus* species has been described by several workers (Sharma, 1965; Cohn, 1970; Cohn and Orion, 1970; Radewald *et al.*, 1969; Bleve-Zacheo *et at.*, 1977 a; Bleve-Zacheo *et al.*, 1984). Apart from some differences reported by these authors, mainly concerning the tissues involved in gall formation, the pattern is essentially similar for all the species.

Necrotic spots, presumably where cells were directly injured by the stylet of the nematode, are often visible on terminal root-tips.

Nematode feeding induces a drastic disturbance of the meristematic tissues. Hyperplasia of the cortical parenchyma cells occurred on bur marigold root tips, attacked by *L. africanus* (Cohn and Orion, 1970). In contrast, not hyperplastic response was observed on celery root tips attacked by *L. elongatus*, but clusters of hypertrophied cells with enlarged nuclei and dense cytoplasm were present around the feeding sites, indicated by rows of necrotic cells (Wyss, 1981). *L. apulus* induced hyperplasia in the cortex and hypertrophy in the cambial cells of root-tip galls on chicory; on the contrary, only hypertrophied cells were observed on swelling roots of celery. Occurrence of syncytia is reported in celery root galls, resulting from the breakdown of cell walls (Bleve-Zacheo *et al.*, 1977 b). The syncytia, later called « lysigenous cavities », represented the feeding sites of

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the nematode (Bleve-Zacheo *et al.*, 1979). The cells, directly affected by the feeding of *L. apulus*, showed numerous protrusions of the cell walls, interpreted as the beginning of the differentiation of transfer cells, similar to that induced by the feeding of endoparasitic nematodes (Bleve-Zacheo *et al.*, 1982). No ingrowths were observed on cell walls of the root tissue of chenopodium attacked by *L. apulus*.

Cells, fed upon by the nematode, became necrotic and several enlarged multinucleated cells were present in neighbouring tissue (Bleve-Zacheo *et al.*, 1984). Hypertrophy of the meristematic tissue occurred in ryegrass root tips at the initial stage of infection by *L. elongatus*, followed by hyperplasia and secondary hypertrophy (Griffiths and Robertson, 1984).

There is much information on the feeding behaviour of *Xiphinema* species, which invariably causes swelling of root tips, gradually transformed into terminal galls (Fisher and Raski, 1967; Cohn, 1970, 1975). Histopathological investigation of root tip galls induced by *X. index* on grapevine, revealed the presence of modified multinucleated cells beneath the necrotic cells, injured by the nematode (Weischer and Wyss, 1976). Multinucleate cells were also observed in ultrathin sections of fig root tips, after *X. index* attack (Wyss *et al.*, 1980) and this tissue reaction was observed to start 12h after feeding was initiated (Bleve-Zacheo and Zacheo, 1983).

The genus *Paralongidorus* includes those species having intermediate characters between *Longidorus* and *Xiphinema* and there is no information about *Paralongidorus* feeding behaviour and host response. This paper reports the morphological development of galls and ultrastructural changes that take place in tomato roots in response to the feeding of *Paralongidorus buchae* Lamberti, Roca *et* Chinappen (Lamberti *et al.*, 1985).

Materials and Methods

Two seedlings of tomato cultivar Roma were transplanted when 2-3 cm tall into clay pots, 5 cm diameter, containing 10 ml sterilized sand. Each pot was inoculated with 5 specimens of *P. buchae* from a population collected from the rhizosphere of declining chilli in Mauritius (East Africa). The pots were maintained in a growth chamber at 25°C. Three days after inoculation the plants were removed and swollen root tips were excised.

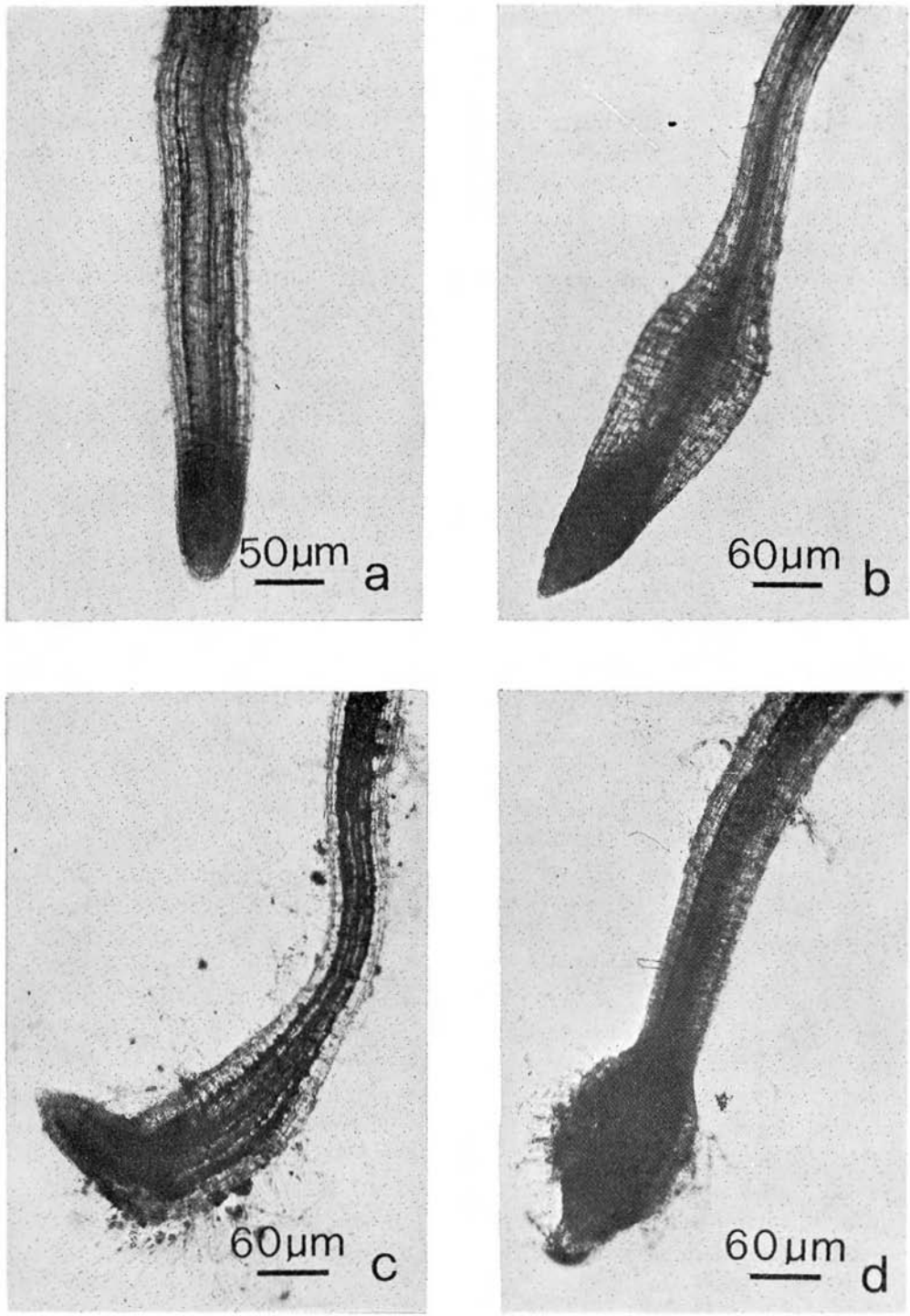


Fig. 1 - a, Healthy root tip of tomato « Roma VF »; b, Swollen root tip of tomato, 24h after *P. buchae* inoculation; c, Swollen root tip, slightly curved, with intense growth of root hairs; d, Root tip gall, five days after nematode inoculation.

The root tips were fixed in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2 for 4 h, rinsed in the same buffer and post-fixed in 2% osmium tetroxide for 4 h at 4°C, then stained in 0.5% uranyl acetate, dehydrated in an ascending series to absolute ethanol and embedded in Spurr's medium. Sections, 2 μm thick, were stained with toluidine blue, mounted in xylene and observed under the light microscope.

Ultrathin sections were cut with a LKB ultratome III, stained with uranyl acetate and lead citrate and examined under a Philips 400 T transmission electron microscope.

Results

Paralongidorus buchae showed a distinct preference for the undifferentiated root tissue of tomato seedlings and induced characteristic terminal galls. Root tip (the healthy one is in Fig. 1 a) was normally attacked by the nematode between the meristematic and the elongation region and slowly swelled within 48 h after nematode feeding started.

A dark spot was evident in the cells of the procambium where the nematode had inserted its stylet and had fed (Fig. 1 b); roots examined 4 days after nematode inoculation showed the galls typical of longidorid feeding, with some tips transformed in typical galls and sometimes they were slightly curved (Fig. 1 c); usually an intense growth of root hairs was observed on the galls (Figs. 1 c, d).

The maximum size of the galls was reached five days after inoculation and after ten days all the gall tissue became necrotic.

A general view of a parasitized root tip, 72h after initiation of nematode feeding, is represented in Fig. 2. Longitudinal sections through the whole root tip showed the possible feeding site, revealed by an area of collapsed tissue which formed an empty space (lysigenous cavity). This area, where the nematode presumably had fed, is delimited between apparently intact meristematic tissue, and hypertrophied cells of the elongation region (Fig. 2). Longitudinal sections showed an area of collapsed tissue (Fig. 3), caused by the necrosis of the cells, probably directly penetrated by the nematode stylet; the plasmalemma was disrupted throughout the cell and separated from the cell wall and the cytoplasm became an amorphous dark material.

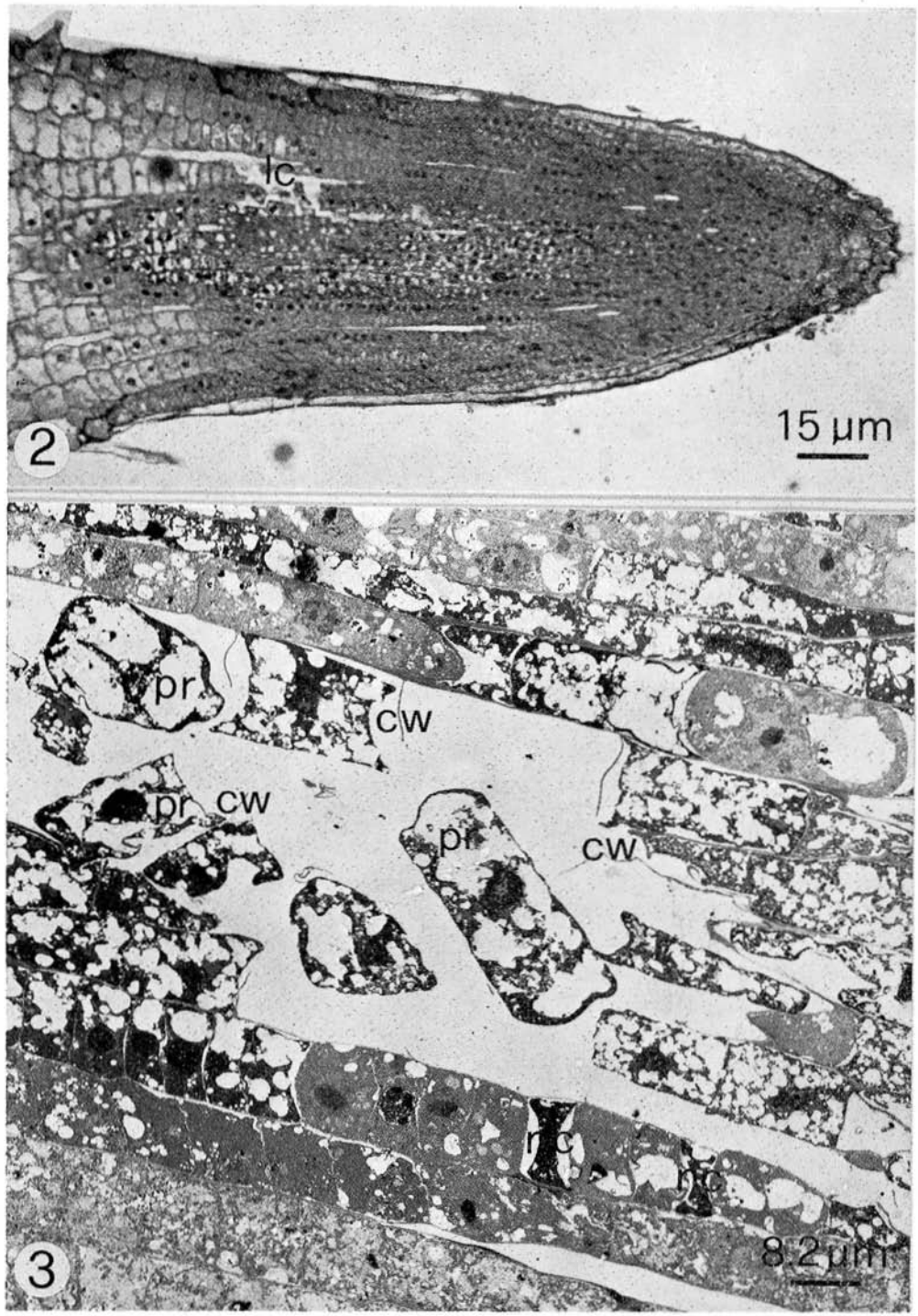


Fig. 2 - Longitudinal section through a swollen root tip of tomato, fed on by *P. buchae*. A lysigenous cavity (lc) is present between the meristematic and the elongation region; cells in the latter show hypertrophy.

Fig. 3 - Longitudinal section through the feeding site of *P. buchae*. Necrotic cells (nc), probably penetrated by the odontostyle, are indicative of a hypersensitive reaction and surround a mass of cells, with degraded cytoplasm; protoplasts (pr) partially or completely isolated in the lysigenous cavity, show intact plasmalemma but digested cell walls (cw).

A very rapid solubilization of the cell wall was noticed in the surrounding cells, not directly penetrated by the stylet and digestion of their content, subsequent to injection of saliva, proceeded more or less synchronously in all of them. An uncontrolled cell mass was involved in this process, stimulating the isolation of the protoplasts free in the lysigenous cavity; in cells partially or completely isolated the plasmalemma remained intact and contained no organelles, apart from the degraded nucleus. The tissue immediately adjacent to the lysigenous cavity contained cells similarly affected but less disorganized, with cytological features indicating unusual metabolic activity as evident from the plasmolysis of the cell walls and the densely packed cytoplasm (Fig. 3).

In the meristematic region further away from the feeding site, there were two layers of cells, located between the cortical and the procambial tissue, clearly suffering for the adjacent lytic process. Cytological examination of these cells revealed deeply stained cytoplasm, as a consequence of excessive metabolic activity. Very large intercellular spaces, lined by dark material which indicated the dissolution of the cell wall along the middle lamella, separated affected cells from the neighbouring cortical cells. The cortical tissue appeared to be normal apart from plasmolysis of a group of cells in the external layer. Procambial cells displayed the typical structure of meristematic cells, with cytoplasm rich in organelles such as mitochondria and plastids but not completely differentiated (Fig. 4).

The most striking and consistent changes occurred in the nuclei of the parenchyma cells, close to the lysigenous cavity. These included synchronous mitosis, hypertrophy of the nucleolus and appearance of fibrillar rings. The mitotic synchrony within parenchyma cells appeared to be a response to a message triggered by the nematode feeding. In this particular instance rows of nuclei were all at the same stage of mitosis (Fig. 6); this was not observed in comparable cells of healthy root tips (Fig. 5). The nuclear membrane appeared partially fragmented, swollen and covered with ribosomes (Fig. 7).

Hypertrophic nucleoli occupied a large part of the nucleoplasm and their granular and fibrillar regions were irregular in shape and more electron dense than healthy nucleoli. The nucleoplasm contained fibrillar bodies, which varied in size and number and were composed of electron-dense and extremely compact fine fibrils. The matrices of fibrillar bodies were more electron-dense than other nuclear components and appeared either as ring shaped or solid spheres (Fig. 7).

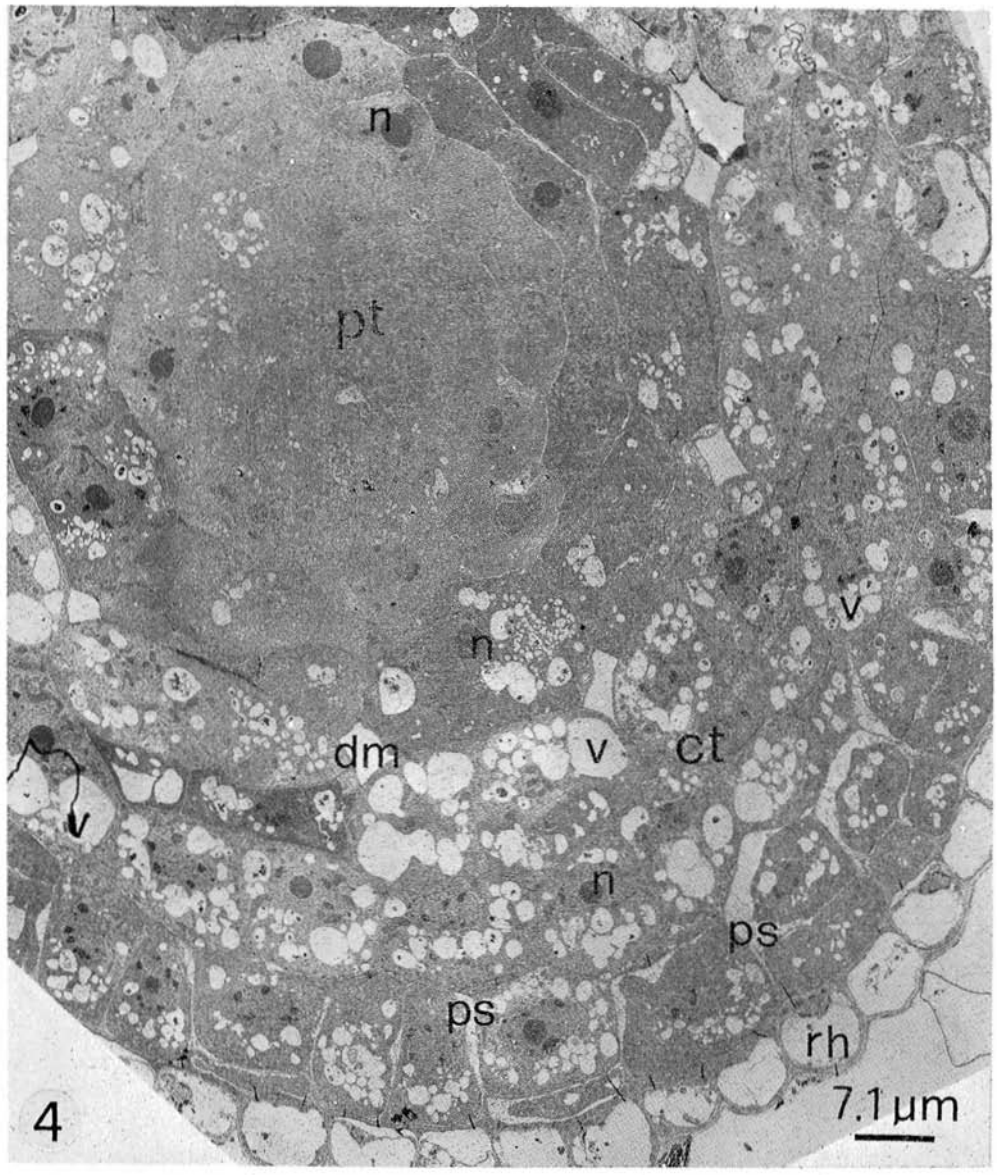
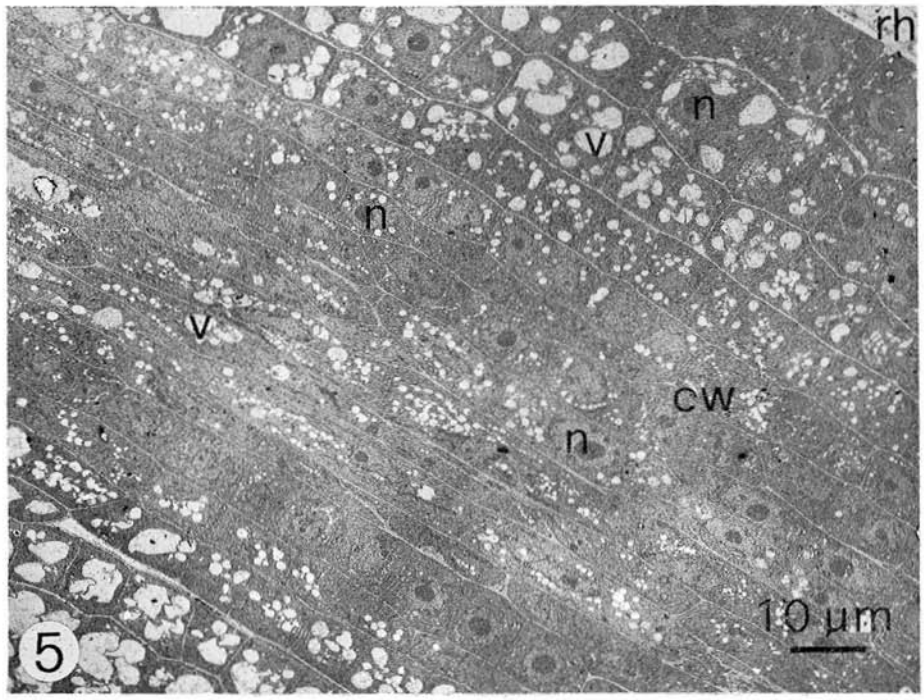


Fig. 4 - Cross section through the meristematic region of a parasitized root tip. Two layers of cells between the procambial (pt) and cortical tissue (ct) show a dense cytoplasm, indicating unusual metabolic activity. They are separated from the cortical tissue by intercellular spaces lined by dark material (dm) resulting from the dissolution of the middle lamella; the procambial tissue is normal; cells of the cortical tissue show many vacuoles (v) which appear to have fused, cytoplasmic organelles and false plasmolysis (ps); rhizodermis (rh); nucleus (n).



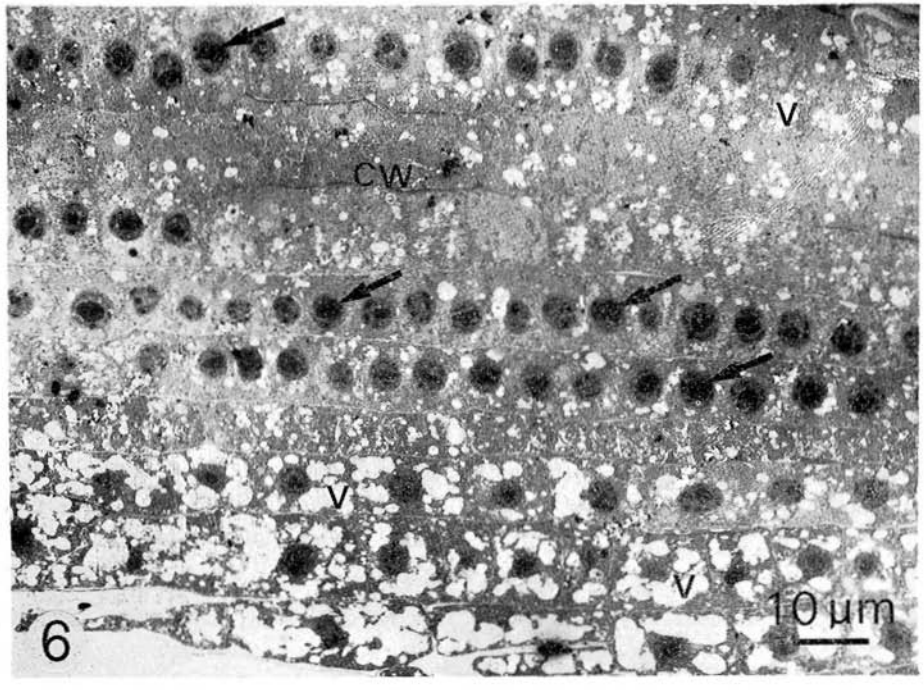


Fig. 5 - Longitudinal section through healthy root tip.

Fig. 6 - Longitudinal section of an infested root tip. The tissue opposite to the lysigenous cavity shows rows of cells with nuclei in synchronous mitosis and hypertrophic nucleoli (arrow).

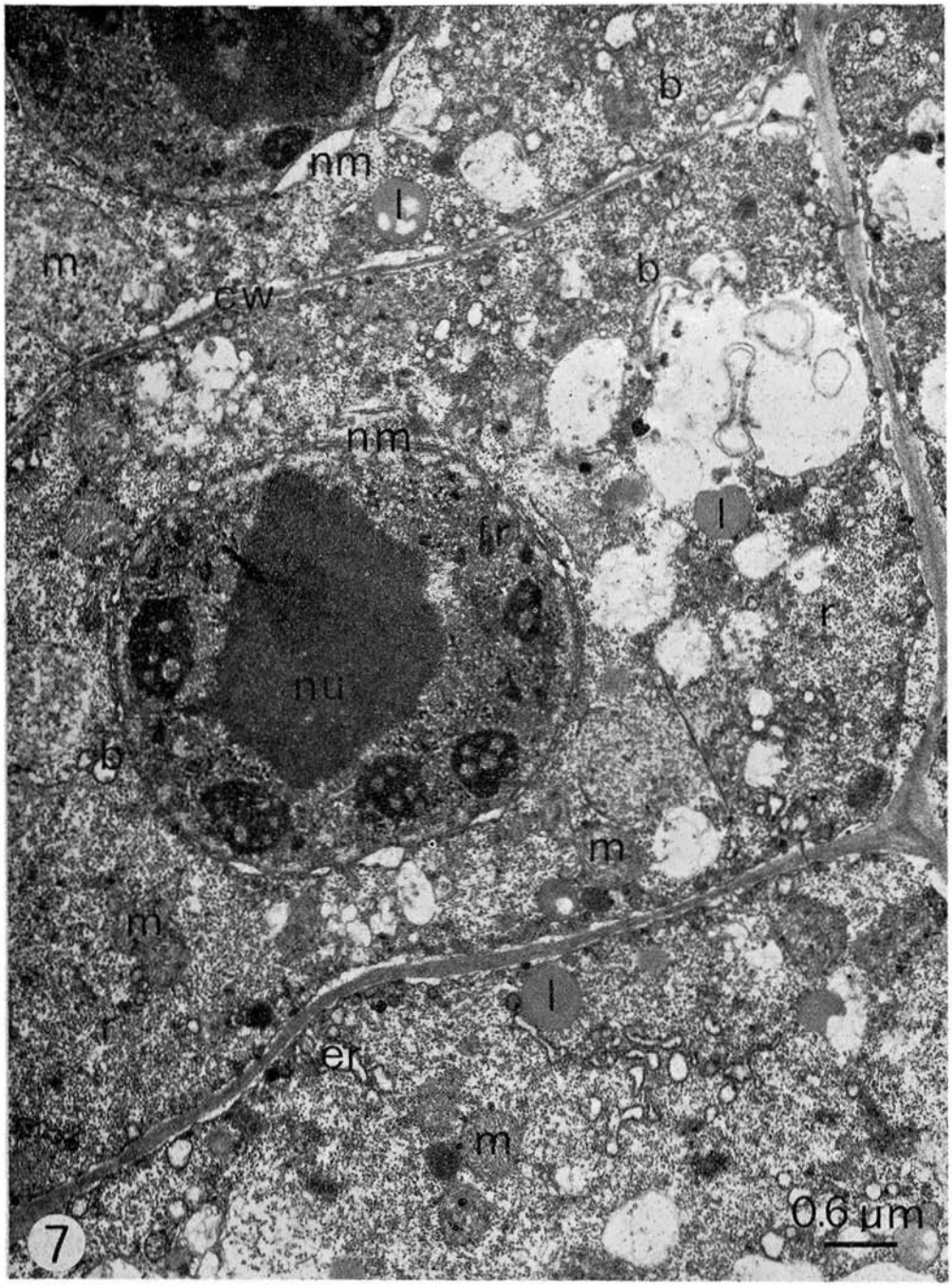


Fig. 7 - An enlargement of a cell in Fig. 6 shows that the disorganization involved both nucleus and cytoplasm. Nuclear membrane (nm) appears swollen and chromatin material is transformed into fibrillar rings (fr) different in size. The matrices of smaller rings are homogenous, whereas the larger ones contain holes. The granular and fibrillar regions of the hypertrophic nucleolus (nu) are indistinguishable and very electron dense. A fibrillar ring is present below the nucleolus (arrow). The cytoplasm is disorganized; conspicuous ribosomes (r) are present in the cytoplasm, attached to the inflated endoplasmic reticulum (er), to spherical bodies (b) and to nuclear membrane; mitochondrion (m); lipid (l).

The cytoplasm was rather disorganized; ribosomes were the most recognizable cytoplasmic organelles in these cells and occurred throughout the cytoplasm, attached to the nuclear membrane, to membrane-bound spherical bodies and to the greatly inflated endoplasmic reticulum (Fig. 7).

Anomalous thickenings and protrusions of cell walls were found in all the meristematic tissue, close to the initial cells of the root tip, parasitized by *P. buchae*. Protrusions sometimes penetrated deeply into the invaginated cytoplasm. Some of them appeared to be isolated pieces of cell wall material surrounded by plasmalemma and cytoplasm. A section in a longitudinal plane through the protruded cell wall showed the cell wall fragment as an elongated bar while in cross section it appeared as a circular body (Fig. 8).

Discussion

Our observations indicate that the ultrastructural changes induced in cells as a result of the feeding behaviour of *P. buchae* are similar to those reported for the related genera *Longidorus* and *Xiphinema* (Cohn, 1975; Wyss, 1981). Root cells, penetrated by the nematode stylet, became necrotic either as a result of the thrusts of the stylet or in response to biochemical stimuli or a combination of both. Each cell, at the site of stylet penetration, showed a traumotactic aggregation of cytoplasm, caused by the withdrawal of the sap from the cells through the odontostyle of the nematode.

Robertson *et al.* (1984) reported presence of holes in the cell wall of cells directly injured by *L. elongatus* and they suggested that these holes resulted from the lytic action of the nematode saliva.

It is well known that higher plants can synthesize polysaccharidases which are involved either in wall loosening (Fan and Machlachlan, 1967) or removal of wall material during differentiation (Sheseldrake and Moir, 1970). Similarly, wall degradation has also been reported in noninfected somatic plant tissues other than developing vascular elements (Bowes, 1972).

Longidorus species could produce cell wall degrading enzymes, capable of attacking each of the major polymeric components of plant cell walls. The contact between extracellular cell wall degrading enzymes of *P. buchae* and cell walls of tomato root could induce the lysigenous cavity, as a result of a complete cell wall degradation.

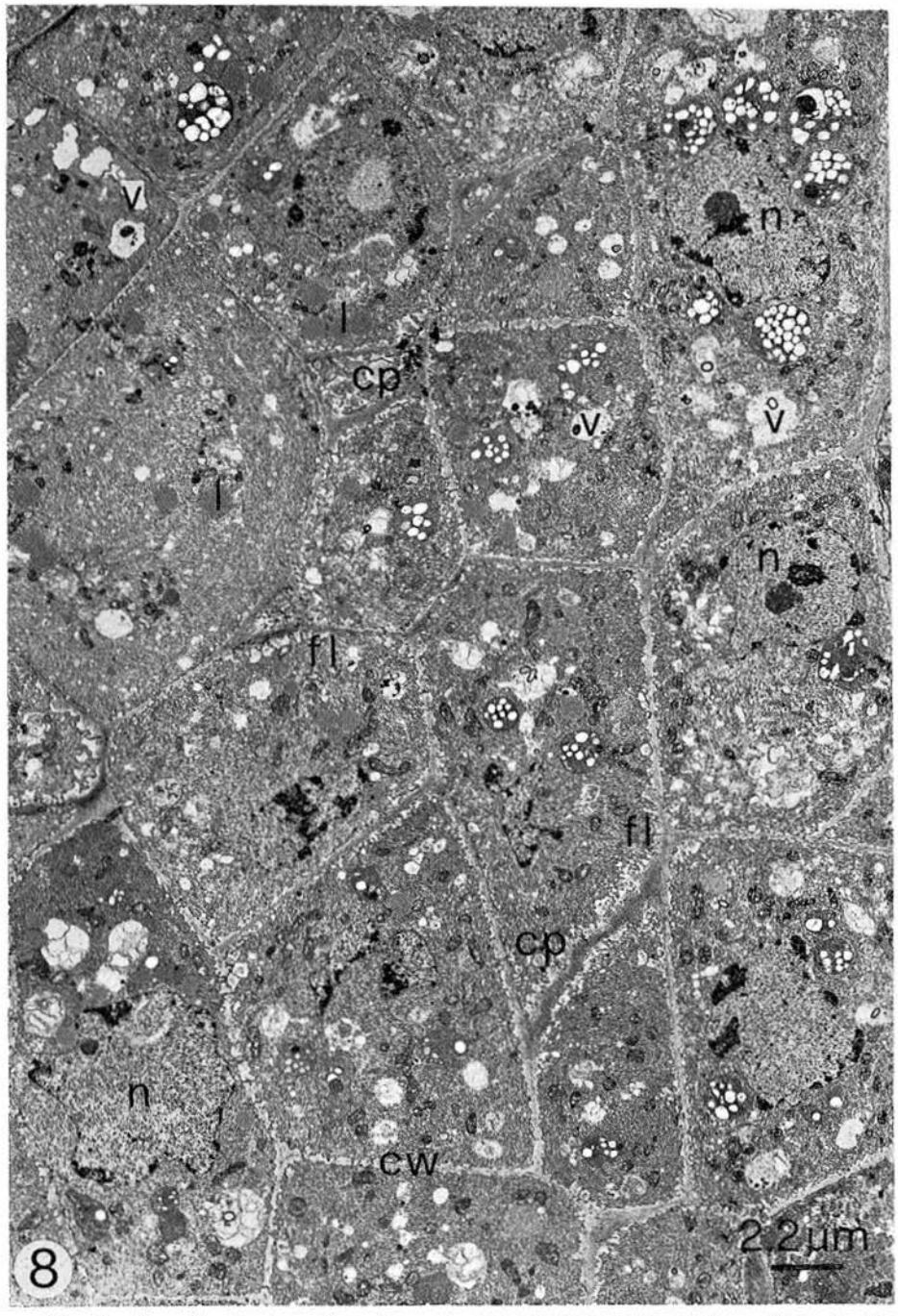


Fig. 8 - Longitudinal section through the meristematic region, close to the initial cells, in an infested root tip. The cells show an unusual intense cell wall growth, in the form of cell wall thickenings and finger-like (fl) or circular protrusions (cp), depending on the plane of the section.

The extensive digestion of the cell wall in the lysigenous cavity contrasted with the occurrence of cell wall ingrowths in the meristematic tissue, adjacent to the lysed area. The phenomenon was extensive throughout the meristematic area, whose cells were healthy in appearance. It has been demonstrated that transfer cells are common in all plant organs but not in roots, and are regarded as analogous of the microvilli that occur in many animal cells, which are specialized absorbing processes. These cells are similar to the « transfer cells » occurring adjacent to vascular elements in roots infected by endoparasitic nematodes (Jones, 1981). Howewer, transfer cells in the meristematic tissue were never observed.

Where transfer cell development has been observed, it invariably appears that formation of wall ingrowth coincides with the onset of intensive solute transport. Cause and effect cannot be distinguished in this relationship, but a plausible explanation is that the growth of the wall-membrane apparatus is a response to the presence of solutes (Pate and Gunning, 1972).

These results suggest that the enzymes injected by the nematode produce changes in osmotic potentials, as a result of the gradient that is established, wall ingrowths form at the furthest extremities of the root tip.

An unusual pattern of nuclei was noticed in plants attacked by *P. buchae*. In the tomato roots, infested by *P. buchae*, rows of more than 20 single cells, not directly fed upon, showed synchronous mitosis. The manner in which nematode achieves this control is unknown; probably the same processes, described for endoparasitic nematodes are involved (Bird, 1974). It seems that in the host-parasite relationship the nematode injects a histone-like basic protein, which controls DNA synthesis and induction of synchronous mitosis (Bird, 1972). At this stage ultrastructural abnormalities were detected in the nucleoplasm; hypertrophied nucleoli more granulated than those of healthy cells and fibrillar bodies scattered in the nucleoplasm seem to be a consequence of the disease.

The nuclear changes described in this paper were closely identical to those induced by ssDNA virus infection (Kim and Fulton, 1984), where cytochemical studies revealed that the nucleoli were composed mainly of ribonucleoproteins while the fibrillar rings were deoxyribonucleoproteins (Kim *et al.*, 1978).

SUMMARY

A population of *Paralongidorus buchae*, Lamberti, Roca *et* Chinappen from Mauritius, fed exclusively on the root tips of tomato seedlings. As a response tips started to swell and became transformed into terminal galls three days after nematode inoculation. Electron microscopy studies of swollen root-tips revealed ultrastructural changes resembling those induced by *Longidorus* species. These included necrotic cells, where nematodes inserted their odontostyle and occurrence of a lysigenous cavity, as consequence of cell wall dissolution in the feeding site. In addition a cytopathic effect on the nuclei of the neighbouring cells was observed, such as synchronous mitosis, hypertrophy of nucleolus and occurrence of fibrillar bodies in the nucleoplasm. Unusual localization of cell wall thickenings was found in the meristematic tissue of the infested root tip, very close to the initial cells and also at a distance from the feeding site.

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