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## DEFENCE RESPONSE OF RICE AND TOMATO TO *XIPHIDORUS MINOR* AND *XIPHINEMA VULGARE* (NEMATODA, DORYLAIMIDA)

by

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**Summary.** Cellular modifications induced by *Xiphidorus minor* and *Xiphinema vulgare* on rice and tomato differed extensively. Root tips of both plants when fed upon were transformed into galls. However, ultrastructural observations showed a gradient of resistant response with callose-like production in rice and an osmotic imbalance in protein and starch metabolism, together with phenolic deposits and lytic processes, in tomato fed upon by *X. minor*, whereas in the presence of *X. vulgare* injured tomato cells were partially digested and rice cells gave a strong hypersensitive reaction.

During a nematode survey carried out in Venezuela, *Xiphinema vulgare* Tarjan was found to be widespread in the rice growing region of Calabozo, Guárico State, although usually in low population densities in the rice fields. *Xiphidorus minor* Rashid, Coomans *et* Sharma occurred in high populations in the rhizosphere of various plants on the campus of the Universidad Central de Venezuela at Maracay, Aragua State.

Dagger nematodes, such as *Xiphinema ifacolum*, can be a major cause of damage to rice crops in the tropics (Lamberti *et al.*, 1987, 1991). *Xiphidorus* is a widespread genus in South America; however, no information is available on its pathological action. The general and evident reaction of the host to *Xiphinema* feeding is the production of a terminal root swelling. However, within the root, cellular responses differ among species in relation to the host reaction. In a good host *Xiphinema* induces coenocytes where cells with partially formed cell walls show dense hyperactive cytoplasm together with amoeboid nuclei. In poor hosts, nutritional re-

quirements for the nematode are provided by rows of cells whose walls are perforated by and cell content removed through the long odontostyle of the parasite (Leone *et al.* 1997).

Occasionally a difference in feeding behaviour may be observed between two distinct species of *Xiphinema* on the same host confirming Dropkin's (1969) view that nematode parasitism can be categorized as either destructive or adaptive, with regard to cell modifications.

The aim of this work was to investigate the feeding behaviour of *X. vulgare* and *X. minor* on tomato and rice.

### Materials and methods

Seed of rice (*Oryza sativa* L., cv. Llanos 5) and tomato (*Lycopersicon esculentum* Mill., cv. Roma), were surface-sterilized by immersion for 2 min in 95% ethanol, 5 min in 1.07% sodium hypochlorite, followed by rinsing three times in sterile water. The seeds were then transferred to

9 cm plastic Petri dishes containing 7 ml nutrient medium comprising 0.1% Gamborg's B5 vitamin solution and 2% sucrose. Germination and growth of the seedlings were maintained at 22 °C and a 16 h light regime.

Ten day-old seedlings were inoculated with specimens of either nematode species. Females and juveniles of *X. minor* were obtained from the rhizosphere of natural vegetation at Maracay and those of *X. vulgare* from the rhizosphere of a coconut palm at Hato Terecay, on the road between San Fernando de Apure and Calabozo. Nematodes were sterilized for 30 min in a 0.05% hibitane solution and washed in sterile water. Batches containing 20 specimens of either species were transferred to rice or tomato seedlings, respectively, in an aqueous suspension.

For electron microscopy, swollen root tips were fixed in 2% paraformaldehyde-glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) for 2 h, rinsed in the same buffer and post-fixed in 2% osmium tetroxide in cacodylate buffer for 2 h at 4 °C. Samples were dehydrated through an ethanol series to absolute ethanol and then embedded in Spurr's medium. Ultrathin sections, cut with a Reichert (Leica) Ultracut E, mounted on formvar-coated grids and stained with a saturated ethanolic solution of uranyl acetate followed by lead citrate, were observed with a Philips 400T transmission electron microscope.

## Results

The histopathological studies under controlled conditions indicated that *X. minor* and *X. vulgare* fed exclusively on the tips of rice and tomato roots. As a response to feeding by the nematodes the tips became swollen and ceased their growth (Fig. 1). However, when parasitized swollen root-tips were examined two days after the first attack by a single nematode or several individuals, distinct cellular modifications were observed between rice and tomato roots and between the two species of parasites.

## Ultrastructural changes associated with *Xiphidius minor* feeding on rice and tomato root cells.

Sections through a fed upon root tip of rice showed rows of necrotic cells. These cells, presumably representing the initial feeding site, and directly injured by mechanical perforation of the nematode odontostyle, were almost empty. Their cytoplasm consisted of a dark remaining of cellular organelles detached from the cell walls, which were thin and stretched (Fig. 2a). As a reaction, neighbouring cells became hypertrophic (Fig. 2b). Although the cytoplasm was not greatly modified in these cells, an active process of dense and electron-transparent vesicle movement outside the plasma membrane occurred. As a consequence, the plasma membrane became very irregular, and callose-like material appeared to be deposited in between the plasma membrane-cell wall (Fig. 2c). Hypertrophied cisternae of smooth endoplasmic reticulum close to the plasma membrane and frequently associated with lightly-stained vesicles appeared also to be involved in secretory components (Fig. 2c). Apart from long profiles of rough endoplasmic reticulum with polysomes, indicating an active protein manufacture (Fig. 2c), extensive changes of proplastids were observed (Fig. 2b). Each structure was highly hypertrophied and contained numerous starch grains, together with large protein bodies (Fig. 2d).

Longitudinal sections through a parasitized root tip of tomato showed the possible feeding site, as indicated by an area of collapsed procambial tissue (Fig. 3a). There was a clear-cut demarcation between fed upon necrotizing and modified cells. The necrotic cells were axially located with respect to the insertion point of the nematode odontostyle, where completely destroyed cells were present. Cell contents moved towards that position, probably because of the removal of cytoplasmic sap during nematode ingestion (Fig. 3a). A very rapid digestion of walls and cytoplasm withdrawal of



Fig. 1 - A typical galled root system as a result of *Xiphidorus minor* and *Xiphinema vulgare* feeding action (*X. minor* on tomato).

an uncontrolled mass of cells, not directly penetrated by the odontostyle, was noticed. Degeneration of the remaining cytoplasmic contents, where only nuclei were recognizable, coincided with critical death of injured cells (Fig. 3b). In addition, there were significant changes in the cytological structure of meristematic cells close to and far from nematode feeding site. A process of plasmolysis with a detachment of the plasma membrane from the cell wall involved almost all the cell layers (Fig. 3a). A common response of these cells was the paucity of cytoplasm, where dictyosomes were very active in producing vesicles of varying size and electron density. Osmiophilic deposits, resembling phenolic material, accumulated between the invaginated plasma

membrane and the cell wall. Additional accumulation producing dense deposits with non-delineated margins occurred in vacuoles (Fig. 3c). In these cells, nuclei had regular profiles and a still unchanged structure. They were subjected to irreversible karyolysis (Fig. 3d) as the degeneration process spread.

#### *Ultrastructural changes associated with Xiphinema vulgare feeding on rice and tomato root cells.*

*Xiphinema vulgare* feeding on rice and tomato roots was more destructive than *Xiphidorus minor*. In rows of cells in the root tip of rice cell walls were breached indicating that the nematode had thrust its odontostyle deeply into the cells by rupturing their walls and in-



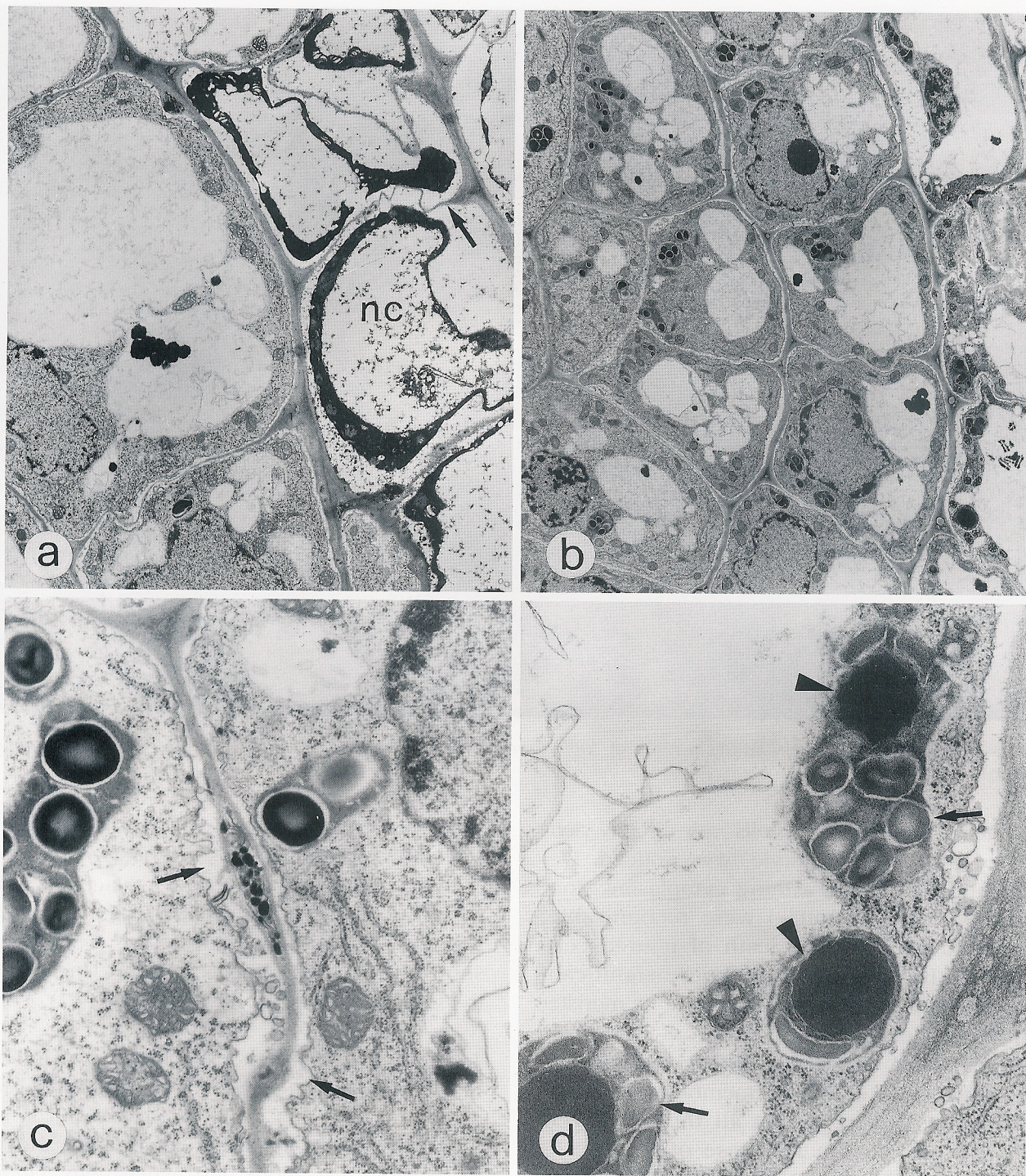


Fig. 2 - Micrographs of rice root cells parasitized by *X. minor*: a) a row of meristematic cells assumed to have been fed on by the nematode shows dark remains of cellular components (nc) and thin and stretched cell walls (arrow).x4600; b) layers of cells adjacent to the feeding site are hypertrophic and their cytoplasm contains anomalous organelles.x 3000; c) detail of plasma membrane showing vesicular and callose-like material (arrow) interposed between its irregular profile and the cell wall.x 19500; d) enlargement of proplastids, anomalous in shape because of starch (arrow) and protein (arrow head) deposits in their stromata. x 25400.



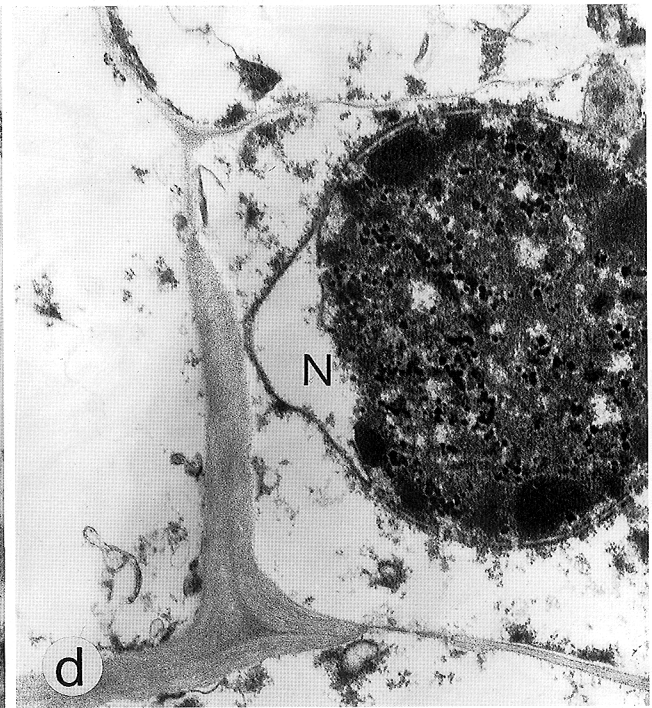
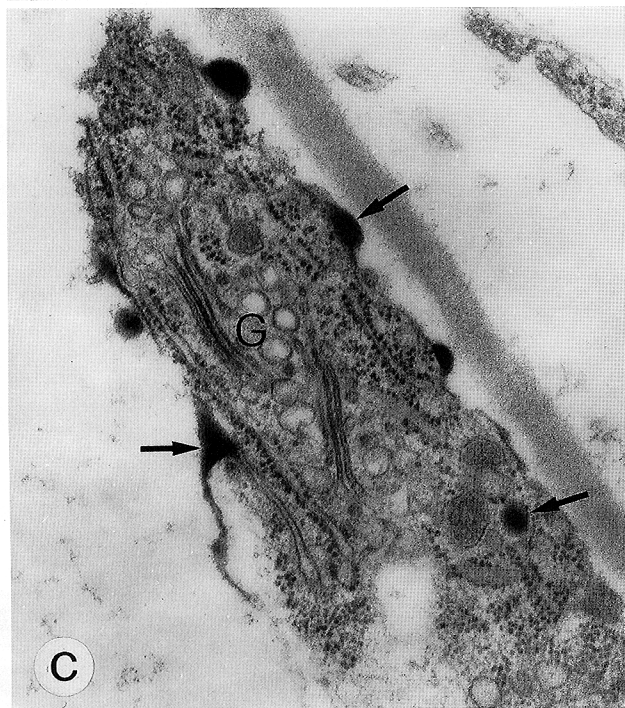
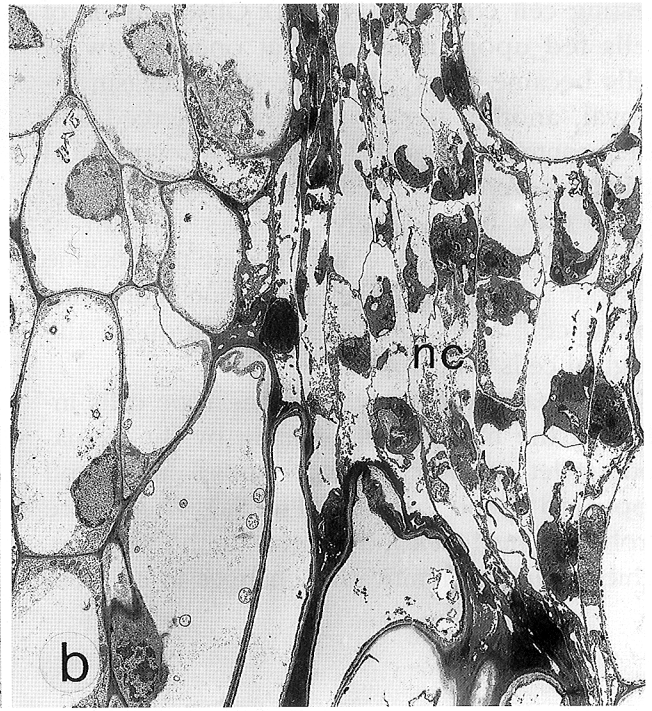


Fig. 3 - Micrographs of tomato root cells fed upon by *X. minor*: a) longitudinal section through a galled root tip where numerous necrotic cells (nc), representing the feeding site, are detectable in the procambial zone.x 950; b) detailed view of digested cells, axially located with respect to the insertion point of nematode odontostyle, where the lytic activity also involves cell walls.x 1950; c) Golgi-bodies (G) in cells not involved in the feeding action produce different kinds of vesicles that seem to be related to osmiophilic (phenolic) deposits (arrow).x 40500; d) irreversible karyolysis of nuclei (N) of cells fed upon by nematode. x 20400.



gesting cell contents (Fig. 4a). Observations of cells fed upon indicated that once a row of cells became empty, because of cytoplasm removal, another tier of them was pierced and their contents ingested (Fig. 4a). No cytological reactions were observed in cells fed upon because they immediately died. As a result of nematode feeding, the whole root tip became necrotic and only partially lysed nuclei and distorted cell walls marked the position of the original cells (Fig. 4b).

In tomato roots only a few necrotic cells indicated the mechanical piercing by the nematode stylet. These cells were almost empty and appeared to be crushed (Fig. 5a). They were probably the first food source of the parasite, which induced significative changes in adjacent

cells. They consisted of a progressive fusion of meristematic cells that formed a syncytium-like structure through the breakdown of cell walls (Fig. 5a). Wall breaching appeared to be a digestive process rather than a mechanical process (Fig. 5b). The multinucleate structure showed that the walls were opened in different areas of the cells. Deposition of electron-dense material at the level of median lamella appeared to be linked in such a way to the reaction (Fig. 5b). Golgi-derived vesicles and hyperactive portions of endoplasmic reticulum suggested that cell productive machinery tried to counteract the lytic message received from the parasite by the secondary wall apposition (Fig. 5c). Nuclei appeared to be not yet affected by both lytic and metabolic processes (Fig. 5c).

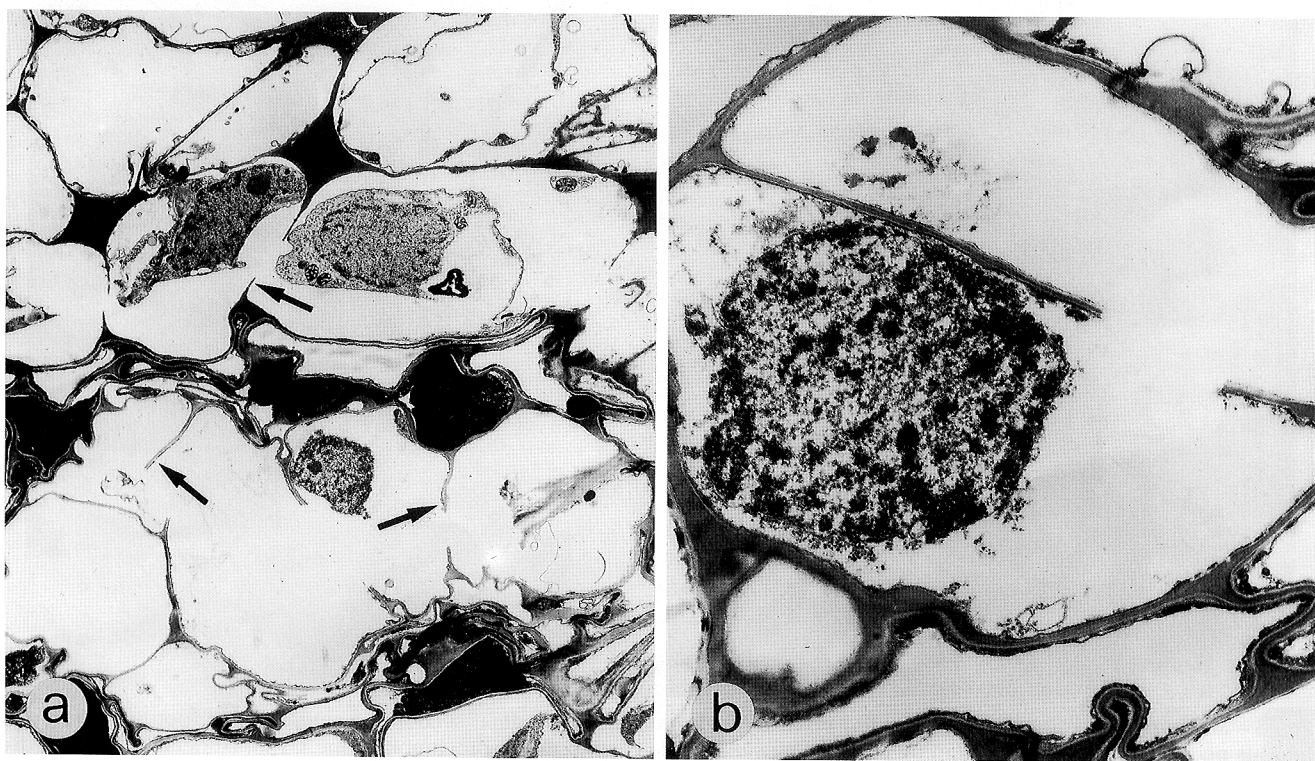


Fig. 4 - Cellular response of rice roots to *X. vulgare* trophic action: a) rice cells perforated (arrow) by nematode odontostyle; wall breakdown and empty cells indicate the destructive feeding behaviour of the parasite. x 5300; b) cells die immediately without showing any reaction. x 19000.



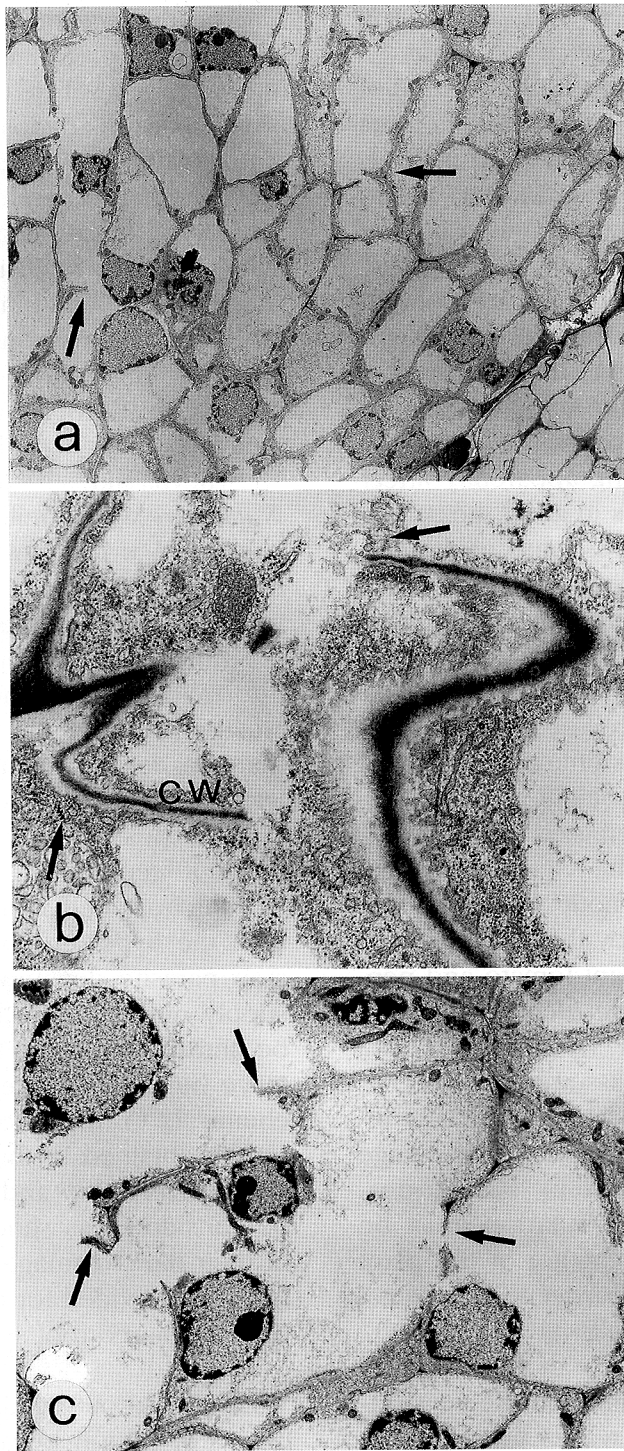


Fig. 5 - Cellular response of tomato roots to *X. vulgare* trophic action: a) tomato root tip fed upon by the parasite shows a row of perforated cells, with breached walls (arrow). x 1400; b) the process of wall breakdown (cw) seems to be more digestive than mechanical and a proliferative process of vesicles (arrow) as a defence response is detectable in the cytoplasm. x 17900; c) cell walls interrupted in different areas (arrow) of the injured cells are indicative of a process involving chemical breakdown. x 3000.



## Discussion

Cellular modifications induced by *Xiphidiorus minor* and *Xiphinema vulgare* in rice and tomato roots differ extensively. Indeed, defence mechanism in rice-*X. minor* relationship and tomato attacked by *X. vulgare* involved a delayed response which took place after the nematode had obtained sufficient food for its nourishment, before moving to a new feeding site. In contrast, the reaction of rice to the feeding of *X. vulgare* and tomato to *X. minor* could be summarised as a hypersensitive response.

In detail, *X. minor* feeding apparently affected some metabolic activities in all of the cells of the rice root tip. The early response of the cells to the presence of nematodes, expressed in vesicular and membranous structures associated with the plasma membrane and in the storage of starch and proteins in the plastids, indicated active responses of host cells to signals from feeding nematodes. There is little doubt that the formation of starch and crystalline structures is the consequence of an increased synthesis induced by nematode stimulus. Accumulation of proteins had an effect of osmotic imbalance due to lytic enzymes produced by the nematode has also been reported in resistant tomato and pepper invaded by *Meloidogyne incognita* (Bleve-Zacheo *et al.* 1982, 1998).

Equally, it is logical to assume that callose-like material occurring in uninjured cells is induced by an unknown biochemical message from cells directly attacked by nematodes. Therefore, callose-like accumulation must be considered a non-specific reaction of the plant to wounding rather than a barrier to counteract the action of the parasite (Bell, 1981).

Tomato root cells, at the site of *X. minor* stylet penetration, showed a traumatotactic aggregation, caused by the withdrawal of the sap from cells selected as feeding sites, dark deposits near the plasma membrane and in vacuoles of unaffected cells, and a progressive lysis of all root tissues.

The defence mechanisms of a plant can be classified as preformed or induced. Examples of the latter type of defence include the hypersensitive reaction, deposition of phenolic material, and synthesis of hydrolytic or other enzymes (Keen, 1992). Therefore, phenolic deposits together with the strong lytic reaction of the whole root tip indicated tomato-*X. minor* as an incompatible combination with related typical hypersensitive response. A similar tendency was detected in rice roots fed upon by *X. vulgare*. They exhibited rapid cell necrosis prior to changes of cytological structures.

Syncytia-like structures induced in tomato by *X. vulgare* were similar to those reported in rice roots fed upon by *Hemicycliophora typica* (Bleve-Zacheo *et al.*, 1987). In both cases they acted as nutrient sinks, but without maintaining the functional integrity of syncytia induced by endoparasitic cyst nematodes (Jones, 1981). Metabolic changes in tomato roots may indicate that the destructive action of the parasite was less drastic than in rice in order to maintain as long as possible the viability of the cells on which it feeds.

The host cell death classically associated with the hypersensitive reaction is believed to stop parasite development *per se* by failing to supply nutrients, but this is now considered to be untrue. Cell death itself may not cause resistance but may induce the synthesis of defence chemicals (Yamamoto, 1995). In any case, the results obtained in this study suggest that both *Xiphidiorus minor* and *Xiphinema vulgare* must be considered as serious pests of rice because of their direct damage to growing roots and the facilities provided for other pathogens to parasitize the injured root system.

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