Nematol. medit. (1987), *15*: 235-251.

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A COMPARISON OF HISTOLOGICAL CHANGES INDUCED BY *XIPHINEMA BASIRI* AND *X. IFACOLUM* IN THE ROOTS OF TOMATO

by
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Xiphinema and *Longidorus* species have long odontostyles which enable them to establish feeding sites well within the young roots. Those species that have been studied in any detail penetrate a column of cells near the apex of the roots, sometimes feeding on the cells progressively, until the tip of the odontostyle is located at the feeding site some 5-7 cells distant from the rhizodermis. The time that the nematode remains at the feeding site varies from a few minutes to several hours, this appearing to depend on the species, the point of insertion of the odontostyle into the root, and various environmental factors affecting the behaviour of the nematode (Cohn, 1970; Weischer and Wyss, 1976; Towle and Doncaster, 1978; Robertson *et al.*, 1984).

The general and externally evident reaction of the plant to feeding by *Xiphinema* or *Longidorus* nematodes is the production of a terminal root gall. However, within the root the cellular responses appear to be quite distinct between the two genera. *Xiphinema index* feeding on grapevine (Radewald and Raski, 1962; Weischer and Wyss, 1976; Cohn and Orion, 1970), or on fig (Rumpenhorst and Weischer, 1978; Wyss *et al.*, 1980) and *X. diversicaudatum* feeding on rose (Davis and Jenkins, 1960) or *Lolium perenne* (Griffiths *et al.*, 1982) induced a hypertrophic reaction of the cells in the vicinity of the odontostyle tip. The cells were metabolically active, as evident from the enlarged, lobed nuclei with hypertrophied nucleoli,

[1] C.E. Taylor was in receipt of a NATO / CNR Senior Research Fellowship. We thank Mr. Lerario for photographic assistance.

the increased cytoplasmic density, the proliferation of the rough endoplasmic reticulum and an increase in the number of mitochondria and plastids. In many respects the cellular modifications resembled those induced by endoparasitic and sedentary nematodes of the Heteroderidae and Nacobbidae (Wyss *et al.*, 1980; Wyss, 1981).

Longidorus apulus feeding on celery initially induced hypertrophy of the procambial cells, in which the numerous protrusions on the walls were interpreted as indicative of the beginning of differentiation of multinucleate transfer cells. However, the cells then became extensively degraded to form a lysigenous cavity which was considered to be a food source for the nematode (Bleve-Zacheo *et al.*, 1979; 1982b). Griffiths and Robertson (1984) reported similarly that *L. elongatus* feeding on perennial ryegrass initially caused hypertrophy of cells at the feeding site, followed by hyperplasia and then the emptying of a large number of cortical and procambial cells. A recent study of *Paralongidorus buchae* showed that the response to feeding in tomato roots is similar to that induced by *L. apulus* in celery, except that in the former species major cytological changes involve all the root tip (Bleve-Zacheo *et al.*, 1985).

Samples of *X. ifacolum* Luc and *X. basiri* Siddiqi obtained in Liberia and Mauritius, respectively, by Professor F. Lamberti (1987a, b) provided the opportunity for investigating the host response to two tropical *Xiphinema* species. The observations were made on tomato (*Lycopersicon esculentum* Mill. cv. Roma), on which both species readily fed and reproduced, and somewhat surprisingly induced markedly different cellular reaction.

Materials and Methods

Tomato cv. Roma seedlings were transplanted singly into 5 cm diameter clay pots containing 10 ml sterilized sand. Each pot was inoculated with 5 adult female *X. ifacolum* or *X. basiri*. The pots were kept in a growth chamber at 24°C. At 2 and 5 days after nematode inoculation seedlings were randomly selected for histological observations of swelling root tips (day 2) and galls (day 5). The affected tissues were excised from the roots, fixed in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.2 for 4 hours, then rinsed in the same buffer and post-fixed in 2% osmium tetroxide for 4 hours at 4°C, followed by staining in 0.5% aqueous uranyl acetate, dehydration in an ascending series to absolute ethanol and embedding in Spurr's (1969) 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

Both *X. ifacolum* and *X. basiri* fed near the root tip and galls began to form within two days. The insertion of the odontostyle into the root was evident from the column of necrotic cells, in which only nuclei were still visible. During the extended feeding period of the nematodes, the tip of the odontostyle remained within a necrotic area formed from one or more cells, some 5 to 7 cells distant from the rhizodermis. Around the feeding site, considerable histological changes occurred in the surrounding cells but differing between the two species. However, the feeding activity of both species destroyed the root tip after about eight days and the nematodes then moved to other roots.

The main cellular change associated with the feeding of *X. ifacolum* was the production of a coenocyte involving all the meristematic tissue of the root tip (Fig. 1). Cells comprising the coenocyte had only partially formed walls and the amoeboid nuclei and the appearance of the cytoplasmic inclusions indicated hypersensitive metabolism. However, the cell wall stubs were of normal thickness and with plasmodesmata present (Fig. 2). Proplastids contained starch granules and protein bodies, which would not be evident in normal meristematic cells (Fig. 3a); crystals of proteinaceous material are quite commonly found in the stroma, particularly when plastids have been subjected to physiological stress (Bleve - Zacheo *et al.*, 1982a).

In the mitochondria the nucleoids were visible, indicating active replication (Fig. 3b); in the Golgi bodies several different types of vesicles were evident and the presence of polysomes indicated active protein synthesis (Fig. 4). Many of the cells were «plasmolysed» on the side nearest the nematode feeding site i.e. the cell membrane was withdrawn from the wall, suggesting a flux toward the coenocyte, induced by secretions from the nematode (Fig. 1).

Feeding by *X. basiri* induced extensive lysis of the procambial cells towards the root apex from the feeding site. This process continued after the withdrawal of the odontostyle from the root until all the procambial cells between the feeding site and the root tip had been lysed (Fig. 5). Lysis of the cells was observed at two days after exposure of the plants to the

nematodes (Fig. 6) and continued for up to eight days, by which time the root tip was totally destroyed.

Cells immediately adjacent to the lysed area contained rounded nuclei, with swollen nuclear membranes and with the chromatin located at the periphery (Fig. 6). Large areas of the nucleoplasm appeared to be structureless and the aggregated heterochromatin was clearly distinguishable from the condensed euchromatin (Fig. 7). Cortical cells near the lysigenous cavity were similar in appearance to normal meristematic cells and thus were apparently unaffected by the feeding of the nematode (Fig. 6). However, in these cells the nuclei had large nucleoli and the heterochromatin was condensed, whereas the euchromatin was diffused throughout the nucleoplasm. In addition, the nuclei in the cells throughout the root tip showed similar modification (Fig. 8), resembling those observed in tomato roots infested by *P. buchae* (Bleve - Zacheo *et al.*, 1985).

Five days after nematode inoculation, the meristematic cells in the root tip, which were not directly affected by nematode feeding, showed evidence of degeneration (Fig. 9). The reticular part of the nucleolus (Fig. 10), the remainder of the nucleus and all the cytoplasmic organelles were severely damaged to an extent that there was no possibility of recovery. The condensed appearance of the cytoplasm was caused by a degradation of the endoplasmic reticulum which resulted in the release of a large quantity of ribosomes and in accumulation of hyaloplasm material; all the events led to the failure of the synthesising processes within the cells (Fig. 9).

Discussion

Syncytia, giant cells or similar structures induced in the roots of host plants by endoparasitic nematodes are large volumes of active cytoplasm which the nematodes tap as a food source (Jones, 1981). Among the ectoparasites, the trichodorids obtain their food by plundering individual cells and do not induce any histological changes other than necrosis of the damaged cells; secretions from the oesophageal glands are involved initially in the formation of a feeding tube (Rumpenhorst, 1984) but the secretions continue to be passed into the cells, presumably to ensure that the cytoplasm is in a state suitable for ingestion by the nematode. *Longidorus* and *Xiphinema* may ingest the content of the individual cells which are penetrated by the odontostyle to reach the feeding site, but their main source of food is from modified cells surrounding the feeding area,

LEGEND OF FIGURES

Figs 1-4 - Longitudinal sections of tomato root tips 2 days after inoculation with *X. ifacolum.*

Fig. 1 - Necrotic cells (nc) representing the feeding site of the nematode, surrounded by modified cells with more than one nucleus. Nucleoli with vacuoles indicate high activity. The cells contain dense cytoplasm and small vacuoles. A process of false plasmolysis involves all the cell layers and extends throughout the root tip (arrows). × 920.

Fig. 2 - Enlarged modified cells with long wall stubs. The incomplete walls are of the same thickness and with plasmodesmata (pd) of normal cell walls. × 2470.

Fig. 3a - Proplastids of modified cells with prominent starch grains (arrows) indicating increased supply of sucrose via the phloem to the root tip and protein bodies (head arrows), as storage of proteins synthetized in the cells. × 4570.

Fig. 3b - Mitochondria in modified cells with numerous cristae and ribosomes in more densely stained parts of the matrix. The nucleoids (N) (electron transparent areas) contain fine DNA-fibrils (arrows). × 54400.

Fig. 4 - Golgi apparatus with numerous discrete dictyosomes between which there are ramifying cisternae of rough endoplasmic reticulum (ER). Transitional vesicles (tv) and numerous other vesicles are present. The larger type (v), more or less spherical, are formed at the margins of cisternae. Many of the free cytoplasmic ribosomes are organized into polysomes (arrows). × 67700.

Figs. 5-10. Longitudinal sections of tomato root tips, 2 days after inoculation with *X. basiri.*

Fig. 5 - Tomato root tip infested by *X. basiri*; necrotic cells (arrow), representing the feeding site, lie in an area of discoloured cells. Staining with toluidine blue showed the diffusion of the cytological damage. × 32.

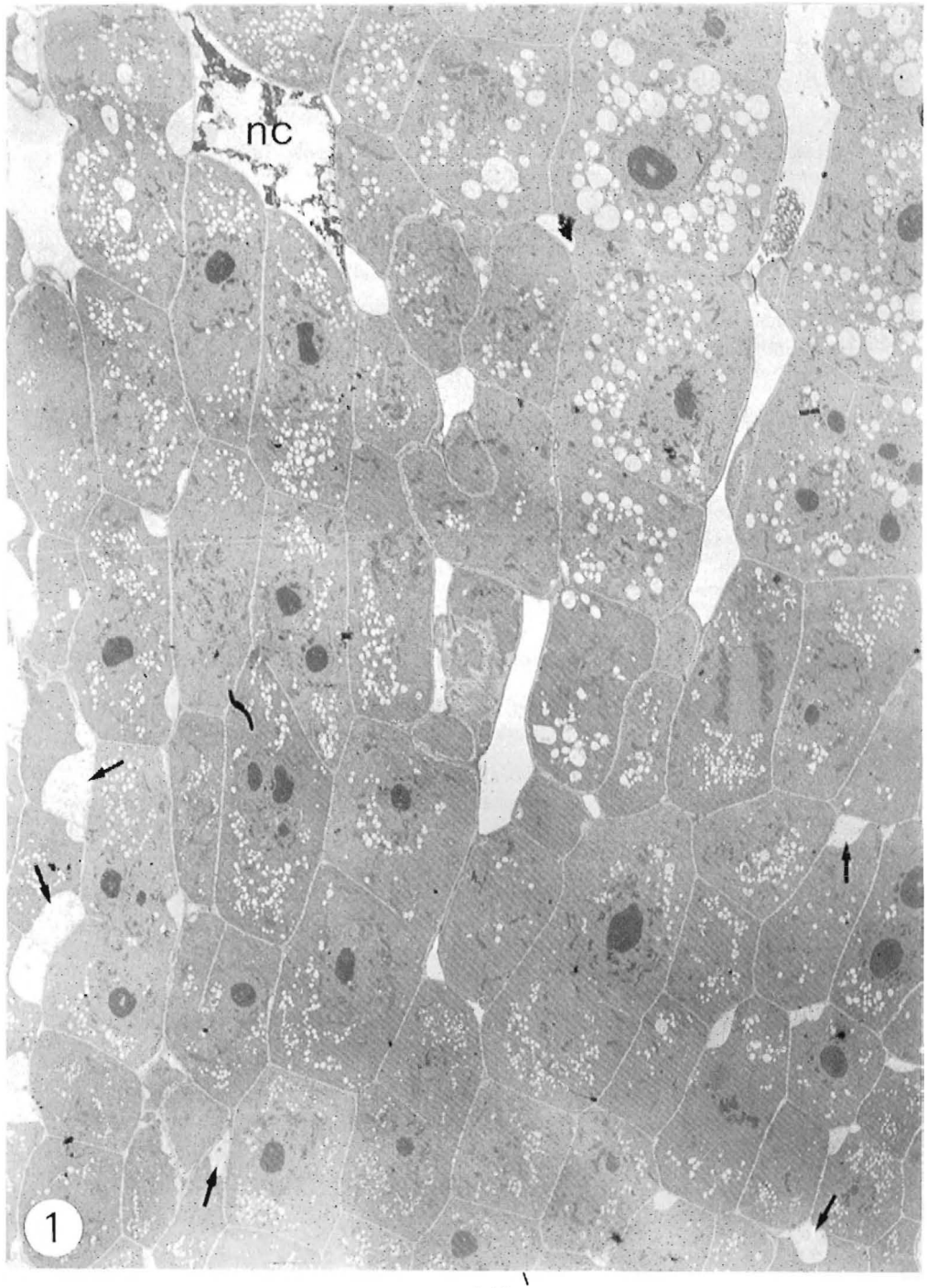
Fig. 6 - Procambial cells, close to the feeding site of the nematode. The phenomenon of lysis is widespread and only nuclei are recognizable, the cytoplasmic contents and walls of the cells having disappeared. The layers of cortical cells appear to be unaffected. × 930.

Fig. 7 - Nuclei of the procambial cells immediately adjacent to the cisternum (lysigenous cavity). The nuclear membranes are swollen; a cleft completely separates the nucleolus from the other components of the nucleus. Euchromatin (et) and heterochromatin (ht) are aggregated and interspersed perichromatin granules (pg) are visible. × 26680.

Fig. 8 - The nuclei of all the root tip cells are in interphase and heterochromatin is aggregated in fibrillar bodies located along the nuclear membranes. × 1700.

Fig. 9 - Cytological and nuclear changes induced by *X. basiri* 5 days after inoculation. Longitudinal section of a root tip showing the complete disorganization of the cytoplasm with no organelles distinguishable. × 1400.

Fig. 10 - The nuclei are more severely deranged than those in Fig. 8 with marginal clumping of the chromatin, dispersion of nucleolar components and clustering of interchromatin granules. × 16000.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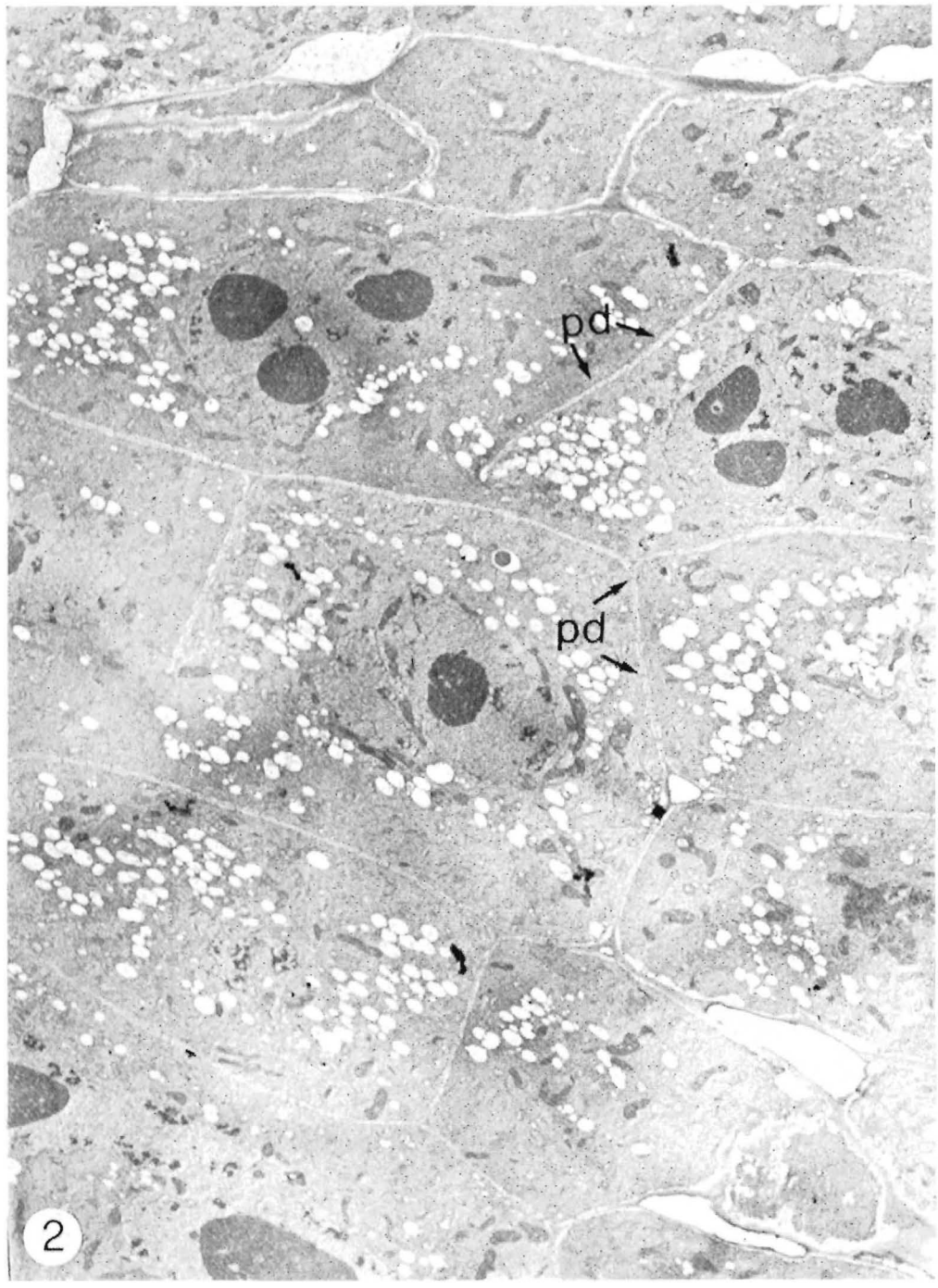
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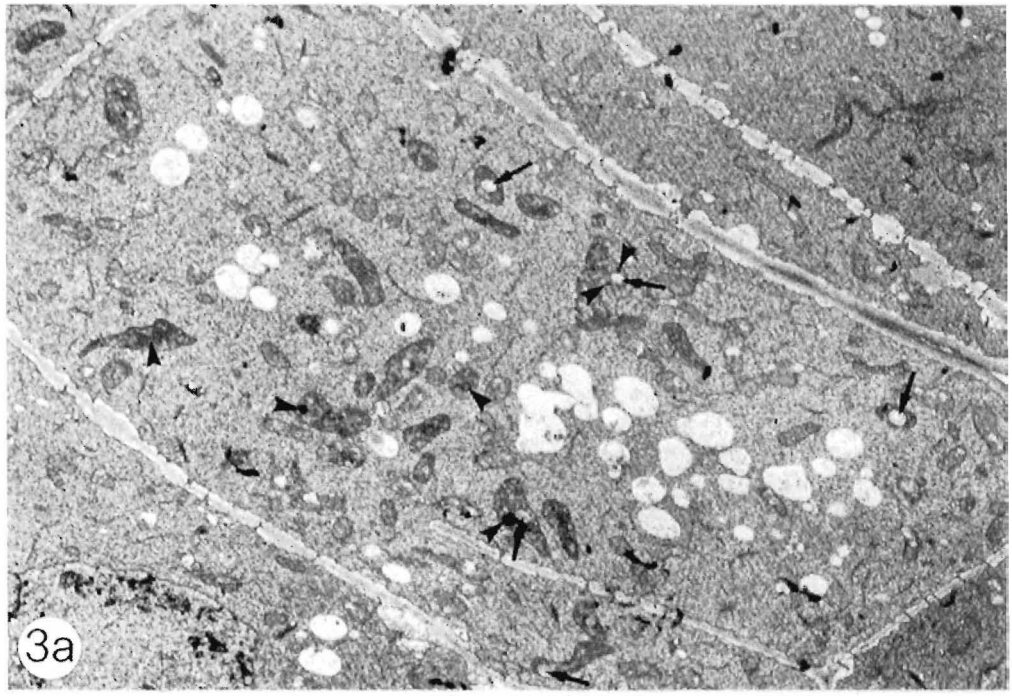
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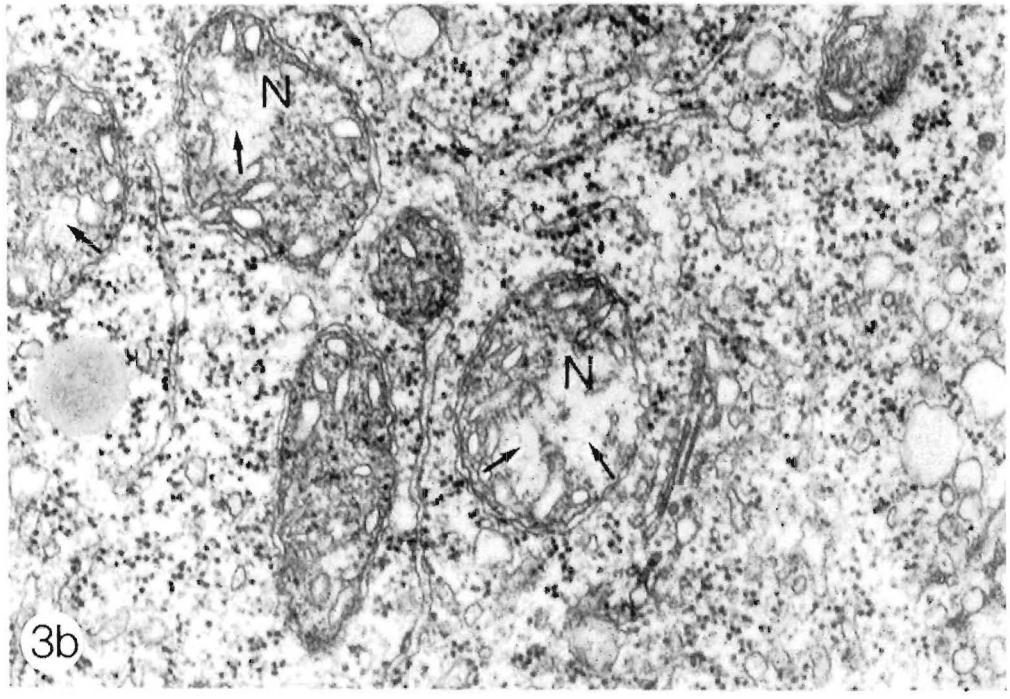
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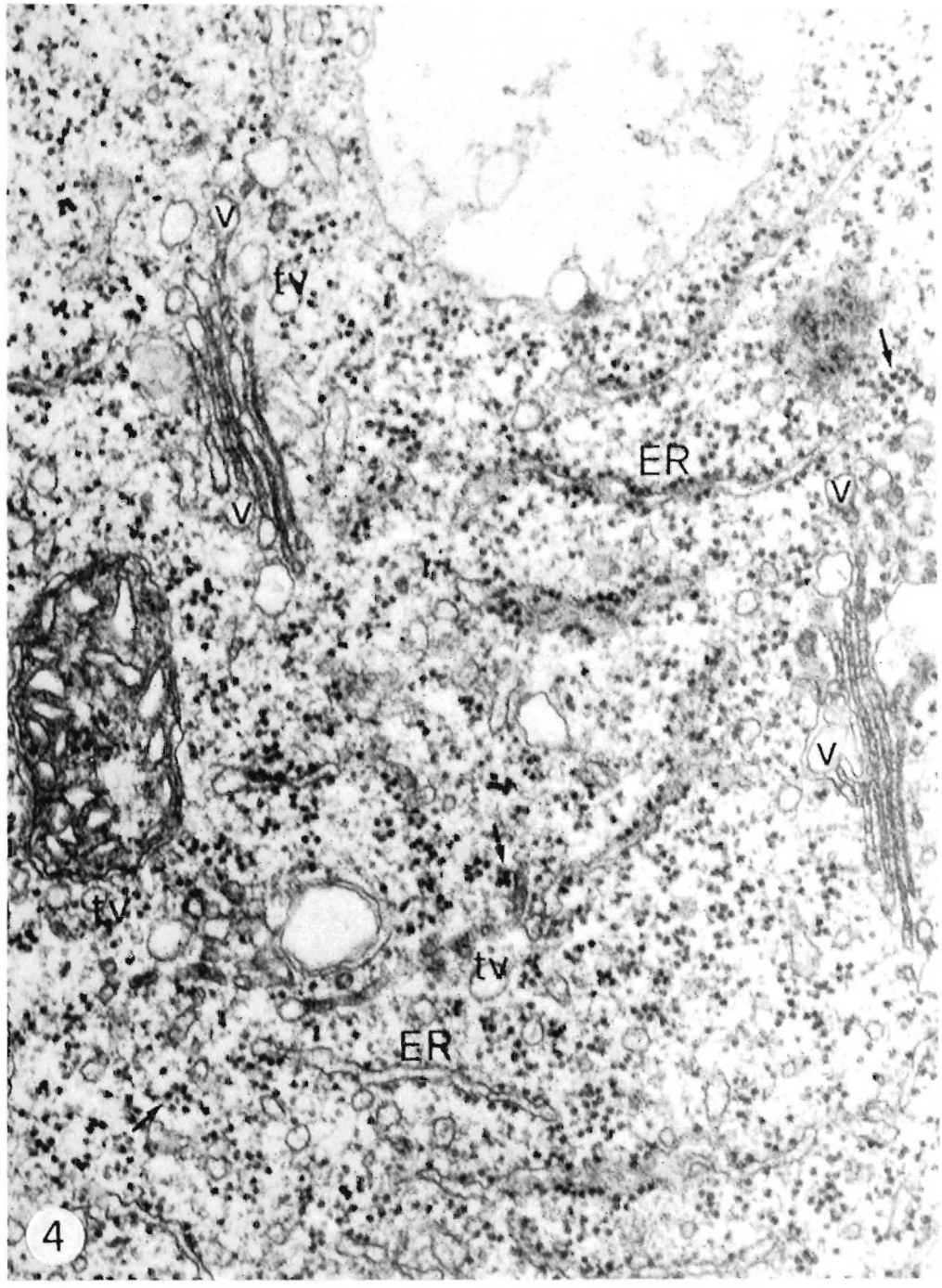
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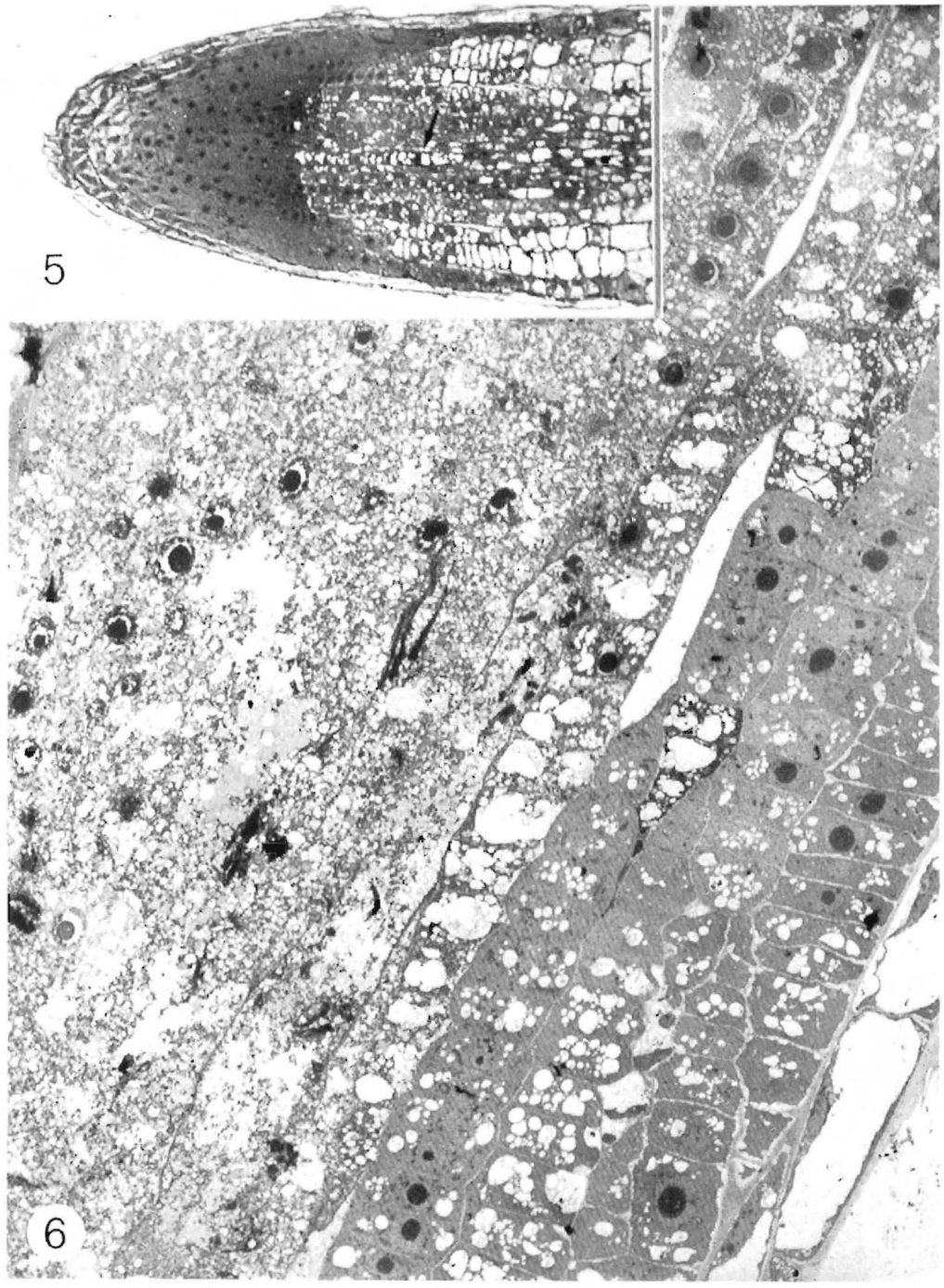
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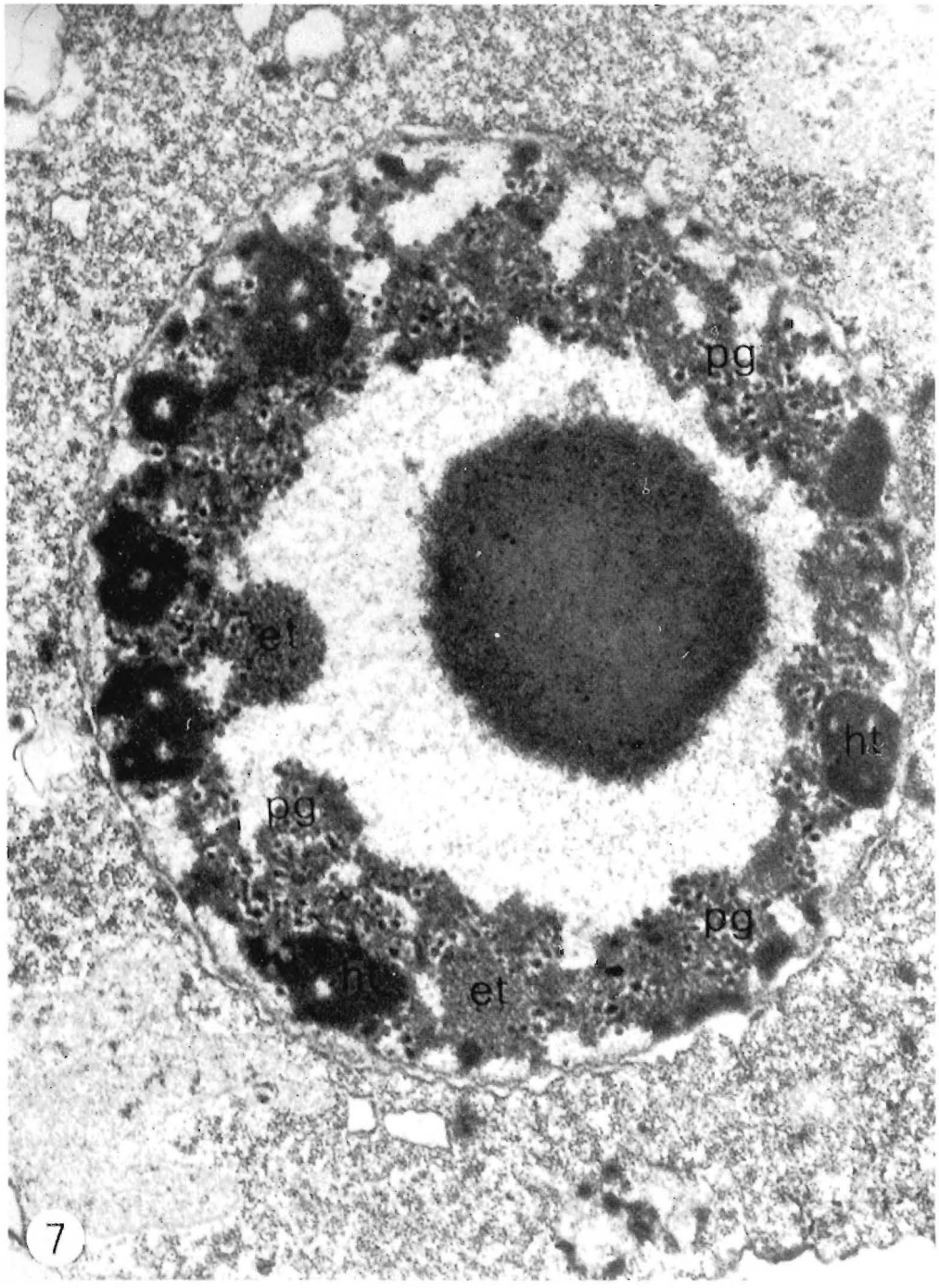
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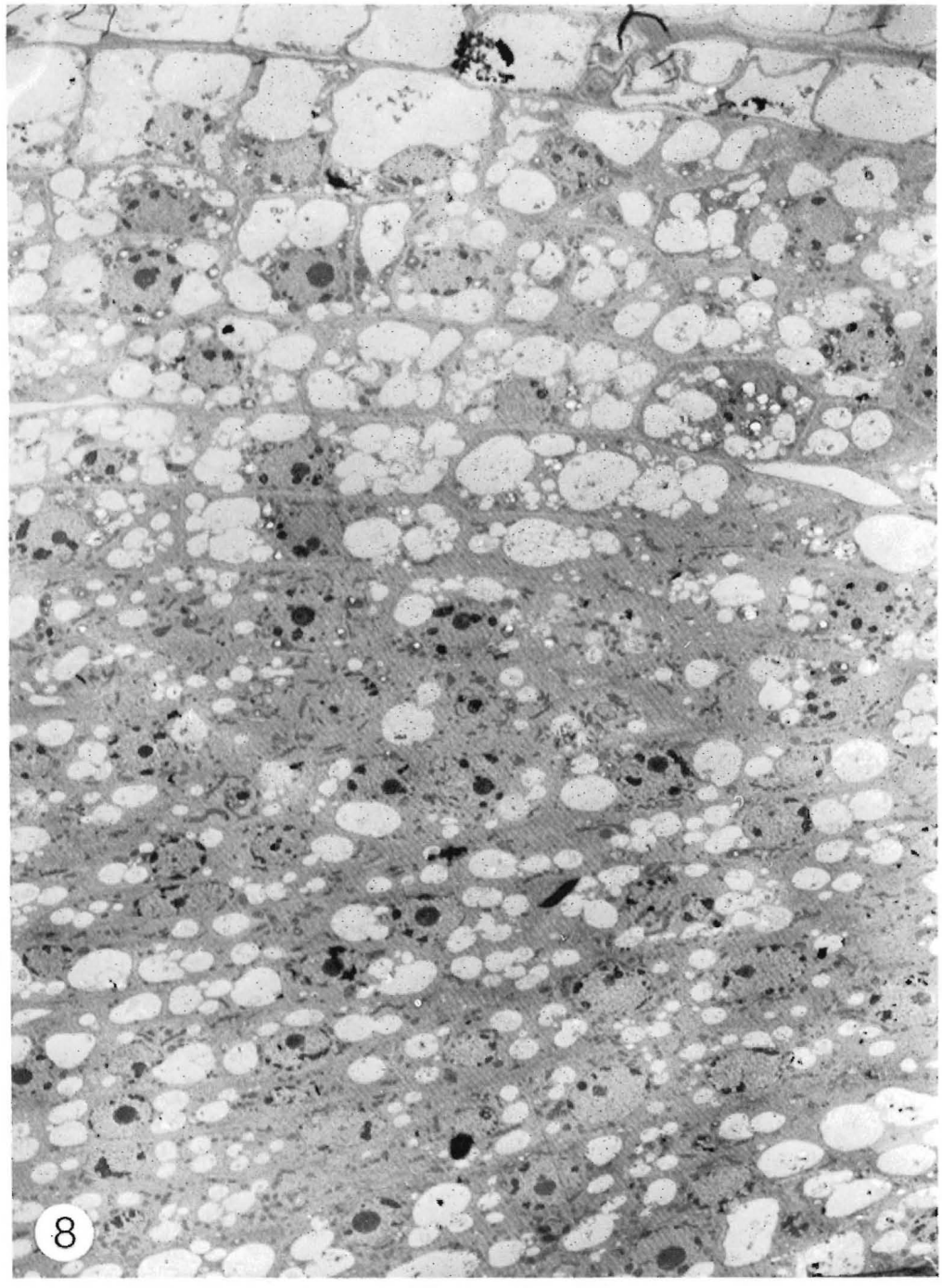
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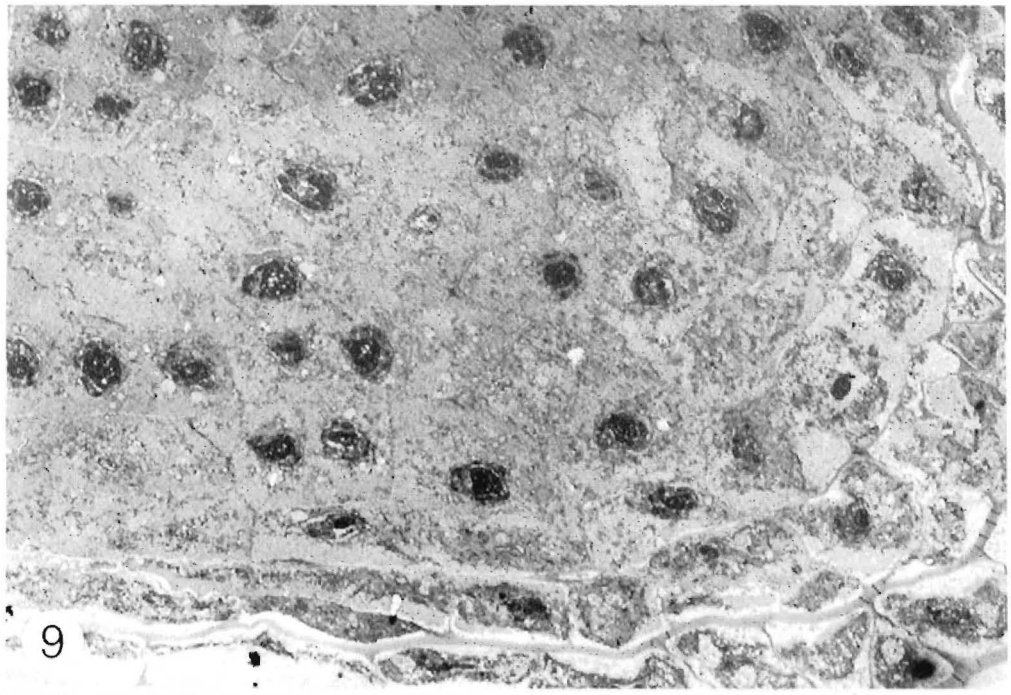

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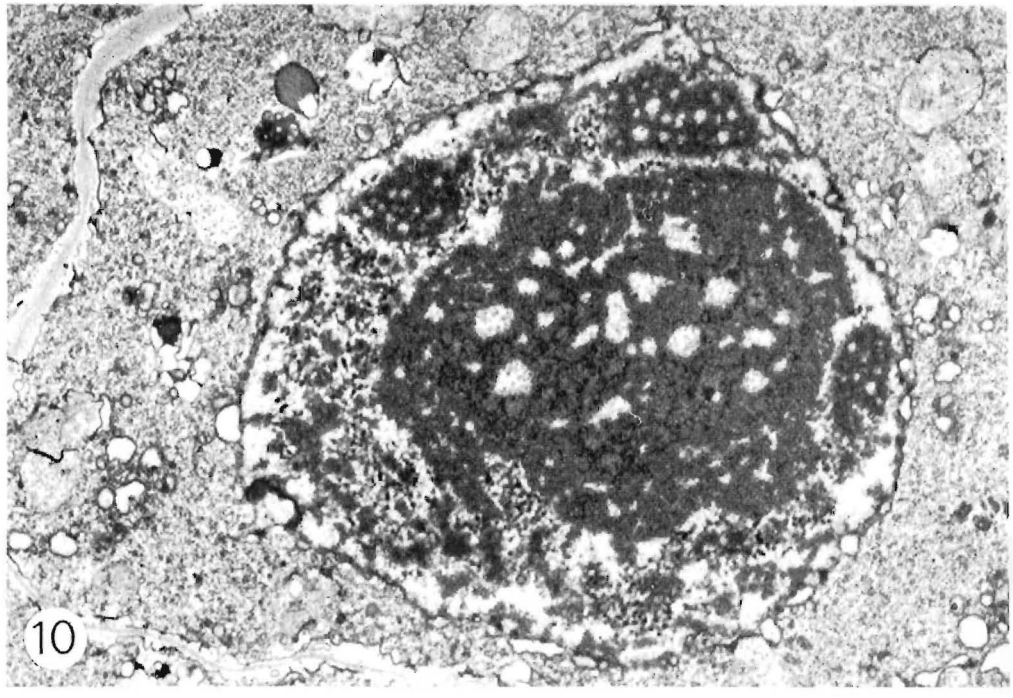

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where the odontostyle may remain located for several hours. The plant response is no doubt induced by secretions from the oesophageal gland and can result either in the formation of a coenocyte or a lysigenous cavity, the former occurring mostly in response to *Xiphinema* and the latter to *Longidorus* and *Paralongidorus* (Bleve-Zacheo *et al.*, 1979, 1985).

The initial stages of feeding in *Xiphinema* and *Longidorus* are similar i.e. a column of cells is destroyed as the odontostyle penetrates the root and a group of cells then become necrotised to form the feeding site. The initial cellular modifications around the feeding site are also similar in the nuclear and the nucleolar enlargement and in the general hyperactive metabolism evident from the increased cytoplasmic inclusions (Griffiths and Robertson, 1984; Wyss, 1981). The events that then follow differ between the two genera.

In *Xiphinema* species, with the exception of *X. basiri* as discussed here, the coenocyte which is formed next to the feeding site provides a source of food and may be maintained for up to two weeks (Wyss, 1981) but not indefinitely as with syncytia induced by *Meloidogyne* spp. Eventually *Xiphinema* species abandon a feeding site, probably because the cells of the coenocyte have been damaged and a flux of nutrients toward the nematode can no longer be maintained. Such damaged coenocytes have been observed within layers of the necrotic and collapsed cells (Wyss *et al.*, 1980). In the case of *X. ifacolum* on tomato, feeding on a root tip was rarely extended beyond eight days by which time the root tip had been destroyed.

Longidorus elongatus feeding on perennial ryegrass induced an enlargment of procambial cells in the root tip, the contents of which were gradually depleted as feeding continued until after 10 to 12 days the galled tissue became necrotic and collapsed (Griffiths and Robertson, 1984). The contents of the cells, which were some distance from the odontostyle tip located in its feeding site, were considered to flow to the nematode through holes in the cell walls produced by a localised lysis (Robertson *et al.*, 1984).

In the case of *L. apulus* feeding on celery (Bleve-Zacheo *et al.*, 1979) and *P. buchae* (Bleve-Zacheo *et al.*, 1985) and *X. basiri* feeding on tomato, lysis was much more extensive and a large lysigenous cavity (cisternum) was formed in the procambial tissue of the root tip. Although the extensive breakdown of cell contents would seem to provide a readily available food source for the nematode, the formation of a lysigenous cavity is assumed to be a «more primitive» condition than the formation of a coenocyte, which resembles the syncytia induced by endoparasitic nematodes.

Whatever the differences between species of *Xiphinema* and *Longidorus* in their effect of feeding on host root tissues, the cellular changes induced result from the introduction and diffusion within the root tip of secretion from the dorsal oesophageal gland. Differences in the chemical constituents of the secretion are not likely to be great between the two genera, or between species, but sufficient to induce significantly different effects.

Differences between coenocyte and lysigenous cavity formation may be more apparent than real and may depend on the quantity of secretion introduced into the root which in turn may depend on the host acceptance by the nematode.

It is interesting to note that the cellular modifications which occur in the root cells outwith the immediate area of the coenocyte or lysigenous cavity are similar whatever the nematode species involved. Similar metabolic activity and cellular changes have been observed in roots infected by some fungal and viral pathogens (Gill and Chong, 1976; Hadwiger and Adams, 1978).

SUMMARY

Xiphinema ifacolum and *Xiphinema basiri* fed on the root tip of tomato, and as a result the tips were transformed into galls. Sections of galls incited by *X. ifacolum* revealed a column of necrotic cortical cells, penetrated by the odontostyle and representing the feeding site. Nematode feeding caused hypertrophy of the cells, which had cytoplasm packed with inclusions, and nuclei and nucleoli larger than those of unaffected cells. Cell wall stubs were present in cells with more than one nucleus, indicating that there were mitoses with failed cytokineses. These modifications are very similar to those induced by *X. index* in its host. Feeding of *X. basiri* induced a drastic disturbance of the physiology of the root tips, which were transformed into galls. All of the procambial tissue was transformed to a cisternum (lysigenous cavity) without cytological structures probably caused by the injection of lytic enzymes by the nematode and their diffusion within the root. Nuclei in the meristematic cells far from the feeding site were stopped in interphase and the structure of their components indicated they had lost the capacity of synthesis.

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Accepted for publication on 25 April 1987.