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DEVELOPMENT OF GALLS INDUCED IN *CHENOPODIUM QUINOA* BY *LONGIDORUS APULUS*

by
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The plant parasitic nematode *Longidorus apulus* Lamberti *et* Bleve-Zacheo (1977) appears to be fairly polyphagous; it has been found in association with various herbaceous plants such as artichoke, potato, chicory, fennel and weeds on which it may cause direct damage by feeding on the roots. Root tip galls induced by *L. apulus* on chicory showed hyperplasia in the cortex and hypertrophy in the cambial cells (Bleve-Zacheo *et al.*, 1977a). In celery roots a lysigenous cavity was associated with the trophic action of the nematode. Cells surrounding the lysigenous cavity showed pronounced formation of paramural bodies and conspicuous callose-like deposition, interpreted as wound response (Bleve-Zacheo *et al.*, 1979, 1982).

Necrosis or other pathological alterations in weed species caused by *L. apulus* have not been investigated. In this paper the development of galled root tips and the ultrastructure of modified root cells are described in *Chenopodium quinoa* parasitized by *L. apulus*.

Materials and Methods

Chenopodium quinoa, chicory (*Cichorium intybus* L.) and celery (*Apium graveolens* L.) seedlings 2-3 cm tall, were transplanted into 5 cm diameter clay pots, containing 10 ml sterilized sand; they were then inoculated each with 5 females of *L. apulus* extracted from a culture maintained at 22 ± 1° C on celery, and placed in a growth

chamber (22° C, 65% RH, 3,000 lux). At weekly intervals, plants were removed and nematodes recovered. Roots and nematodes were observed under a stereomicroscope and the number of galls and nematodes recorded.

Chenopodium seedlings, inoculated as described above, were removed three days after. Seedlings showing necrosis on the root apices were kept in water, without nematodes; after 24 and 48 hours the swollen root tips were excised. In other experiments galls were excised ten days after inoculation.

Swollen root tips and galls were fixed in 3% glutaraldehyde in 0.05M uranyl acetate, dehydrated in a graded ethanol series and embedded in Spurr's medium. Sections 2 μm thick were stained in toluidine blue and observed with a light microscope. Ultrathin sections were cut with a LKB ultratome III, stained in uranyl acetate and lead citrate and examined in a Philips 400 T electron microscope.

Results

The feeding of *L. apulus* caused terminal galls on the root tips of chenopodium, chicory and celery. However, more galls were produced on chicory and celery than on chenopodium (Fig. 1). The number of galls increased progressively till 55 days after the inoculation, when also the plants became senescent (Fig. 1). The fewer galls on chenopodium, are a reflection of this plant being a poor host compared with chicory and celery. Generally, all the nematodes survived well in the chicory and celery pots, while on chenopodium survival was estimated to be about 60% of the original population.

Galls on chenopodium, generally were localized on the lateral root-tips. In response to nematode feeding, the root-tip (Fig. 2) started to swell and became transformed into a terminal gall (Fig. 3). A necrotic area, presumably where cells were directly injured by stylet insertion, was often visible at the end of the gall. Necrosis was already evident on the roots of plants three days after nematode inoculation. When these root tips were placed in water without nematodes about 24 hours later they began to swell, and continued to do so for the next two to three days. Sections, of the root-tip swellings showed a cluster of hypertrophied cells at sites where the nematodes probably had fed and rows of necrotic and collapsed cells, reaching several cell layers, deep into the gall (Fig. 4).

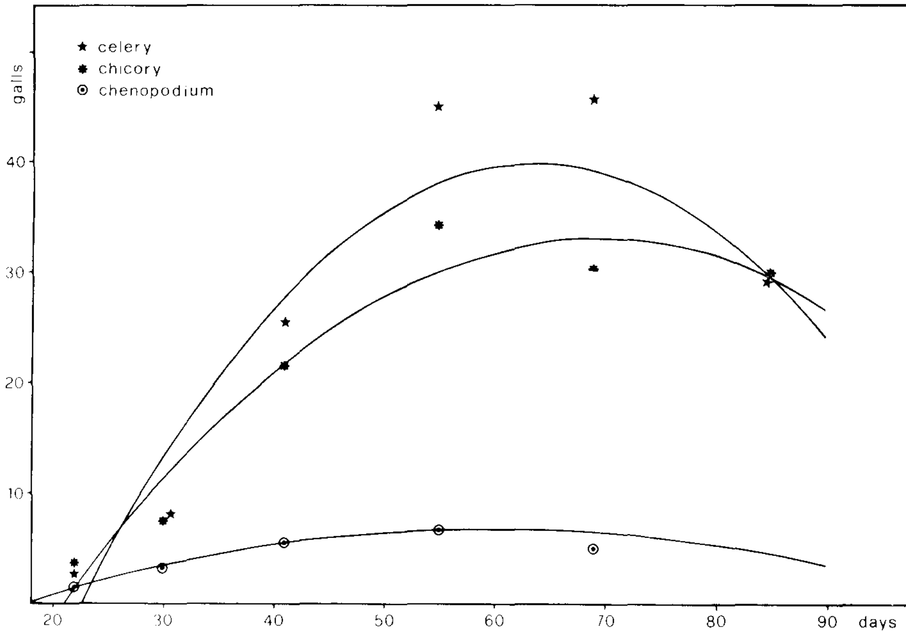


Fig. 1 - Gall formation induced by *L. apulus* feeding on three hosts. Chenopodium: $y = -6.38x + 0.43x^2 - 0.003$; chicory: $y = -34.85x + 1.96x^2 - 0.014$; celery: $y = -55.26x + 2.97x^2 - 0.023$.

A clear demarcation was evident between necrotic and adjacent hypertrophied cells; the walls of cells that were affected by the nematode feeding became coated with material that stained deeply with toluidine blue. In thin sections electron-dense material also was similarly distributed in those cells that were still partially filled with degraded contents (Figs 4, 7); disorganized nuclei were more electron-dense than in adjacent cells; organelles were no longer recognizable while adjacent cells were densely packed with cytoplasm, indicating high metabolic activity (Fig. 4).

In section of 4-5 day old galls there were enlarged cells with 6 to 8 nuclei per cell (Fig. 5). It was difficult to count the actual number of nuclei in the cells because of the different planes of the sections. Multinucleate cells did not show any irregularities of their cell walls, such as distinct wall ingrowths, and had the typical appearance of meristematic cells, with dense cytoplasm, rich in organells, and without intercellular spaces (Figs 5, 6). Sections of 10 day old galls showed that the necrotic area had been absorbed, this apparently being a

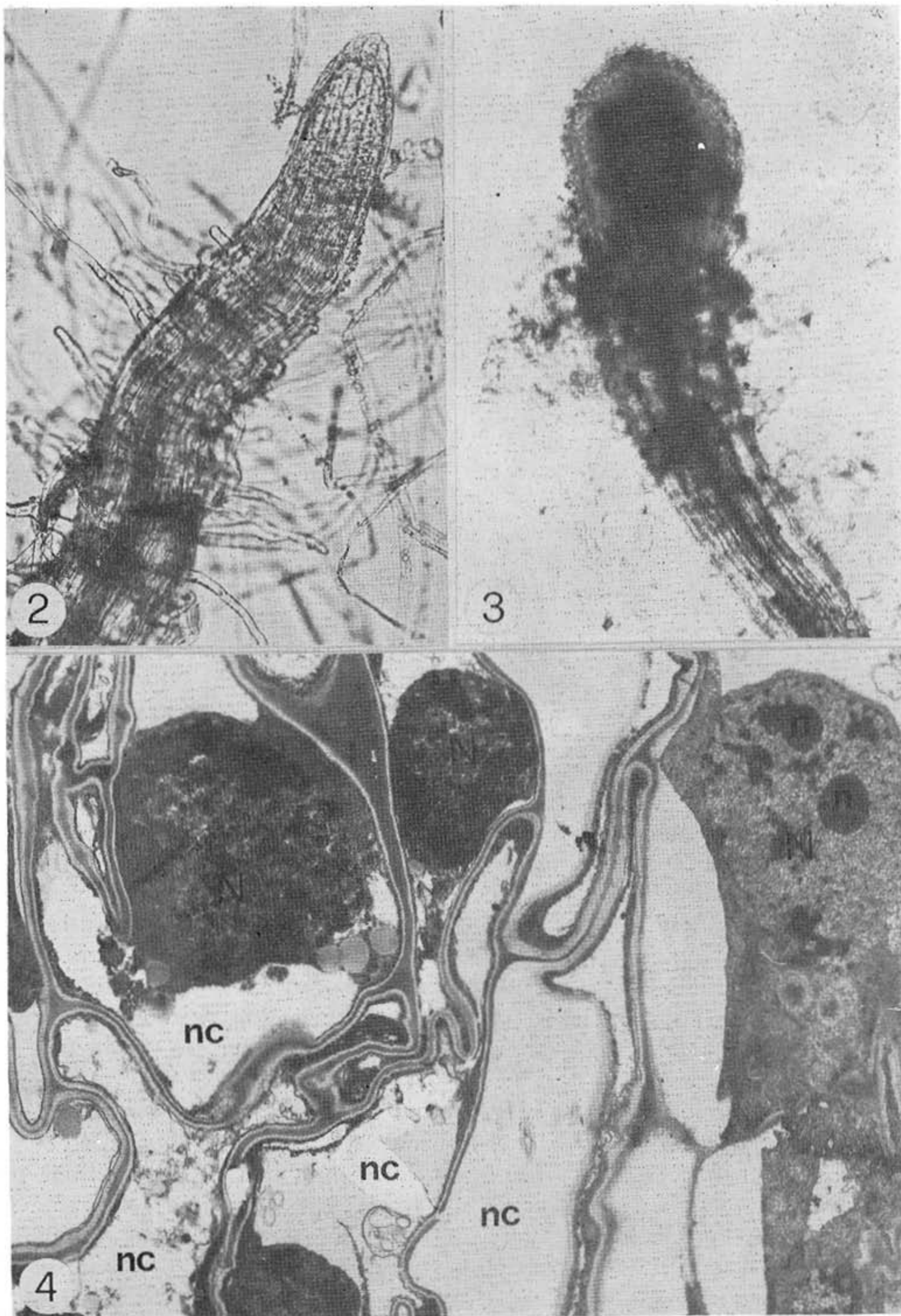
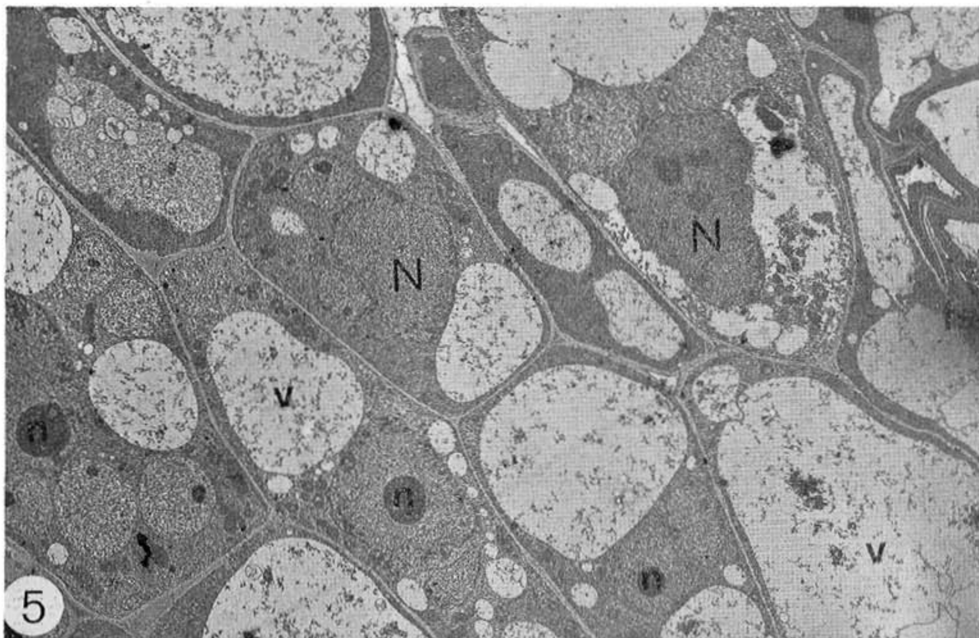


Fig. 2 - Healthy root-tip of *C. quinoa.*
Fig. 3 - Root-tip gall induced by *L. apulus* on chenopodium, one week after inoculation.
Fig. 4 - Photomicrograph of a longitudinal section through the feeding site of *L. apulus* on chenopodium. Note the clear-cut demarcation between the 2 cell types. The necrotic cells (nc) contain no cytoplasm and nuclei (N) are more electron dense than those of the adjacent cells which show a large nucleus with two nucleoli (n). × 8400.



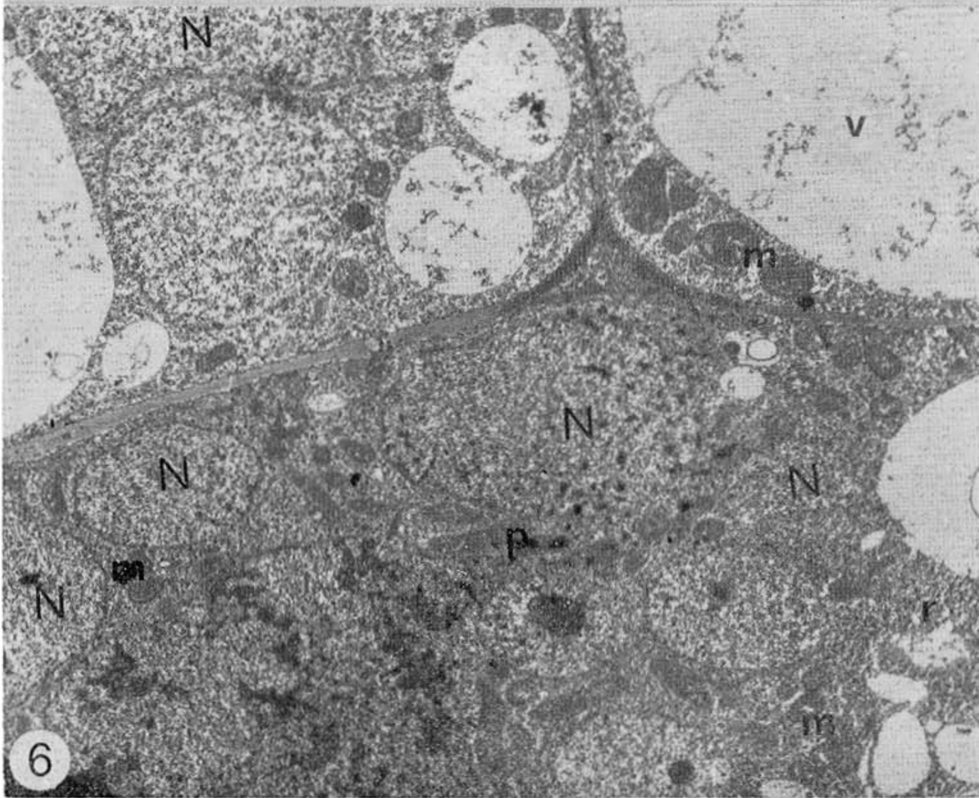


Fig. 5 - Longitudinal section through the feeding site (fs) of *L. apulus*. Necrotic cells and meristematic multinucleate cells are present. × 2300.

Fig. 6 - Longitudinal section of two multinucleate cells. Note the many mitochondria (m), ribosomes (r) and proplastids (p), indicating intense metabolic activity. Small or confluent vacuoles (v) are visible but no intercellular spaces. × 7600.

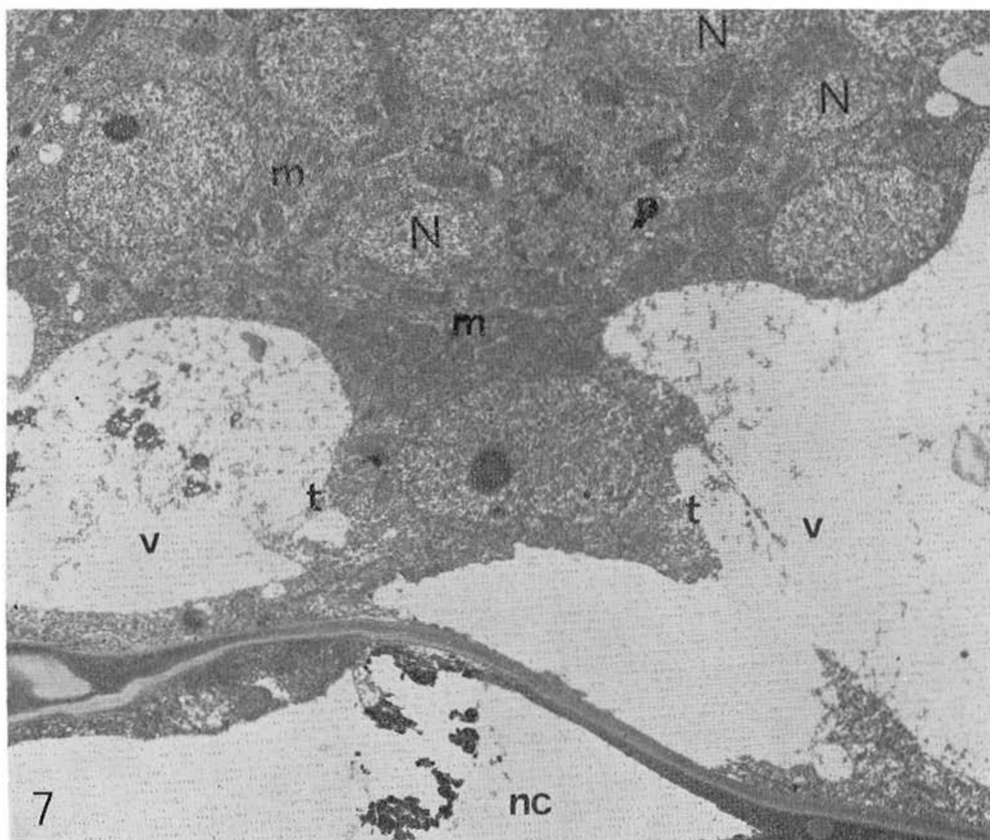
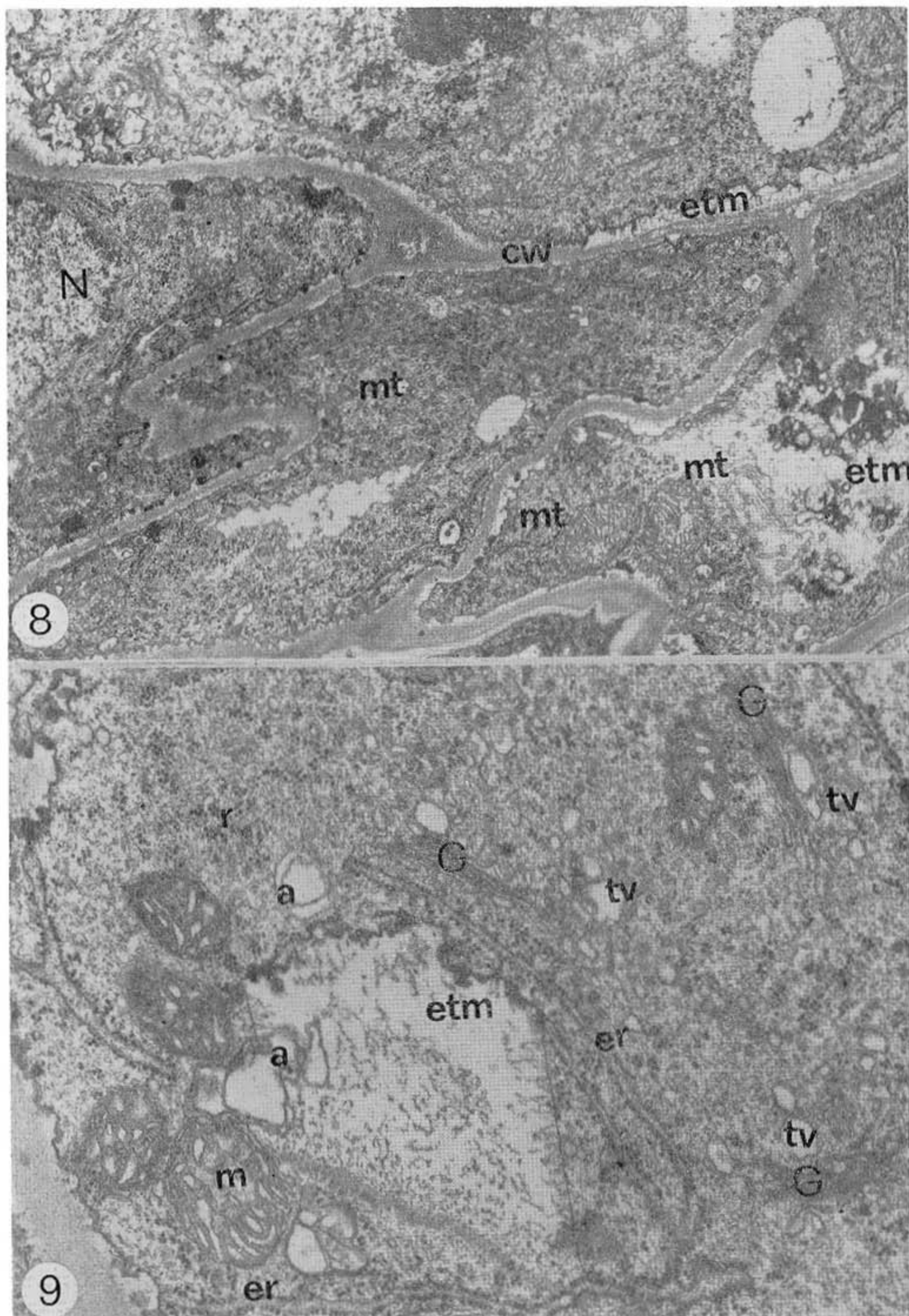


Fig. 7 - Longitudinal section of 10 day old gall; a multinucleate cell is adjacent to the necrotic ones; the tonoplasts (t) are lysed but nuclei and other organelles are well preserved. × 6950.

gradual process involving the adjacent cells and the multinucleate ones. The cytoplasm of the multinucleate cells adjacent to the necrotic areas showed an initial process of dissolution; the tonoplasts were swollen and disintegrated, and the cytoplasm had mixed with vacuolar material; nuclei and organelles were still clearly recognizable (Fig. 7). Tissue immediately adjacent to multinucleate cells showed no changes in shape or size, but some cytoplasmic organelles were altered. The Golgi apparatus and the rough endoplasmic reticulum appeared to have been involved in an abnormal synthesis of electron translucent material. Golgi bodies had given rise to numerous transitory and coated



Figs. 8-9 - Meristematic cells neighbouring the multinucleate cells. Fig.8. Electron translucent material is evident on cell walls (cw) and in the cytoplasm (etm). Macrotubules (mt) in longitudinal and cross section are in groups or scattered in the cytoplasm. × 18,500. Fig. 9. Cell showing Golgi bodies (G) transformed into large, irregular transitory vesicles (tv), with altered membranes (a). Long and actively synthetizing profiles of endoplasmic reticulum (er) are closely associated with an electron translucent area. Mitochondria show very dense matrix and enlarged cristae. × 34,800.

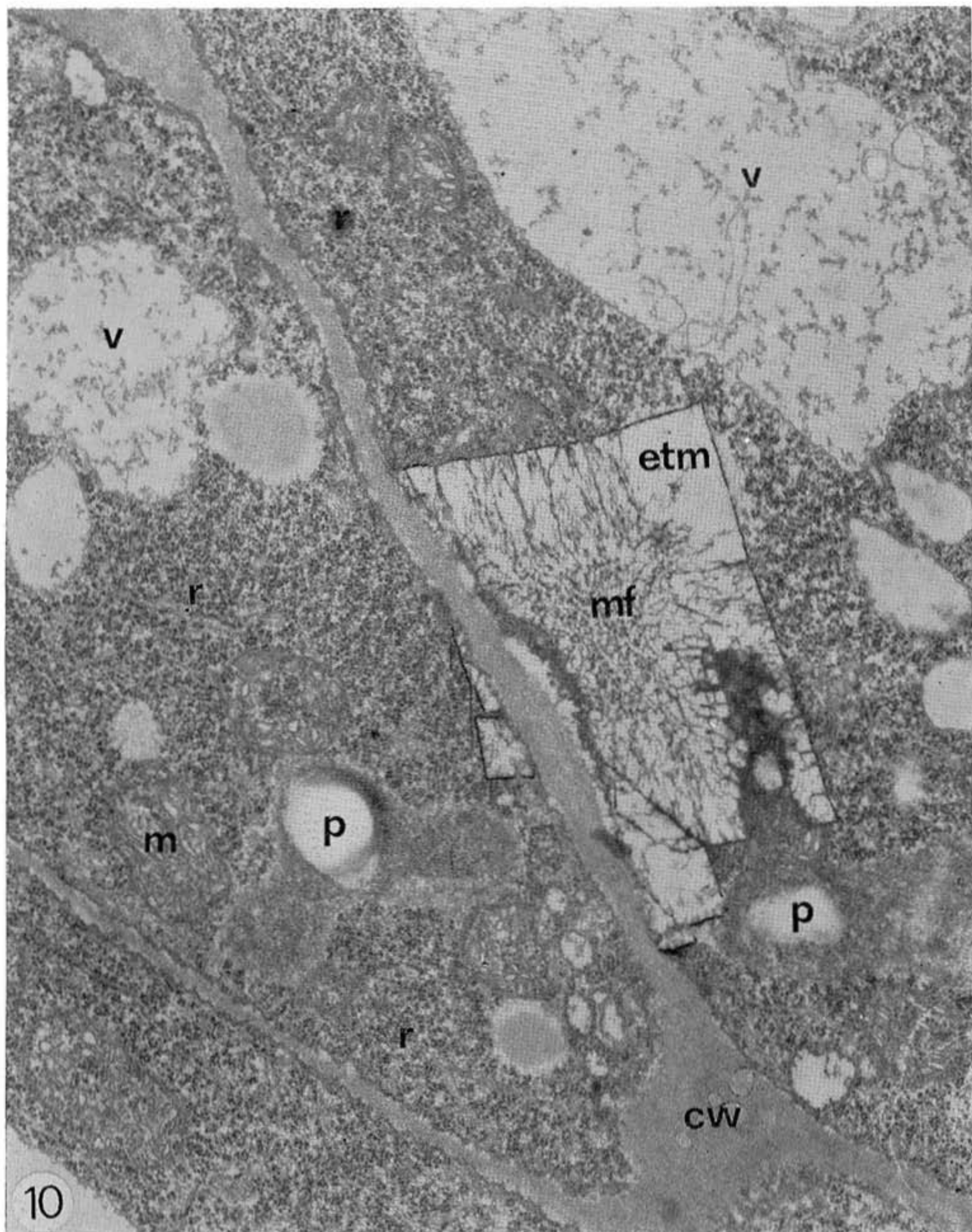


Fig. 10 - Cells as in Fig. 9, showing typical cytoplasm of meristematic cells. Note electron translucent material with a dark line of demarcation, filled with longitudinal and transverse microfibrils (mf). × 24,500.

vesicles, that separated from the Golgi stack and formed loose groups of variably shaped and sized vesicles (Figs 8, 9). The endoplasmic reticulum showed long profiles of enlarged membranes, rich in ribosomes, full of synthetizing material. Profiles were strictly related to the plasma membrane and to the electron translucent material present in the cytoplasm (Figs 8 and 9). Numerous tubular structures were visible either in a group or scattered in the cytoplasm; some of them probably were involved in the transport of material into the electron translucent areas (Fig. 8). The translucent areas were associated with the cell wall (Fig. 10) and delimited by a dark line, on which microfibrils appeared to be attached (Figs 9, 10).

Discussion

Longidorus apulus did not multiply as well on chenopodium as it did on celery or chicory (personal data). However, although chenopodium may be considered as a relatively poor host, apical galls were induced by the nematode's feeding and in general these were similar to those described for several longidorid nematodes on other host plants (Cohn, 1970, 1975; Cohn and Orion, 1970; Wyss, 1970; Bleve Zacheo *et al.*, 1977a, b; Griffiths *et al.*, 1982), except that the ultrastructural response in the feeding site differed, because of the presence of hypertrophic multinucleate cells. A similar response has not been described in roots attacked by other *Longidorus* species.

It has been reported that *L. africanus* induced hyperplasia of the cortical parenchyma and the formation of lateral root initials (Cohn and Orion, 1970). Clusters of hypertrophied cells with enlarged nuclei were observed at the feeding site of *L. elongatus* in celery but not multinucleate cells (Wyss, 1981). Similarly, root tip swellings of *Lolium perenne*, caused by the feeding of *L. elongatus*, contained only a single, but slightly enlarged amoeboid nucleus per cell (Griffiths *et al.*, 1982). Hyperplasia in the cortex and hypertrophy in the cambial cells were described in root tip galls of chicory and hypertrophied cells in root tip galls of celery, attacked by *L. apulus* (Bleve Zacheo *et al.*, 1977a). Multinucleate cells, adjacent to the lysigenous cavity, were occasionally observed in celery (Bleve Zacheo *et al.*, 1977b). The nuclear modifications induced by *L. apulus* in chenopodium roots resemble in many respects those induced by the ectoparasite *Xiphinema index* and by specialized endoparasitic and sedentary nematodes.

In chenopodium *L. apulus* induced the formation of specialized cells. Meristematic cells normally form specialized cells of different types with specific functions in response to various repressors and inducers operating within the plant. Since the nematode can repress part of the genetic coding of the cell and activate other parts, a special type of cell is induced (Bird, 1974). In chenopodium these highly specialized cells are induced by a stimulus from the migratory ecto-parasitic *L. apulus*, but this process is only temporary because, as reported by Bird (1974), multinucleate cells are maintained and completely depend on a continuous stimulus from the nematode; removal of the stimulus leads to their disorganization and subsequent degradation.

No ingrowths were observed on cell walls. Considerable changes in Golgi apparatus and rough endoplasmic reticulum activity occurred in the cells, directly adjacent to the multinucleate ones. In these cells, Golgi bodies breakdown progressed from increased production of transitory vesicles, which resulted in loose collections of vesicles with distorted and irregular unit membranes. In close association with the electron lucent areas tubules, larger and more variable (30-40 μm) than microtubules (25 μm), were found in groups or scattered throughout the cytoplasm; these tubules, in contrast to the microtubules, remained intact after treatment at 4° C. It has been reported that macrotubules in the leaf gland of *Phaseolus vulgaris* were confluent with the membranes of the endoplasmic reticulum from which they appeared to have arisen (Steer and Newcomb, 1969). We assume that transitory Golgi vesicles, macrotubules and endoplasmic reticulum are engaged in the production and transport of the electron lucent material. The purpose of this production could be to advantage the roots by synthetizing considerable amounts of metabolites antagonistic to the nematode and may thus function as a biochemical barrier which discourages the nematode from feeding again at the same site.

SUMMARY

Longidorus apulus feeding on the root tips of *Chenopodium quinoa* induced the formation of galls, but relatively fewer than on celery and chicory. Examination of the swollen root-tips in ultrathin sections, showed that the cytological response was found different from that in other hosts parasitized by *Longidorus* species. Cells fed upon by *L. apulus* became necrotic and neighbouring cells contained several enlarged multinucleate cells, containing 6 to 8 nuclei. The modified cells showed increased cytoplasmic density and abundance of mito-

chondria and proplastids, indicative of high metabolic activity. Cells adjacent to the multinucleate cells contained electron translucent structures, which were closely associated with the transitory Golgi vesicles, rough endoplasmic reticulum and macrotubules.

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