Ultrastructural response of potato roots susceptible to cyst nematode *Globodera pallida* pathotype Pa 3

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SUMMARY

Syncytia were examined by light and electron microscopy in the roots of three susceptible potato cultivars four and twelve days after inoculation with juveniles of *Globodera pallida* pathotype Pa 3. The syncytia formed in cv. Diamant developed into the stele, occupying most part of the central cylinder. The nematodes established their feeding site in the endodermal cells. In cv. Anosta the syncytia developed particularly in the cortical region and ultimately involved only the outer region of the vascular cylinder. The nematodes were localized in the cortical cells. In cv. Irene the syncytia were formed outside the stele. The only major difference observed between the syncytia was, apart from the larger area in Diamant, in the metabolic activity of the cells involved. Syncytial cells in cv. Diamant roots showed considerable proliferation of smooth endoplasmic reticulum, less in cv. Anosta and almost none in cv. Irene. Differences in cytoplasmic activity may be indicative of a variation in the susceptibility of the plant due to the nutritional quality of the food. No wall ingrowths were observed in the three syncytia examined.

RÉSUMÉ

Ultrastructure de la réaction des racines de pommes de terre sensibles au nématode à kyste Globodera pallida *pathotype Pa 3*

Les syncitia induits par *Globodera pallida* pathotype Pa 3 sur les racines de trois cultivars de pomme de terre sensibles, quatre et douze jours après l'inoculation de larves du nématode, ont été observés en microscopies optique et électronique. Les syncitia formés sur le cv. Diamant se développent dans le cylindre central dont ils occupent la majeure partie. Le nématode établit son site d'alimentation sur les cellules de l'endoderme. Sur le cv. Anosta, les syncitia sont plus particulièrement développés dans la région corticale et ne s'étendent qu'à la partie extérieure du cylindre central. Les nématodes se localisent dans les cellules corticales. Sur le cv. Irene, les syncitia se forment à l'extérieur du cylindre central. Les plus importantes différences entre syncitia, mise à part une plus grande surface (cv. Diamant), ont trait à l'activité métabolique des cellules impliquées. Dans les racines du cv. Diamant, les cellules syncitiales montrent une prolifération considérable du reticulum endoplasmique lisse, phénomène moins développé chez le cv. Anosta et presque absent chez le cv. Irene. Ces différences dans l'activité du cytoplasmique peuvent indiquer une variabilité de la sensibilité de l'hôte causée par la qualité de la nourriture. Dans les trois syncitia examinés, il n'a pas été observé d'excroissances internes de la paroi cellulaire.

Globodera rostochiensis and *G. pallida* produce similar physiological responses after they have invaded the potato roots. During the early stage of infestation apparently identical cell changes, expressed in the formation of syncytia, occur in susceptible and resistant cultivars. Distinct ultrastructural differences between modified cells of susceptible and resistant roots are evident within a few days of nematode invasion. At this time syncytia start to degenerate in resistant roots (Hoopes, Anderson & Mai, 1978; Jones, 1981; Rice, Leadbeater & Stone, 1985; Rice, Stone & Leadbeater, 1987).

The results reported contribute to the information on the histological changes associated with susceptibility, particularly with respect to two Italian potato commercial cultivars, Diamant (mutant from Cardinal) and Anosta (parentage Ostara × Provita). The cytological changes induced by *G. pallida* pathotype Pa 3 on the two potato cultivars were compared with those in cv. Irene (a Dutch cultivar) infested by the same pathotype and reported by Seinhorst (1985) as susceptible. The response of the same cultivars to *G. rostochiensis* pathotype Rol are presented elsewhere (Bleve-Zacheo, Melillo & Zacheo, 1990).

Materials and methods

Cysts of *G. pallida* pathotype Pa 3 (Dutch population) were placed in 0.6 mM sodium meta-vanadate in water and one week later second-stage juveniles were collected. Potato roots of cvs. Diamant, Anosta (Italian) and cv. Irene (Dutch) were grown from sprouted potato tuber pieces at 17°. They were then transferred into clay pots containing 10 ml of sterilized sand and simultaneously a suspension of 100 second-stage juveniles of Pa 3 pathotype was added to each pot. The experiments were conducted in growth chambers at 17 °C.

Four and twelve days after nematode inoculation,

roots were removed and washed. The root tips (four days) and portions of infested roots (twelve days) were fixed in 3 % glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) for 4 h, rinsed in the same buffer, post-fixed in 2 % osmium tetroxide for 4 h at 4°, then stained in 0.5 % uranyl acetate, dehydrated in an ascending series to absolute ethanol and embedded in Spurr's medium (Spurr, 1969). Sections, 2 µm thick, were cut with a LKB ultratome IV, stained with toluidine blue and observed under a light microscope to verify the syncytial location. Ultrathin sections were cut in that region and stained with uranyl acetate and lead citrate and examined under a Jeol 100 B transmission electron microscope at 80 kV.

Results

The response to nematode invasion was virtually the same in each of the three potato cultivars and similar to that reported elsewhere (Hoopes, Anderson & Mai, 1978). The first phase involved cells mainly of the root cortex, which were subjected to mechanical damage by the invading nematode as it moved towards the stele. Upon reaching their feeding positions the heads of the juveniles were within or close to the endodermal cells and orientated toward the provascular system. Four days after nematode inoculation, the modifications of the provascular tissue were clearly evident, with the parenchyma tissue transformed into a syncytium through cell

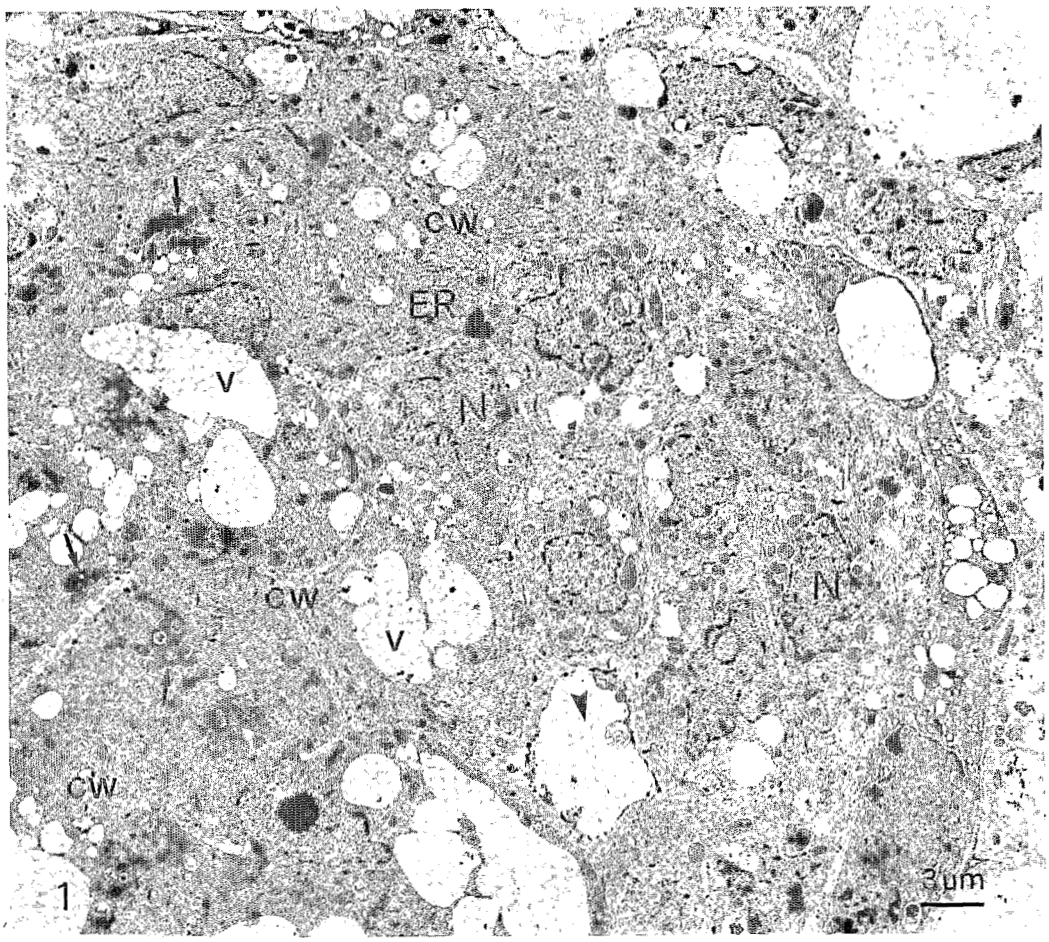

Fig. 1. Micrograph of a portion of a syncytium in cv. Diamant, four days after inoculation. The parenchymatous cells of the procambial tissue show a process of wall dissolution, with wall stubs and gaps in the cytoplasm. The metabolic activity of the cytoplasm is very high, nuclei have amoeboid profiles and a protoxylem vessel is included in the syncytium (head arrow). Starch grains and protein bodies are present in the proplastids (arrow).

List of abbreviations : cw = cell wall; ft = feeding tubes; ER = endoplasmic reticulum; m = mitochondrion; mi = microtubules; N = nucleus; nc = necrotic cell; ne = nematode; nu = nucleolus; p = plastid; ph = phloem; SER = smooth endoplasmic reticulum; sl = nematode stylet; st = starch; sy = syncytium; v = vacuole; x = xylem.

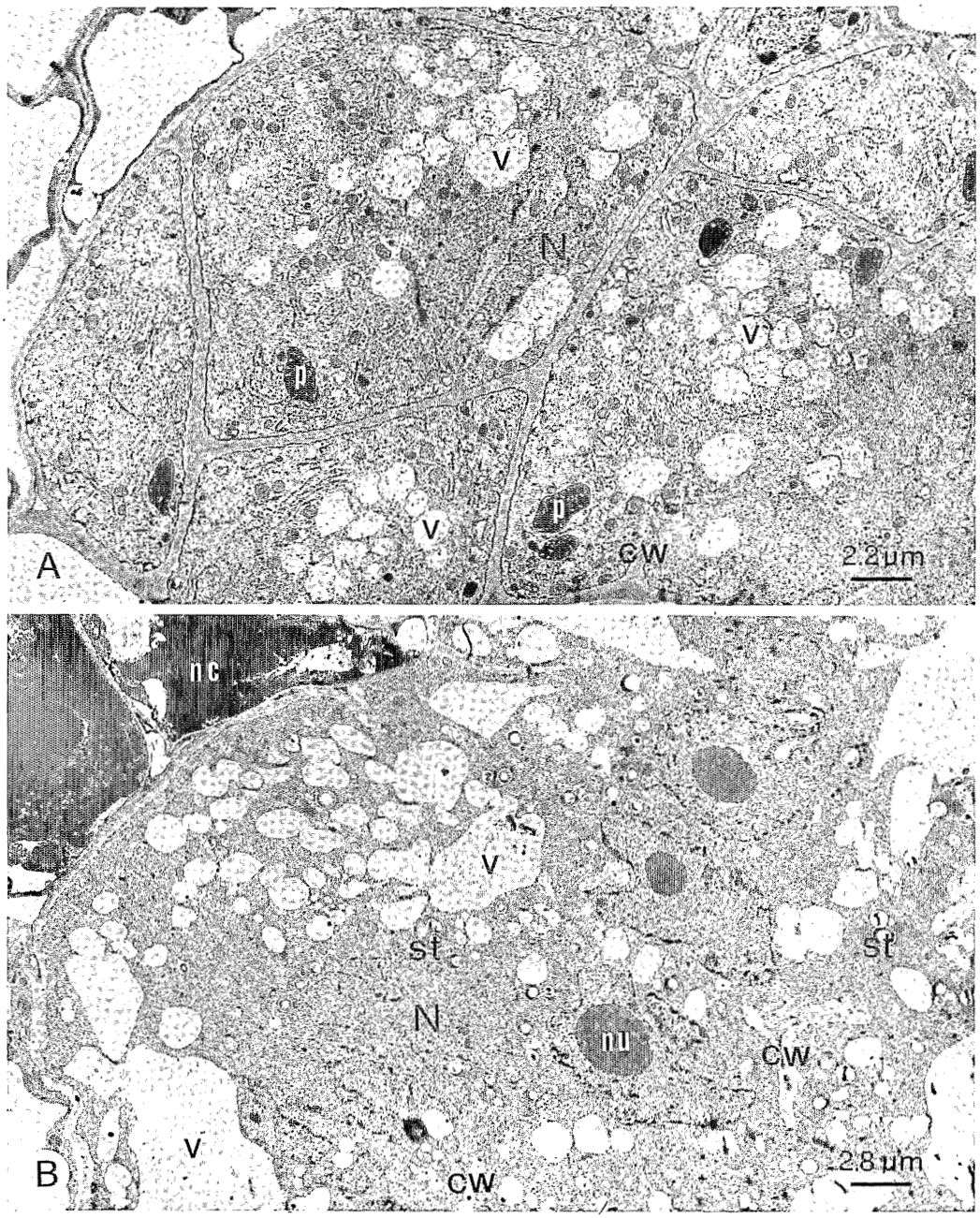

Fig. 2. A : Cross section through syncytial cells in cv. Anosta, four days after inoculation. Cell wall breakdown is in progress. The ground substance of the cytoplasm is granular; endoplasmic reticulum, ribosomes and polysomes have the appearance of active synthesis. The cells still maintain the features of meristematic tissue. — B : Cross section through part of a syncytium induced in cv. Irene root, four days after inoculation. Large areas of cell walls have been digested. Cytoplasm is dense and contains many vacuoles and proplastids rich in starch grains. Nuclei are highly amoeboid.

Abbreviations : see Fig. 1.

wall dissolutions (Figs 1, 2 A, B). Progressive cell wall breakdown in adjacent cells, stimulated by nematode feeding, extended the syncytium. Some cells of the endodermis were necrotic, indicating where the nema-

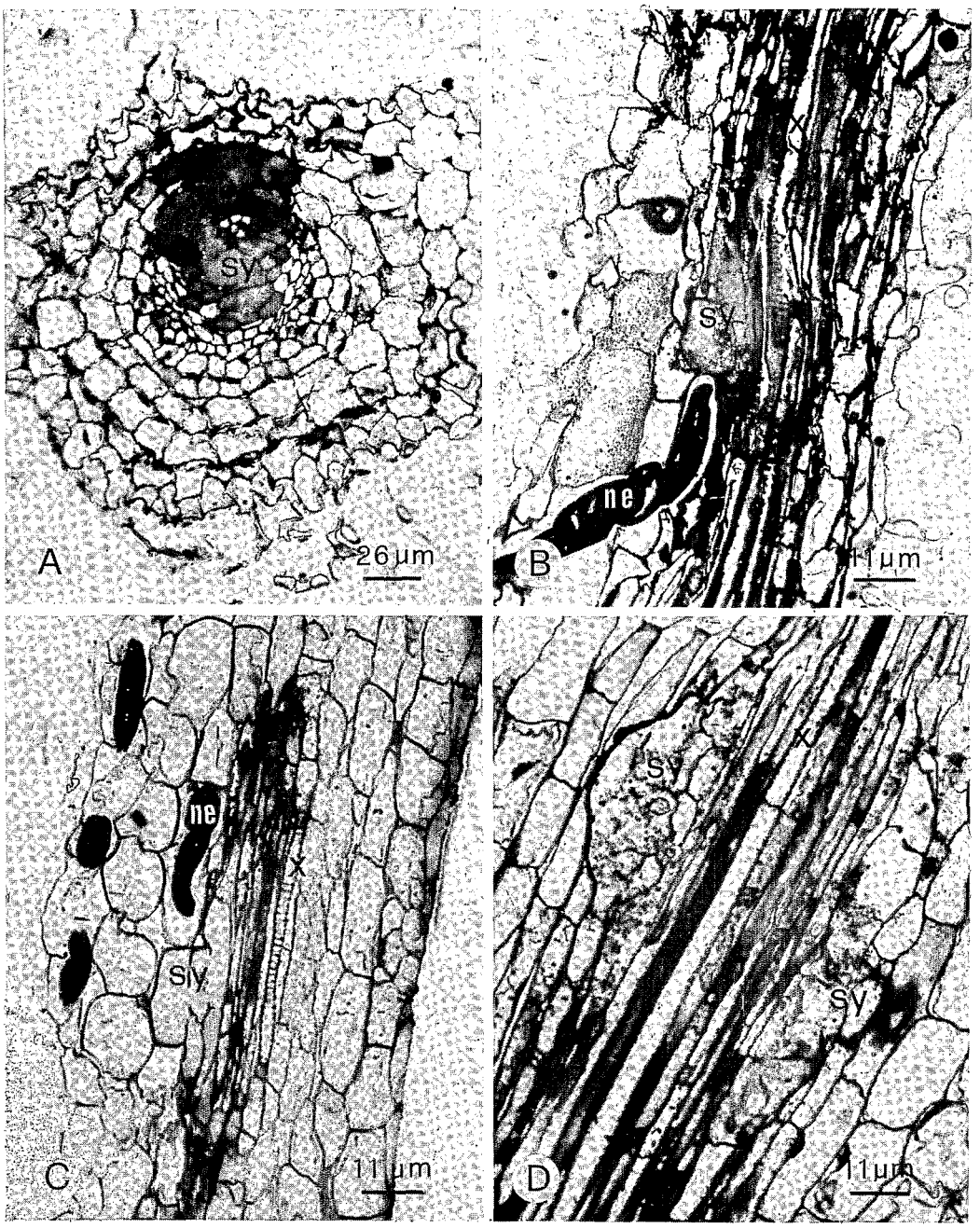

Fig. 3. Light micrograph of root tissues showing syncytia formed twelve days after inoculation with *Globodera pallida* pathotype Pa 3. — A : Transverse section through a cv. Diamant root showing a very large syncytium. The major part of the syncytium is located in the central portion of the stele enclosing xylem vessels. — B : Longitudinal section through a cv. Diamant root. The head of the nematode is located in the endodermis, where the syncytium is initiated. The xylem vessels are crushed between the syncytial cells. — C : Longitudinal section through part of a syncytium in cv. Anosta root. The syncytium is located in the cortex, where a nematode is feeding and extends to the outer portion of the vascular bundle. Another nematode is entering through the rhizodermis. Note the difference in staining of the cytoplasm compared with Fig. 3 A. — D : Longitudinal section through an Irene root containing two syncytia, located on both sides of the vascular bundle. Cell wall breakdown is evident in both syncytia with less dense cytoplasm.

Abbreviations : see Fig. 1.

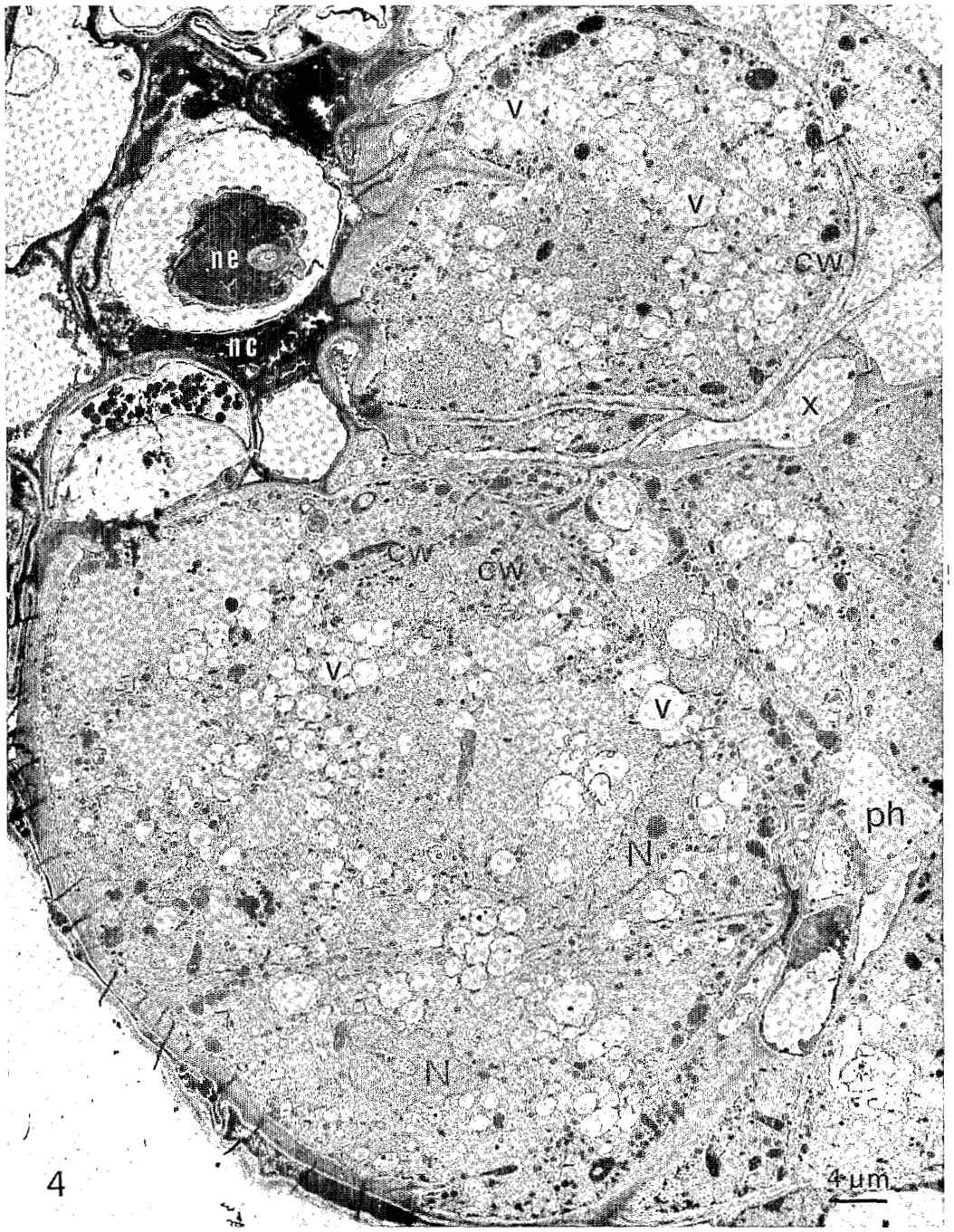

Fig. 4. Cross section through a syncytium induced in cv. Diamant twelve days after inoculation. The head region of the nematode is enclosed in a tunnel of necrotic cells. Syncytial cells are expanded into the vascular cylinder and enclose xylem and phloem elements. Large areas of the cell walls have been dissolved and the cytoplasm is dense containing many small vacuoles. There are no wall ingrowths.

Abbreviations : see Fig. 1.

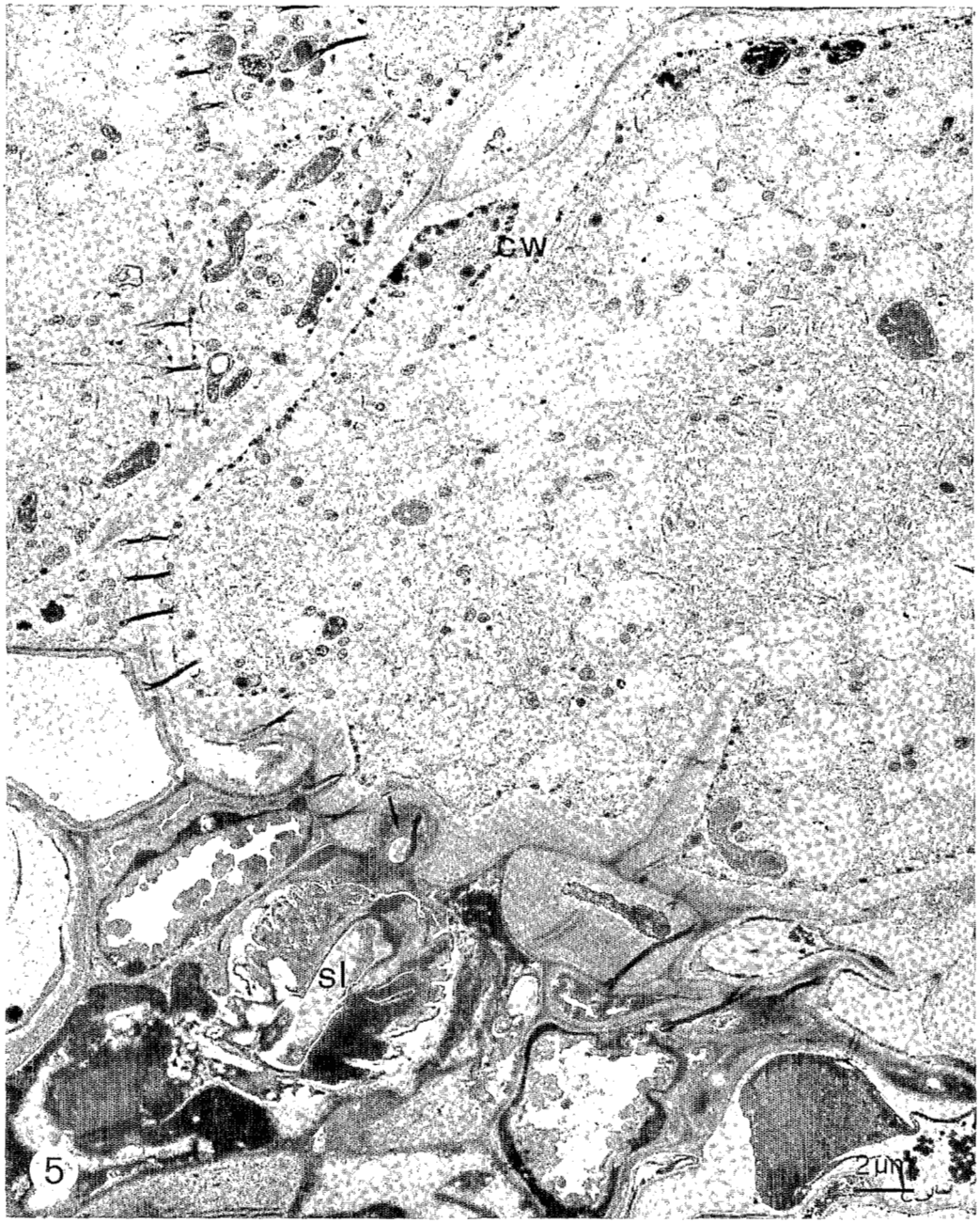

Fig. 5. Cross section through the feeding site of the nematode in cv. Diamant. Tip of the nematode stylet is inserted in the cell wall and surrounded by the feeding plug (arrow). Plasma membrane is intact; the cytoplasmic zone close to the feeding plug is free of larger organelles and has fewer ribosomes than in the surrounding cytoplasm. Osmiophilic material is present along the cell wall.
Abbreviations : see Fig. 1.

tode had penetrated the root cells and had established its feeding site (Fig. 2 B). At this time of root infestation no differences were detectable between the three cultivars.

The modifications of the fine structure of the syncytium consisted of thickened cell walls and some walls reduced to gaps and stubs due to wall breakdown.

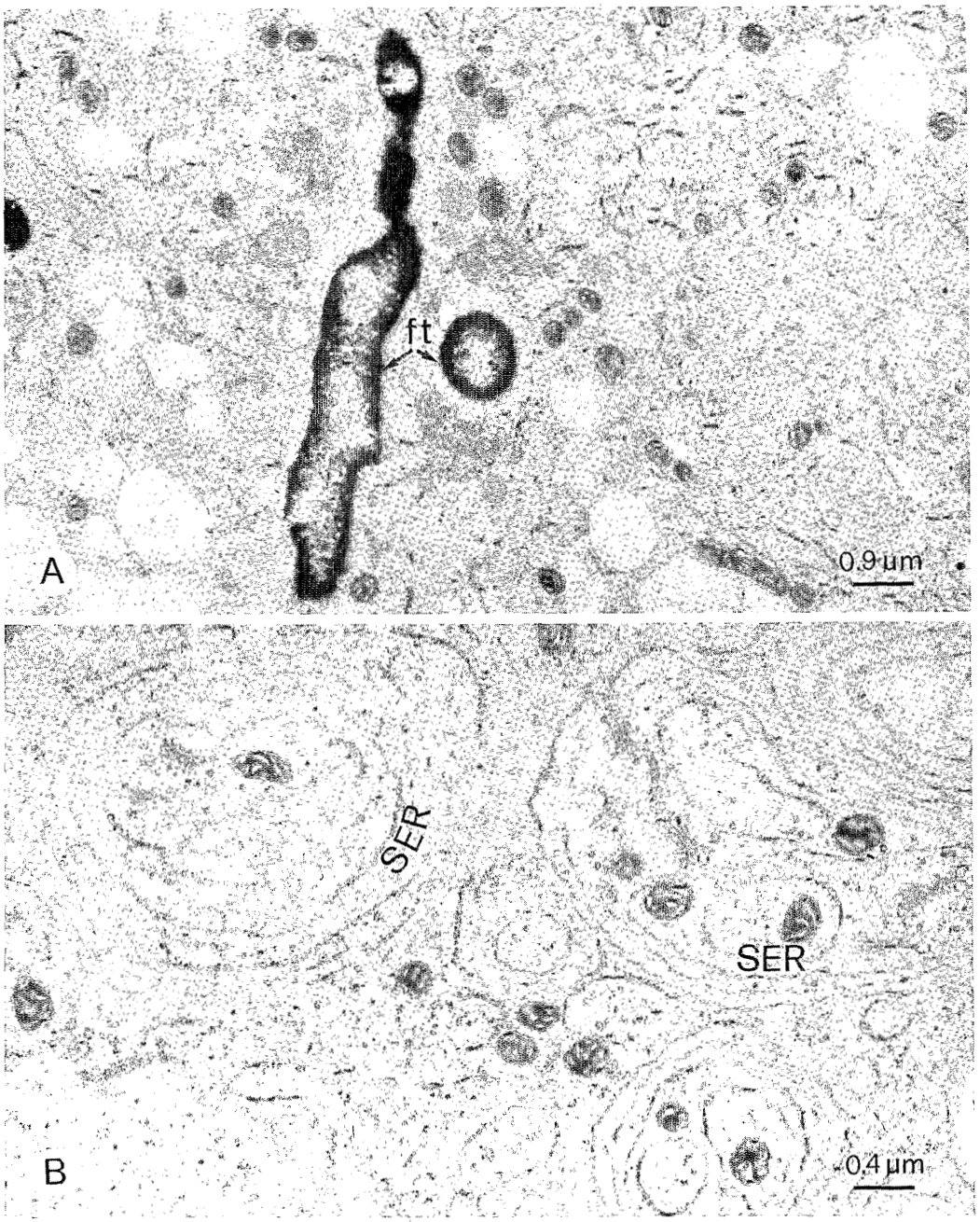

Fig. 6. A : Feeding tubes in longitudinal and transerve section in the syncytium of cv. Diamant. Note the membranous structures in the dense cytoplasm near the feeding tubes. — B : Detail of the cytoplasmic content of a syncytium in cv. Diamant. The main feature of the cytoplasm is the system of smooth endoplasmic reticulum arranged in whorls. The tubules, in cross section, are branched and enclose other organelles such as Golgi bodies, polyribosomes, and mitochondria with heavily stained cristae.
Abbreviations : see Fig. 1.

Syncytia showed increased cytoplasmic density with numerous organelles and abundance of membranous material, particularly in cvs Diamant and Anosta roots (Figs 1, 2 A). Sections of cv. Irene roots showed that the cytoplasm of modified cells in the syncytium was less dense, endoplasmic reticulum was scarce and vacuoles

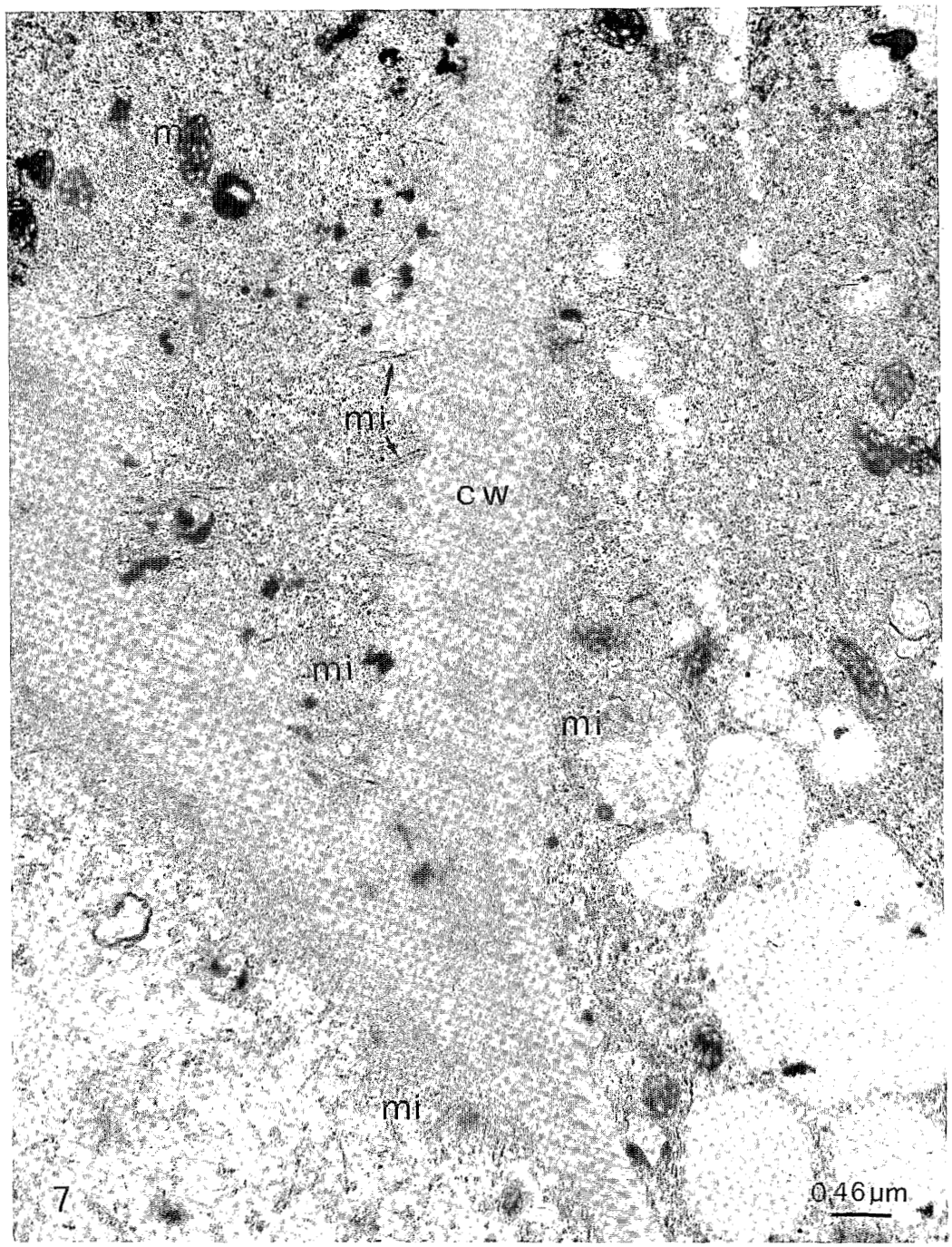

Fig. 7. A portion of a cell wall in a syncytium in Diamant root. Large numbers of microtubules are present along the cell wall, where rapid wall synthesis is thought to occur.
Abbreviations : see Fig. 1.

were more numerous and larger than in the other two cultivars (Fig. 2 B). Proplastids contained more starch grains in cv. Irene than in cvs. Diamant and Anosta. The nuclei were highly amoeboid in the three syncytia and

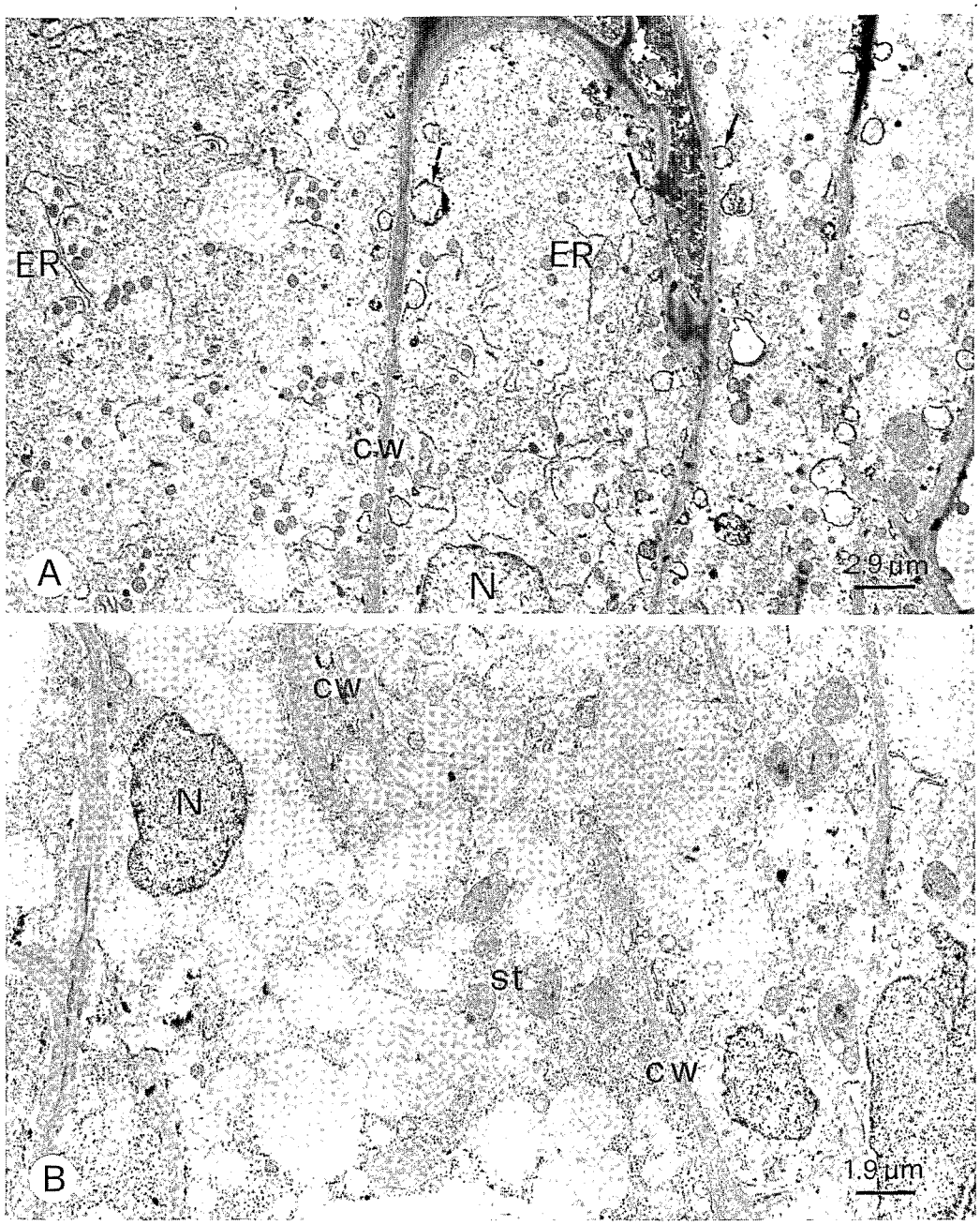

Fig. 8. A : Cross section through a syncytium in cv. Anosta root, twelve days after inoculation. Paramural bodies delimited by osmiophilic membranes are present along the cell wall (arrow). The ground cytoplasm is granular and contains profiles of rough, and tubules of smooth, endoplasmic reticulum. The metabolic activity in those cells is clearly less than in the syncytial cells of Diamant. — B : Portion of a syncytium in cv. Irene twelve days after inoculation. Many cells have been incorporated into the syncytium. The cytoplasm is highly vacuolated and contains many ribosomes, mitochondria and plastids and profiles of rough but not smooth endoplasmic reticulum.

Abbreviations : see Fig. 1.

appeared to enclose portions of cytoplasm (Fig. 1) or in some sections a single nucleus appeared as several small nuclei because its lobes had been separately sectioned (Fig. 2 B).

Syncytial cells were well developed in all three susceptible cultivars twelve days after nematode inoculation. However, there were differences in the structural features of the syncytia, as observed by light microscopy. In cv. Diamant the syncytia had denser cytoplasm (Fig. 3 A, 3 B) than in cv. Anosta roots (Fig. 3 C); in cv. Irene roots the syncytial cells were highly vacuolated containing scarce cytoplasm and the evident wall fragments indicated the shape of the original cells prior to their incorporation (Fig. 3 D).

The sites of syncytium formation differed between the cultivars. In Diamant, they were formed inside the vascular tissue; in transverse sections the syncytial cells were located in the central portion of the stele, stating from the endodermis and enclosing xylem elements (Fig. 3 A). The nematodes appeared to feed on endodermal cells where the anterior part of the parasite was often located (Fig. 3 B). In cvs Anosta and Irene the major portion of the syncytium was located outside the central area of the vascular bundle. Therefore, in longitudinal sections the central vascular portion was always devoid of syncytia (Fig. 3 C, D) and the nematodes were located in cortical cells, adjacent to the endodermis (Fig. 3 C).

Figure 4 shows the ultrastructural features of a syncytium induced in cv. Diamant, twelve days after nematode inoculation. The head of the nematode was localised in a tunnel of necrotic cells. Syncytial cells were greatly enlarged and vascular bundles were sandwiched in between. The structure of the cytoplasm was essentially the same as in syncytia four days after inoculation; all organelles were well preserved and there were many small vacuoles scattered throughout the cytoplasm. Nuclei maintained their amoeboid profiles and intact cell walls as well as fragments were thickened. No wall ingrowths were observed along the xylem vessels (Fig. 4).

The cell wall adjacent to the nematode lip region increased in thickness. In some sections cells directly fed upon by the nematode were easily recognisable due to the presence of an electron dense feeding plug in the syncytial wall, through which the nematode inserted its stylet (Fig. 5) and by portions of the feeding tubes (cut in different planes) dispersed in the cytoplasm (Fig. 6 A). In the vicinity of the feeding tubes, electron dense material was scattered in the cytoplasm, in which there was proliferation of smooth endoplasmic reticulum (Figs 5, 6 A).

The enlargement of sections of syncytia showed that the ground substance of the cytoplasm was granular and dense, with rather sparse number of ribosomes, usually in clusters and presumably, polyribosomes. The smooth endoplasmic reticulum tubules were arranged in concentric whorls, enclosing portions of the cytoplasm, mitochondria, Golgi bodies and plastids (Fig. 6 B). All the organelles showed structural evidence of a high synthesizing activity. No wall ingrowths were observed, but microtubules were associated with the cell walls in localised areas, as the microtubule involvement in secondary thickenings of differentiating xylem elements (Fig. 7).

In sections of syncytia from cv. Anosta roots, twelve days after inoculation, the cytoplasmic ground material was granular but less dense than in cv. Diamant. Many organelles, including mitochondria, endoplasmic reticulum and plastids were widely distributed within the cytoplasm (Fig. 8 A). The mitochondria were of similar appearance to those in unaffected cells, apart from the slightly enlarged cristae. Endoplasmic reticulum was present in both smooth and rough forms (Fig. 8 A). The smooth tubules appeared to be arranged in parallel arrays (Fig. 8 A). Membrane-bounded vesicular bodies occurred at frequent intervals on the walls of the cells within the syncytia. Protuberances in the form of vesicular aggregates or boundary formation were associated with the plasmalemma or with mitochondria and smooth endoplasmic reticulum (Fig. 8 A) (Huang & Maggenti, 1969). The aggregates were bounded by plasma membranes whose size was emphasized by the deposit of electron dense material; a similar deposit was also localised along the cell wall between the wall itself and the plasmalemma (Fig. 8 A).

At the same stage (twelve days after inoculation) syncytia in cv. Irene roots differed somewhat from those in cvs Diamant and Anosta. The differences were mainly changes in the nature of the endoplasmic reticulum and in the degree of vacuolization. The cytoplasm contained predominantly rough-surfaced endoplasmic reticulum and free ribosomes. Plastids contained protein crystals and starch grains, mitochondria were well preserved and the vacuoles were larger and more numerous than in the syncytia of the other two cultivars (Fig. 8 B). Nuclei showed that heterochromatin was condensed and lined the nuclear membranes, whereas the euchromatin was diffused throughout the nucleoplasm (Fig. 8 B).

Discussion

The variation of nematode and syncytia location may be related to the different degree of susceptibility between the three cultivars. These differences suggest the existence of an array of genes in both nematodes and potatoes that control the host-parasite interaction. The establishment of the nematode and the development of its feeding site must result from the interaction of many genes in both host and parasite (Acedo, Dropkin & Luedders, 1984). Kim, Kim and Riggs (1986) reported that there are differences in morphology and location between syncytia induced by *H. glycines* in soybean, Cleome and Lespedeza and they suggest that these

differences may be related to the degree of tolerance and susceptibility of the plants. From our observation the roots of cv. Diamant show the typical response of susceptible roots invaded by *Heterodera* spp. The syncytial cells contain dense cytoplasm and smooth endoplasmic reticulum indicating high secretory activity as reported in susceptible soybean roots attacked by *H. glycines* (Riggs, Kim & Gipson, 1973), and in oil radish and sugarbeet invaded by *H. schachtii* (Wyss, Stender & Lehmann, 1984; Bleve-Zacheo & Zacheo, 1987).

In most plant cells the smooth endoplasmic reticulum is inconspicuous and it is difficult to decide whether the smooth portions are biochemically and morphologically specialized in a permanent fashion, or whether they are merely short-lived, perhaps produced by a temporary loss of ribosomes, as has been reported in sugar beet syncytia induced by *H. schachtii* (Bleve-Zacheo & Zacheo, 1987). Whorls of smooth cisternae surrounding dictyosomes and mitochondria in the syncytia of cv. Diamant roots (Fig. 6 B) suggest that the tubules serve in the collection of raw material and/or energy-rich compounds and the resultant flux into the base of the dictyosomes. The finished products, assembled in vesicles, are discharged into the cytoplasm. Mitochondria in the syncytium have densely packed cristae which almost completely obscured the nucleoids. The high rate of respiration, indicated by the number and conformation of these mitochondria, may be connected with the consumption of energy in pumping solutes across the plasma membrane. The changes in the structure and the physiological function of cell organelles are clearly influenced by the nematodes, which induce the production of specific compounds required as nutrients. What is surprising is the absence of the development of cell wall ingrowths, that usually occur in syncytia close to the xylem vessels (Jones & Northcote, 1972). In our study we did not notice any change in the cell walls apart from a pronounced thickening. We only found microtubules (Fig. 7) and microfibrils which would bring about an extension of the plasmalemma, perhaps some time after nematode infestation. Kim, Kim and Riggs (1986) suggest that differences in the number of cell wall ingrowths relate to the degree of susceptibility of plants to infestation by *H. glycines*. In our case the syncytium induced in Diamant roots was well preserved twelve days after nematode inoculation, indicating that it was an effective nutrient source, but without wall ingrowths.

In cvs Anosta and Irene the syncytium appears to begin in the cortical cells outside the stele, which is not seriously influenced by the infestation. This is in agreement with the observations of Kim, Kim and Riggs (1986) who related the location of the syncytia to the degree of damage to plants. In addition, with time, the cytoplasmic contents of the syncytial cells in cvs Anosta and Irene appear to be reduced. In cv. Anosta the vacuolization of the cytoplasm is not extensive, but the major part of the cisternae of the endoplasmic reticulum are rough surfaced, indicating that they operate differently from those observed in syncytia induced in cv. Diamant. The cristae of the mitochondria were not densely packed and nucleoids were evident, indicating an absence of high respiration activity.

The presence of vesicular and membranous structures, whose delimiting membranes are very osmiophilic (phenolic substances?), along the syncytial cell wall, accords with the observation of Riggs, Kim and Gipson (1973), and Rice, Leadbeater and Stone (1985). They suggest that the function of paramural bodies is to thicken the wall and seal off the syncytium from the rest of plant, and this appears to be so in cv. Anosta. The syncytia in cv. Irene had large vacuoles and scarse cytoplasm, indicating that they were not functioning as a good supply of nutrients for the nematode.

The sequence of changes in syncytia in the roots of the three potato cultivars studied suggest that the susceptibility of the plants varies in relation to the nutritional quality of the host plant, without impeding nematode reproduction. Cultivar Diamant proved to be more susceptible to the nematode than cvs. Anosta and Irene, the latter being reported as a good host of Pa 3 pathotype (Seinhorst & Oostrom, 1984).

Our observations of histological changes may be useful in the selection of potato cultivars that can influence not only the proportion of invading juveniles that become females but also the average number of eggs per female.

References

Acedo, J. R., Dropkin, V. H. & Luedders, V. D. (1984). Nematode population attrition and histopathology of *Heterodera glycines*-soybean associations. *J. Nematol.*, 16 : 48-57.

Bleve-Zacheo, T. & Zacheo, G. (1987). Cytological studies of the susceptible reaction of sugar beet roots to *Heterodera schachtii*. *Physiol. molec. Pl. Pathol.*, 30 : 13-25.

Bleve-Zacheo, T., Melillo, M. T. & Zacheo, G. (1990). Ultrastructural response of potato roots resistant to cyst nematodes *Globodera rostochiensis* pathotype Rol. *Revue Nématol.*, 13 : 29-36.

Hoopes, R. W., Anderson, R. E. & Mai, W. F. (1978). Internal response of resistant and susceptible potato clones to invasion by potato cyst-nematode *Heterodera rostochiensis*. *Nematropica*, 8 : 13-21.

Huang, C. S. & Maggenti, A. R. (1969). Wall modifications in developing giant cells of *Vicia faba* and *Cucumis sativus* induced by root-knot nematode, *Meloidogyne javanica*. *Phytopathology*, 59 : 931-937.

Jones, M. G. K. (1981). The development and function of plant cells modified by endoparasitic parasites. In : Zuckerman, B. M. & Rohde R. A. (Eds) *Plant Parasitic Nematodes, Vol. III.* New York, Academic Press : 225-279.

Jones, M. G. K. & Northcote, D. H. (1972). Nematode induced syncytium -a multinucleate transfer cell. *J. Cell Sci.*, 10 : 789-809.

KIM, Y. H., KIM, K. S. & RIGGS, R. D. (1986). Morphological characteristics of syncytia in susceptible hosts infected by the soybean cyst nematode. *Phytopathology*, 76 : 913-917.

RICE, S. L., LEADBEATER, B. S. & STONE, A. R. (1985). Changes in cell structure in roots of resistant potatoes parasitized by potato cyst nematodes. I. Potatoes with resistance gene H derived from *Solanum tuberosum andigena*. *Physiol. Pl. Pathol.*, 27 : 219-234.

RICE, S. L., STONE, A. R. & LEADBEATER, B. S. (1987). Changes in cell structures in roots of resistant potatoes parasitized by potato cyst nematodes. II. Potatoes with resistance derived from *Solanum vernei*. *Physiol. Molec. Pl. Pathol.*, 31 : 1-14.

RIGGS, R. D., KIM, K. S. & GIPSON, I. (1973). Ultrastructural changes in Peking soybeans infected with *Heterodera glycines*. *Phytopathology*, 63 : 76-84.

SEINHORST, J. W. (1985). The development of individuals and populations of cyst nematodes on plants. In : Lamberti, F. & Taylor, C. E. (Eds) *Cyst Nematodes*, New York & London, Plenum Press : 101-117.

SEINHORST, J. W. & OOSTROM, A. (1984). Comparison of multiplication rates of three pathotypes of potato cyst nematodes on various susceptible and resistant cultivars. *Meded. Fac. Landbouww. Rijksuniv. Gent.*, 49 : 605-610.

SPURR, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.*, 26 : 31-43.

WYSS, U., STENDER, C. & LEHMANN, H. (1984). Ultrastructure of feeding sites of the cyst nematode *Heterodera schachtii* Schmidt in roots of susceptible and resistant *Raphanus sativus* var. *oleiformis* cultivars. *Physiol. Pl. Pathol.*, 25 : 21-38.

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