Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by *Heterodera glycines*

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SUMMARY

Early responses of susceptible and resistant soybean cultivars to infection by the soybean cyst nematode *Heterodera glycines* are the accumulation of smooth (ER) and rough (RER) endoplasmic reticulum within a thick-walled initial syncytial cell (ISC). Nematode secretions are surrounded by a narrow region of ER that integrates with the RER of the host cytoplasm. The ISC and adjacent cells forming the syncytium show slight enlargement at 18 h after inoculation but have definite disruptions in their connecting cell walls. Although the ER closest to the stylet and associated secretions were not associated with mitochondria, the surrounding cytoplasm had RER interspersed among numerous mitochondria, plastids and other organelles. In most resistant cultivars, the syncytial cytoplasm had extensively distributed wide cisternae extending throughout the RER. These cisternae were less prevalent in syncytia induced in susceptible cultivars. Secretions varying greatly in electron density were found in 18 h to 2 day infections. The secretions nearest the nematode stylet appeared uniform in distribution, whereas, others had darkened peripheral cylindrical boundaries with clear to partially occluded centers. Syncytia in susceptible and resistant cultivars 18 h to 4 days after inoculation were hypertrophied with hyperplasia, expanded regions of cell wall dissolutions, some with lysosome-like particles, and cell wall depositions of callose.

RÉSUMÉ

Ultrastructure des réactions précoces des racines de sojas sensibles et résistants à l'infestation par Heterodera glycines

Chez les cultivars de soja sensibles et résistants à *Heterodera glycines,* les premières réactions à l'infestation consistent en une accumulation de reticulum endoplasmique lisse (REL) et granulaire (REG) à l'intérieur de la cellule initiale du syncitium (CIS), à paroi épaisse. Les sécrétions du nématode sont entourées par une plage étroite de REL qui s'intègre au REG du cytoplasme de l'hôte. Dix-huit heures après l'infestation, la CIS et les cellules adjacentes formant le syncytium ne montrent qu'un léger accroissement mais les ruptures des parois les séparant sont nettes. Bien que le REL le plus proche du stylet et les sécrétions connexes ne soient pas associés à des mitochondries, le cytoplasme entourant cette zone montre un REG entremêlé de nombreux mitochondries, plastides et autres organites. Chez les cultivars les plus résistants le cytoplasme syncytial montre d'importants saccules largement répartis dans le REG. Ces saccules sont moins abondants dans le syncytium induit chez les cultivars sensibles. Des sécrétions, très variables quant à leur opacité aux électrons, ont été observées dans les infestations datant de 18 heures à 2 jours. Les sécrétions les plus proches du stylet du nématode ont une apparence uniforme, tandis que d'autres ont des limites périphériques sombres, en cylindre, avec une partie centrale claire ou partiellement obstruée. Chez les cultivars susceptibles et résistants, 18 heures à 4 jours après l'inoculation, les syncytia sont hypertrophiés, ce qui s'accompagne d'hyperplasie, de zones étendues à parois cellulaires dissoutes — certaines comportant des particules semblables à des lysosomes — et de dépôts de callose sur les parois cellulaires.

The initiation and development of syncytial nurse cells in host tissues is critical for the survival of infective cyst nematodes. The host-parasite relationship of the soybean cyst nematode (*Heterodera glycines* Ichinohe) in soybean (*Glycine max* [L.] Merr.) roots has been observed through light microscopy (Ross, 1958; Endo, 1964, 1965; Endo & Veech, 1970; Veech & Endo, 1970; Acedo, Dropkin & Luedders, 1984), transmission electron microscopy (Gipson, Kim & Riggs, 1971; Riggs, Kim & Gipson, 1973; Endo, 1978; Kim, Riggs & Kim, 1987), and scanning electron microscopy (Jones & Dropkin, 1976). The feeding plugs, formed at the feeding sites of soybean roots infected by the soybean cyst nematode (Endo, 1978) and other host-parasite interfaces (Wyss, Stender & Lehmann, 1984), function as a seal between the stylet and host syncytium during feeding and at molt. The fine-structural observations of *H. glycines* infecting susceptible and resistant cultivar roots provide important data on the mechanisms of resistance and susceptibility (Gipson, Kim & Riggs, 1971; Riggs, Kim & Gipson, 1973; Kim, Riggs & Kim, 1987). Syncytia induced by cyst nematodes contain an

increased number of organelles compared to normal cells from which they are derived. At advanced stages of infection, syncytium walls adjacent to xylem vessels are modified into finger-like in-growths that resemble those of transfer cells (Jones & Northcote, 1972; Jones & Gunning, 1976; Stender, Lehmann & Wyss, 1982; Wyss, Stender & Lehmann, 1984).

Recent light microscope observations of syncytia and giant cells emphasize the presence of feeding tubes near the feeding site of these nurse cells (Rumpenhorst, 1984). *In vivo* studies of the feeding process showed the interactions of stylet movement and secretion, cytoplasmic streaming, and food ingestion (Wyss & Zunke, 1986). The ultrastructure of feeding tubes shows that the hardened salivary secretions are attached to the stylet of *H. schachtii* and indicates the continuity between the secretion emanating from the stylet and the feeding tube. Wyss and Zunke (1986) and Wyss, Stender and Lehmann (1984) also report that continuous food uptake lasts for up to 1 h with short pauses during which the nematode salivates. Similar feeding tube formation and accumulation of secretion granules in the dorsal gland ampulla occur in *H. glycines* during infection of soybean roots (Endo, 1987).

Although light and electron microscopic observations have provided new information of the soybean cyst nematode - soybean interactions, the initial stages of infection and their relation to subsequent cytological modifications of host tissues have not been systematically examined. This paper describes the ultrastructure of the initial stages of host-parasite interactions between the soybean cyst nematode and roots of susceptible and resistant cultivars with emphasis on nematode secretions (salivation) and host responses in the initial syncytial cell and adjacent cells.

Materials and methods

Juvenile stages of *H. glycines* in infected soybean roots were prepared for electron microscopy by previously described procedures (Endo & Wergin, 1973; Wergin & Endo, 1976; Endo, 1978). Soybean seedlings of susceptible cultivar, Lee, resistant cultivars Bedford and Pickett 71, and a resistant plant introduction line, PI 88788, were raised in vermiculite and inoculated with race 3 or race 4 of the soybean cyst nematode. Samples taken at 18 h to 4 days after inoculation represent material from a series of experiments conducted over several years. Nematode infected root segments were fixed in buffered 3 % glutaraldehyde (0.05 M phosphate buffer, pH 6.8) at 22 °C for 1.5 h; washed for 1 h in six changes of the same buffer; postfixed in 2 % osmium tetroxide in the same buffer for 2 h; dehydrated in an acetone series; and infiltrated with a low viscosity medium (Spurr, 1969). Silver-gray sections of selected nematodes were cut on a Sorvall MT-2 or MT-5000 ultramicrotome with a diamond knife and mounted on uncoated 75 × 300-mesh copper grids. The sections were stained with 2 % aqueous uranyl acetate for 10 min, then with lead citrate for 5 min. Thin sections were viewed in a Philips 301 or 400 electron microscope operating at 60 kV with 20-µm objective aperture.

Results

Infective juveniles of the soybean cyst nematode (SCN) penetrate the roots of both susceptible and resistant cultivars of the soybean. After the establishment of a feeding site and secretion of a feeding plug in the outer wall of a cortical, endodermal, pericyclic, or phloem parenchyma cell, a syncytium is formed by dissolution of adjacent cell walls and the coalescing of their cytoplasm. The fine-structural response to nematode penetration and initial feeding are described in two parts, the susceptible reaction and the resistant reaction. Not all cultivar and race combinations are compared, but selected samples illustrate the nature of the initial responses of susceptible and resistant roots to infection by the soybean cyst nematode.

Susceptible reactions

Lee soybean roots 18 h to 24 h after inoculation with race 4 of SCN.

Among the initial reactions of susceptible Lee soybean to SCN feeding were localized increases in cytoplasmic density and formation of small vacuoles (Fig. 1 A). Stylet insertion in the initial syncytial cell (ISC) was supported by an electron-dense feeding plug and an unusual electron-lucent mass of cell wall material limited by a membrane (arrows) that was continuous with the plasmalemma of the cell (Fig. 1 B). The inner surface of cell walls of the ISC and immediately adjacent cells was irregular in transection. Some cell wall curvatures occurred in cells where wall perforations were associated with cytoplasmic and nuclear movement between affected cells (Fig. 1 C). During early stages of feeding, nematode secretions accumulated in the initial syncytial cells that had thickened walls and had dense accumulations of endoplasmic reticulum (ER). Although the nematode secretions were more electron-dense than the surrounding cytoplasm, no definite membranes separated the secretions from the adjacent ER (Fig. 2). Syncytium development was initiated by cell wall dissolutions and wall separations between the ISC and the adjacent cells.

Cortical cells adjacent to the ISC were affected by the nematode, as demonstrated 1 day after infection. Some cortical cells were destroyed by intracellular migration and others by apparent lack of host response during stylet probing (Fig. 3). Similarly, xylem parenchyma cells were destroyed adjacent to the protoxylem pole

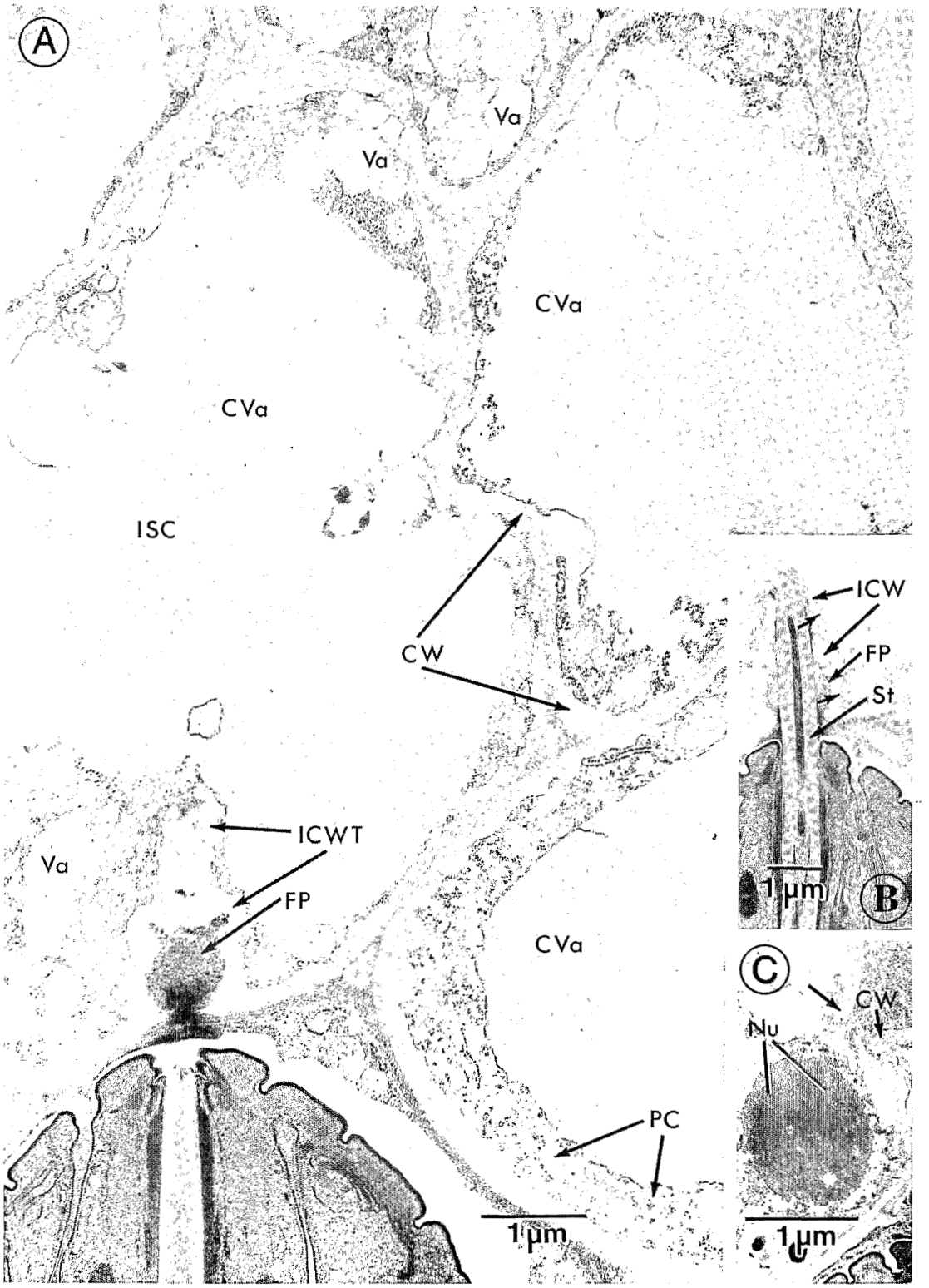

Fig. 1. Cross section of susceptible Lee soybean root 18 h after inoculation with *Heterodera glycines* race 4-A. The syncytial area. Invaginated cell wall thickening (ICWT) corresponds to stylet penetration site (Fig. 1 B). The ICWT is, however, not a usual formation. Small vacuoles (Va) near feeding site and adjacent cell are indicative of initial stages of host response. The two cells anteriad to the nematode are part of a syncytium, but the cell into which the stylet has penetrated is called the initial syncytial cell (ISC). CW : cell wall; CVa : central vacuole; FP : feeding plug; PC : parietal cytoplasm. — B. Longitudinal section through the stylet (St) of the nematode shown in Figure 1 A at a different level. The stylet cone is surrounded by a feeding plug (FP) at the cell surface region and, in this case, by a deposition of cell wall material that appears as an invaginated cell wall (ICW) — C. Section of root and nematode located lower left of Figure 1 A. The cell wall (CW) is discontinuous (arrow) and provides cytoplasmic continuity between the ISC and an adjacent cell with enlarged nucleolus (Nu).

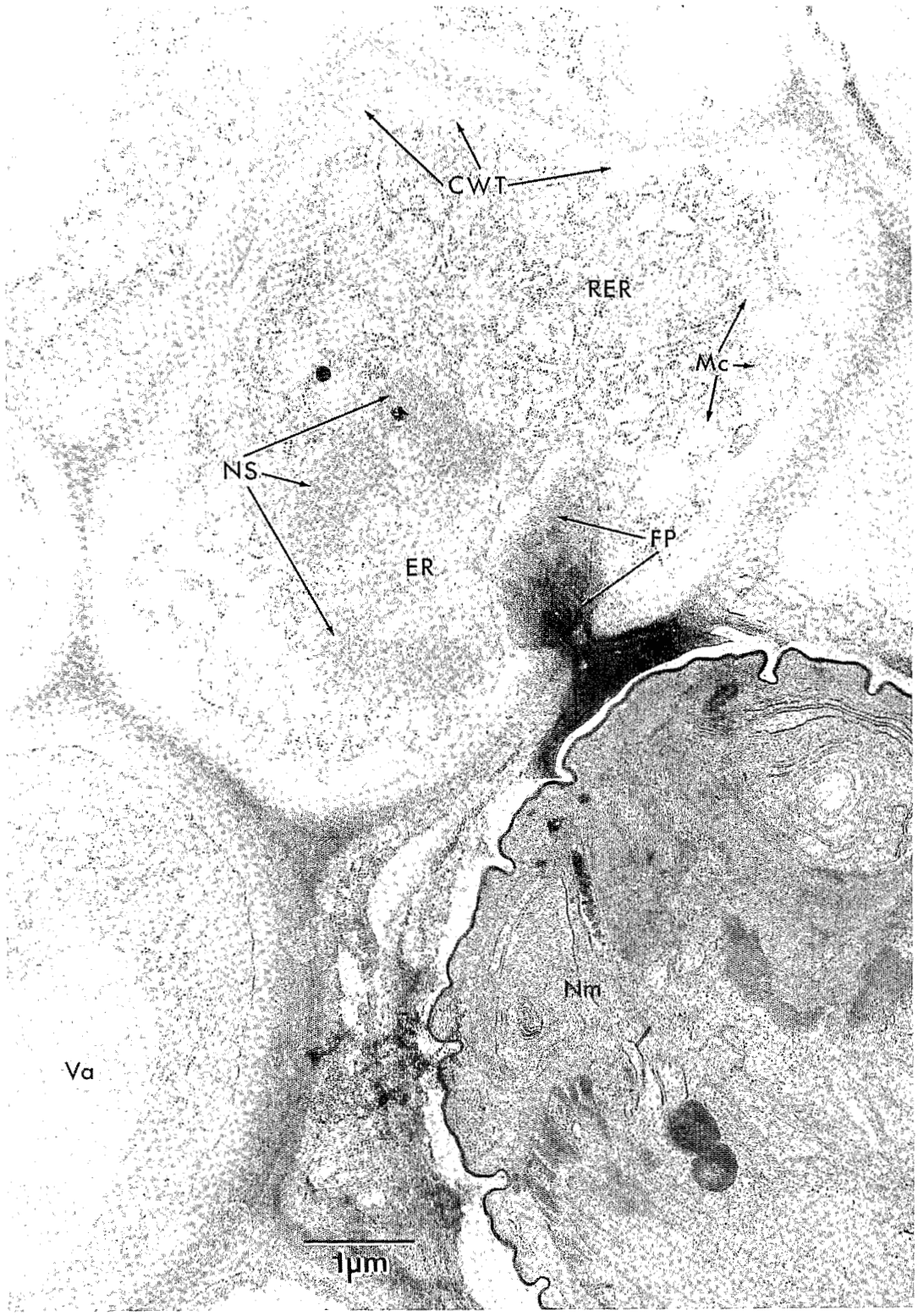

Fig. 2. Nematode secretions (NS) in the initial syncytial cell of Lee soybean root, 18 h after inoculation with *Heterodera glycines* race 4. Secretions are not bound by a membrane, but the secretion-cytoplasm interaction retains a cylindrical shape of the secretions. Irregular wall thickenings (CWT) in the form of deposits occur on the ISC and adjacent cells. These deposits do not appear to have the fibrillar structure of cellulose microfibrils of established cell walls. ER : endoplasmic reticulum; FP : feeding plug; Mc : mitochondria; Nm : nematode; RER : rough endoplasmic reticulum; Va : vacuole.

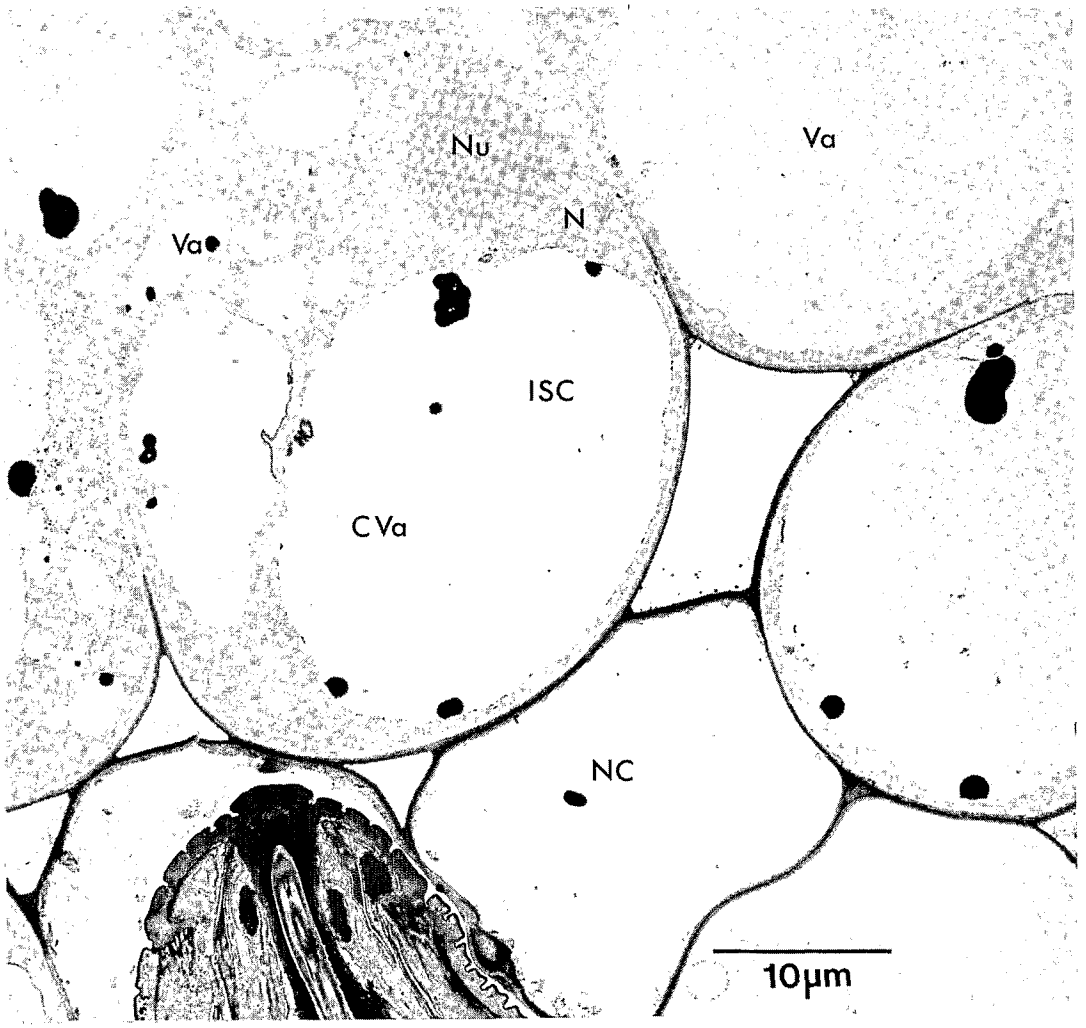

Fig. 3. Cross section of Lee soybean root, 1 day after inoculation with *H. glycines*, race 4. Initial root response to the breakdown of the central vacuole (CVa) and hypertrophy of the nucleus (N) and nucleolus (Nu) of the initial syncytial cell (ISC). NC : necrotic cell; Va : vacuole.

after deep penetration of the host (Fig. 4). At both locations, feeding sites were observed with stylets extended into initial syncytial cells (Figs. 3, 4).

Two days after inoculation with race 4 of SCN

In soybean cultivar Lee, nematode secretions in the ISC were surrounded by electron-lucent zones of smooth ER which in turn occurred in a cytoplasmic matrix of RER. The secretions near the nematode were asymmetrical in outline, whereas the secretion further from the nematode was circular (Fig. 5 A). A similar, circular secretion with a dark periphery was observed from a different nematode on the same host (Fig. 5 B). Sections through other parts of the secretions showed outlines that differed markedly from the more homogeneous masses of secretions observed in earlier stages of infection (Figs 2, 4).

Three days after inoculation with race 3 of SCN

Three days after inoculation, host responses were diverse among cells near the feeding site of the nematode (Fig. 6). Within 3 days, the ISC was greatly hypertrophied and was the base for a syncytium that included several layers of cells opposite and adjacent to the protoxylem poles. The walls of component cells of the syncytium appeared irregular in outline and discontinuous where apparent cell wall dissolutions had occurred

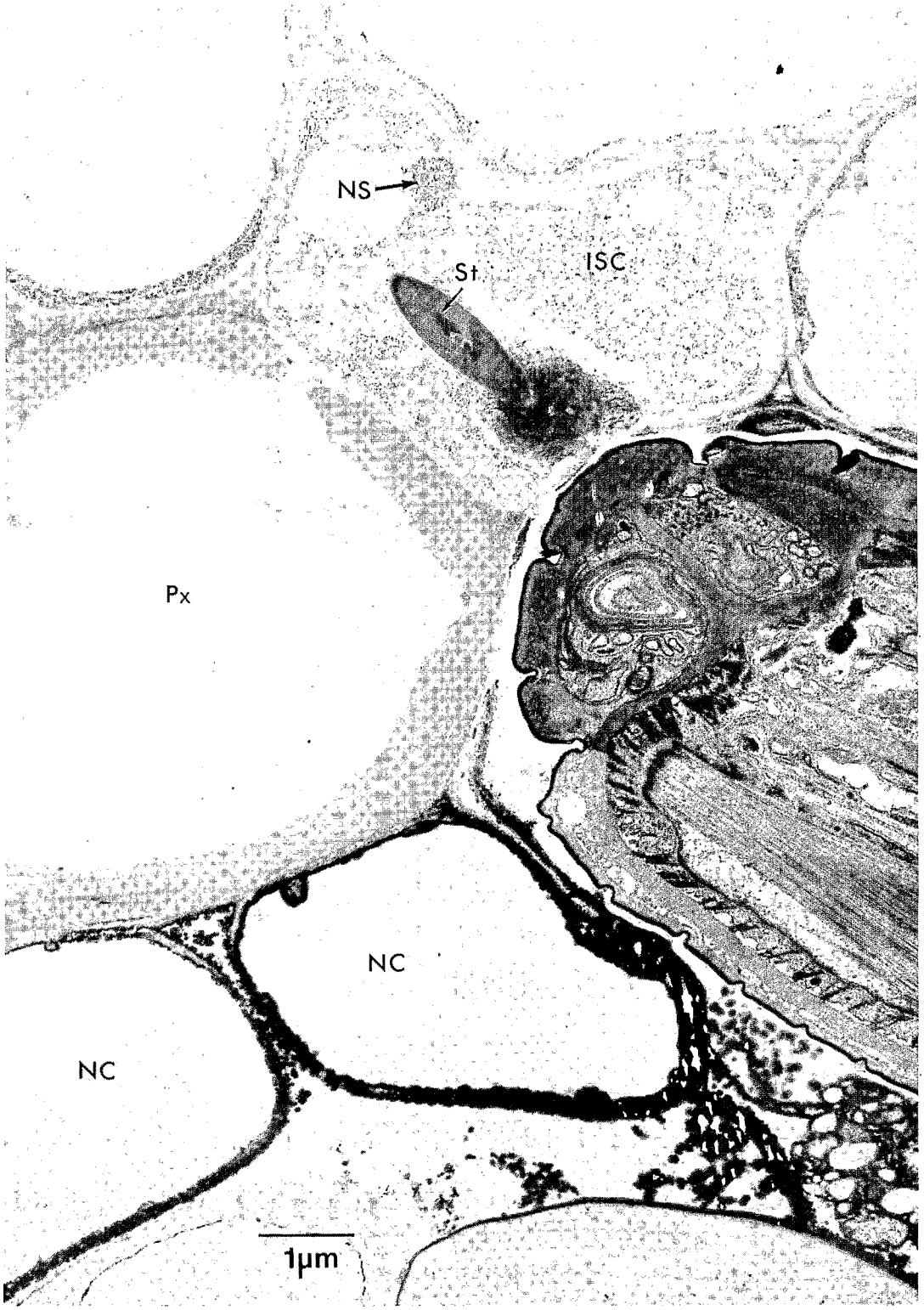

Fig. 4. Cross section through feeding site of *H. glycines* in Lee soybean root 1 day after inoculation with race 4. Stylet (St) located in initial syncytial cell (ISC) adjacent to the protoxylem (Px). Necrotic cells (NC) on opposite side of protoxylem indicate possible sites of destructive stylet probings with lack of host response. NS : nematode secretion.

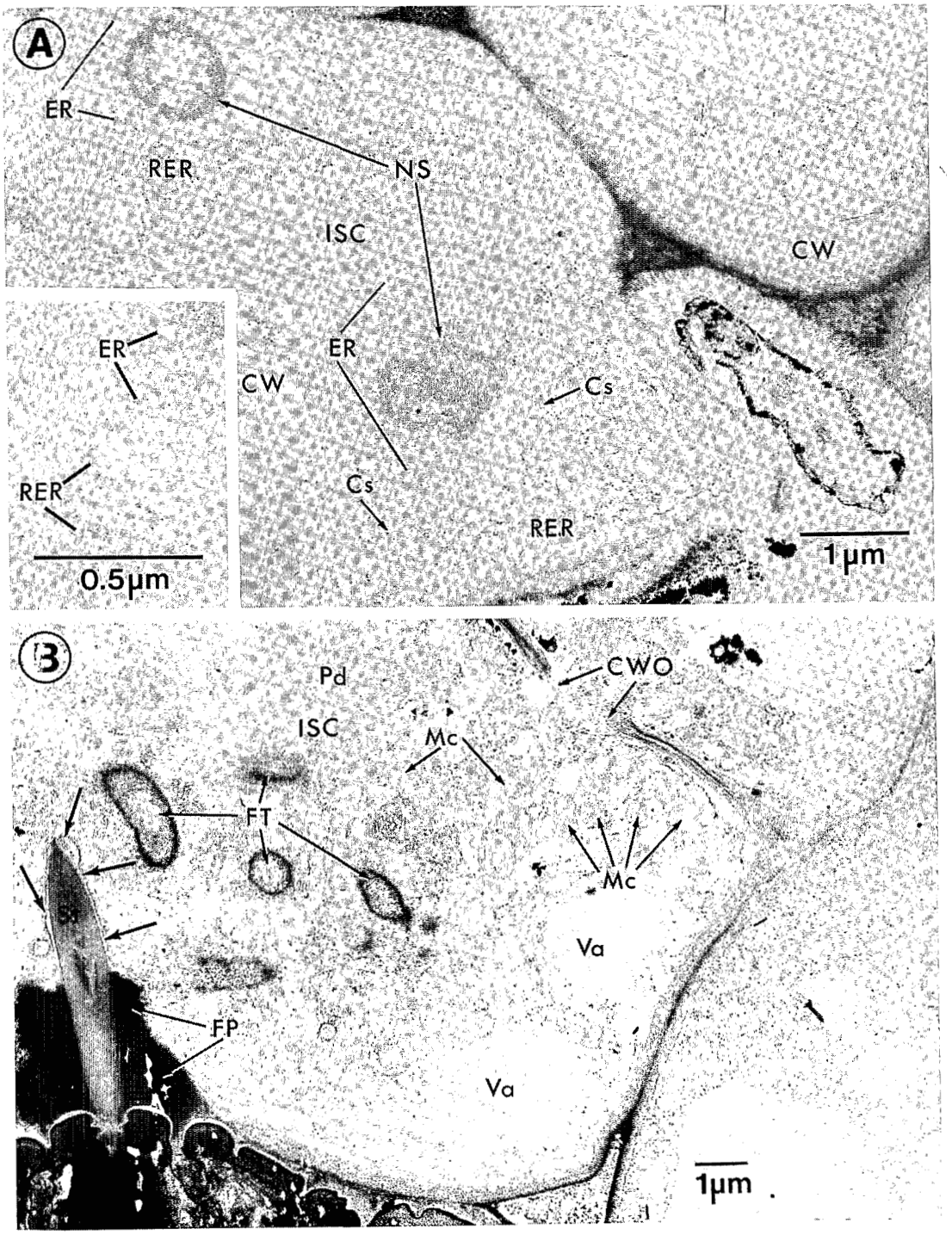

Fig. 5. Section through the initial syncytial cell (ISC) of a syncytium induced in Lee soybean roots, 2 days after inoculation with *Heterodera glycines* race 4. — A. Cross section of nematode secretions (NS) shows the opaque nature of the secretion, surrounded by a perimeter of ER which integrates with the host RER. Note higher mag. inset. Cisternae (Cs) are present. CW : cell wall. — B. Cross section through a stylet (St) tip surrounded by a plasmalemma and initial syncytial cell (ISC). Secretions or feeding tubes (FT) have perimeters of smooth ER. Central region of secretions appear electron-lucent while the perimeters are electron-dense. CWO : cell wall opening; FP : feeding plug; Mc : mitochondria; Pd : plastid; Va : vacuole.

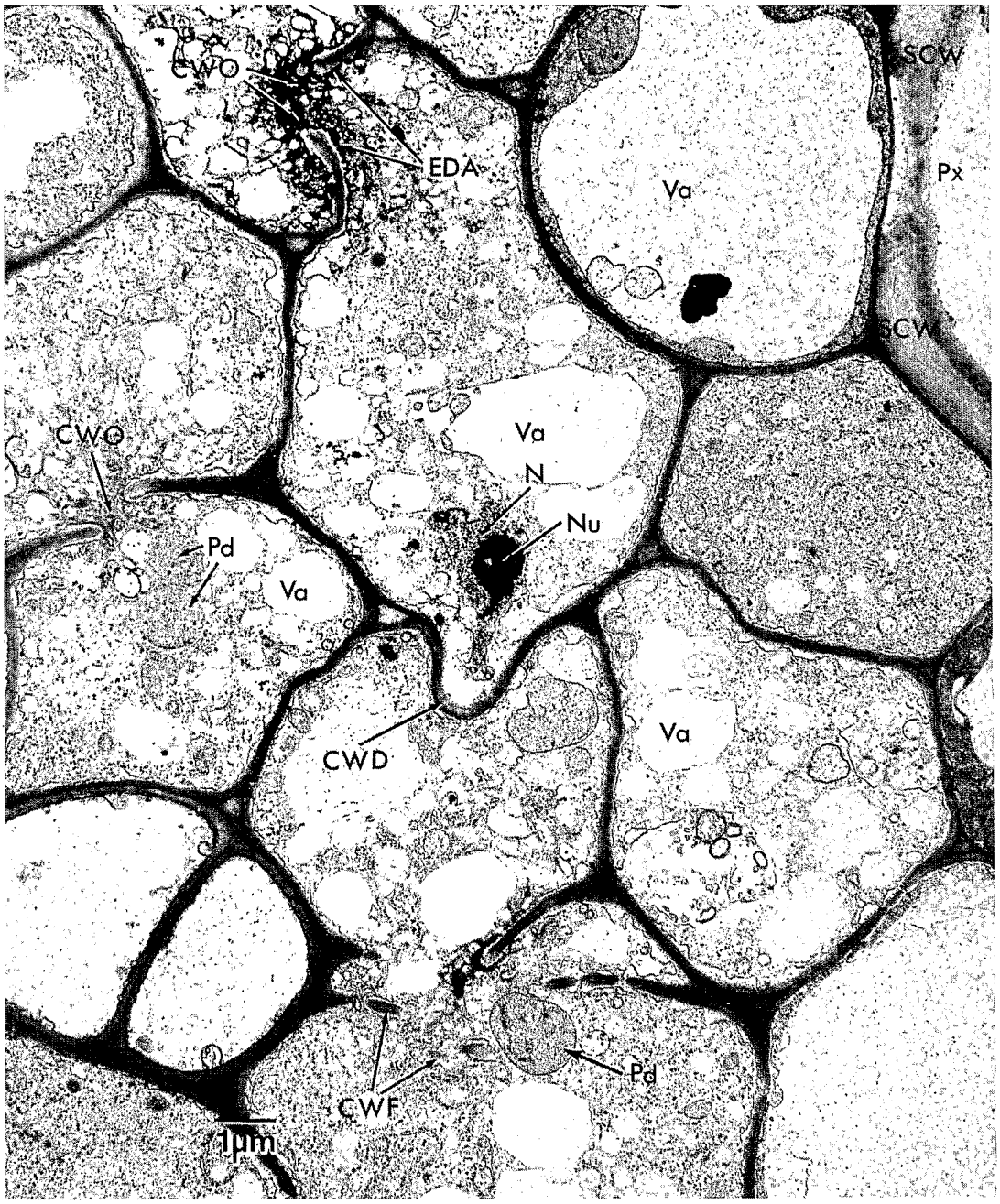

Fig. 6. Cross section of Lee soybean root, 3 days after *H. glycines* race 3 inoculation shows a general tissue reaction to nematode feeding. Cell wall distortions (CWD) and dissolutions are integral parts of the syncytial development. Using the secondary cell wall (SCW) of the protoxylem (Px) as a marker with the ISC located at lower left of figure, the syncytium encompasses the tissues near the protoxylem and involves several layers of cells. Electron-dense accumulations (EDA) occur along the wall separations at the central regions of the developing syncytium. CWF : cell wall fragments; CWO : cell wall opening; N : nucleus; Nu : nucleolus; Pd : plastids; Va : vacuole.

(Fig. 6). One of the fragmented cell walls became thickened terminally, the other was associated with membranes that appeared to form a cell wall-plasmalemma complex (Fig. 7 A). An isolated circular membrane complex (MP) appeared to resemble the cell wall-plasmalemma complex (Fig. 7 A) and was probably part of it. In some cases a nucleus occurred near an invaginated cell wall (Fig. 7 A), or even stretched across the semi-dissolved wall of adjacent cells of a syncytium (Fig. 7 B). The nucleoli of these cells were often hypertrophied (Figs 6, 7 B). Spherical to polyhedral shaped lysosome-like vesicles were observed near the termini of cell walls that were discontinuous (Fig. 8 A). The vesicles were held as an aggregate along the termini of the cell walls by the plasmalemma of adjacent syncytium cells. This was especially evident for the wall between an ISC and an adjacent cell. Both cells had nuclei but large central vacuoles were not visible at this level of sectioning. In lieu of the central vacuole, small vacuoles were dispersed throughout the cytoplasm of the syncytium.

Although hypertrophy and increased cytoplasmic density of the ISC was common in susceptible hosts, hyperplasia of adjacent parenchymatous cells also occurred (Fig. 9). Cell wall dissolution was not observed in the hyperplastic region and the syncytial wall adjacent to the hyperplastic cells was thickened.

Four days after inoculation with race 3 of SCN

The syncytium, and especially the ISC, had numerous small to medium sized vacuoles that contrasted with the large central vacuoles of the non-syncytial cells and ISC of earlier stages of infection. Nematode secretions were observed near the stylet penetration site and at more internal locations. Prominent nuclei with convoluted nuclear membranes occurred at various parts of the syncytium, and its component cell walls had extensive deposits of callose-like material (Fig. 10). " Callose " was deposited in localized areas such as walls opposite cell junctions and along walls that were reduced in thickness (Fig. 10). Most enlarged cells of the internal region of syncytia had cell walls that appeared distended (Fig. 10) particularly in regions where cell wall dissolutions occurred. Sections of the syncytium at a different plane revealed large clusters of plastids and vacuoles.

RESISTANT REACTIONS

Bedford soybean roots, 18 h after inoculation with race 4 of SCN

In one example of a resistant reaction, the ISC contained nematode secretions surrounded by an intertwining layer of ER that merged with a larger region of RER (Fig. 11). Moderate to wide cisternae occurred throughout the ER and RER. Mitochondria were numerous in the cytoplasm of the ISC and adjacent component cells (Fig. 12). The moderately dense accumulation of ER, RER and polysomes of the ISC (Fig. 11), contrasted with the apparently deteriorating electron-dense nucleoplasm of the ISC. The relationship of an ISC of a resistant host and adjacent cells is shown in Figure 12. Figure 13 A is from a serial section of the same host tissue as Figure 12 and shows the localization of nematode secretion and host response. The nematode secretion (NS) shown as Figure 13 B coincides with the secretion site illustrated in Figure 12. Thus, the ISC is shown as an integral part of the syncytium consisting primarily of pericycle that occurs opposite the protoxylem (Fig. 12). The secretions are not membrane bound and merge with the surrounding ER. Cisternae are extensive throughout the ISC.

Pickett 71 soybean roots, 2 days after inoculation with race 3 of SCN

The hypertrophied ISC had a broad cell wall opening that led to an adjacent cell. RER clusters were part of the matrix of the ISC cytoplasm and vacuoles. Nematode secretions (NS) and electron-dense material (Fig. 14 A) were apparently interspersed among the RER near the feeding site of the nematode but were lacking near other RER in Figure 14 B.

PI 88788 3 days after inoculation with race 4 of the SCN

Section through the nematode lip region and stylet penetration of the cell wall (Fig. 15 A) indicated that the nematode had established a stable relationship with the host by stimulation of an initial syncytial cell (ISC). Syncytial cells centrad from the feeding site showed signs of cell wall deterioration, depositions and hyperplasia near the protoxylem (Figs 15 B, 16). Cells located between the necrotic cell and the cluster of hyperplastic tissue showed an apparent high level of activity with accumulations of ER and RER and clusters of Golgi (Figs 15 B, 16). The apparently hyperactive cells may have thickened cell walls with numerous electron-dense deposits (Fig. 16).

Pickett 4 days after inoculation with race 3 of SCN

The resistant reaction of Pickett is characterized by a large, virtually empty outline of a syncytium (Fig. 17). Host cells were stimulated initially by the infective juvenile and the host responded with hypertrophy followed by cytoplasmic deterioration.

Discussion

Among the most salient features of soybean cyst nematode behavior during early infection stages on soybean roots is the feeding process, during which the nematode stylet is inserted through the host cell wall to induce a syncytium. The site of stylet penetration has electron-dense deposits produced primarily by the amphids but with possible secretions originating from the

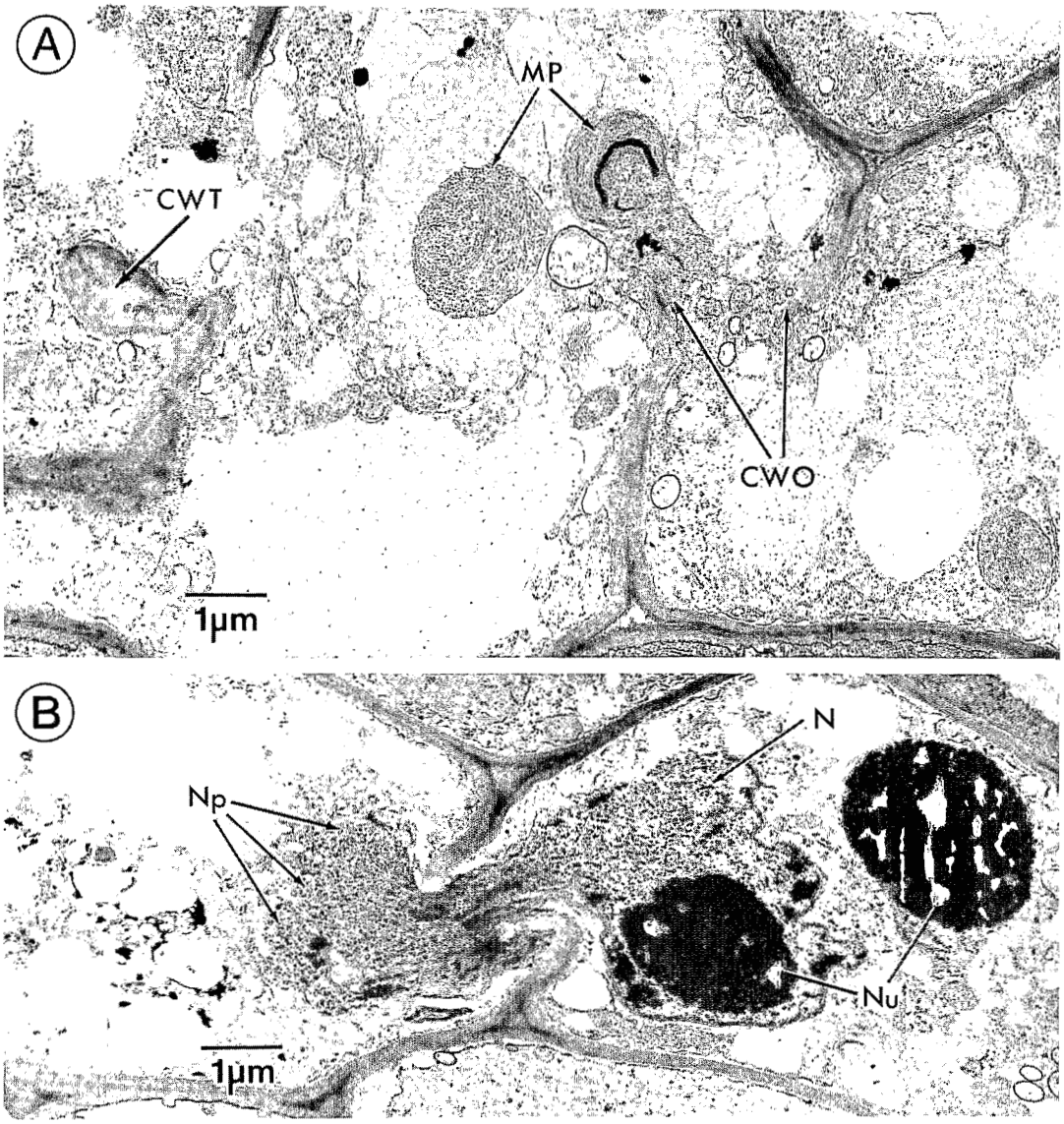

Fig. 7. Section of Lee soybean roots, 3 days after inoculation with *Heterodera glycines* race 3. — A. Masses of membrane proliferations (MP), one of which has an attachment to the cell wall and associated plasmalemma. The central membrane mass may have wall attachments at a different level of sectioning. CWO : cell wall opening; CWT : thickened cell wall. — B. A section through adjacent cells of a syncytium where the nucleoplasm (Np) of a nucleus (N) extends across two cells. The cell at the right contains two hypertrophied nucleoli (Nu).

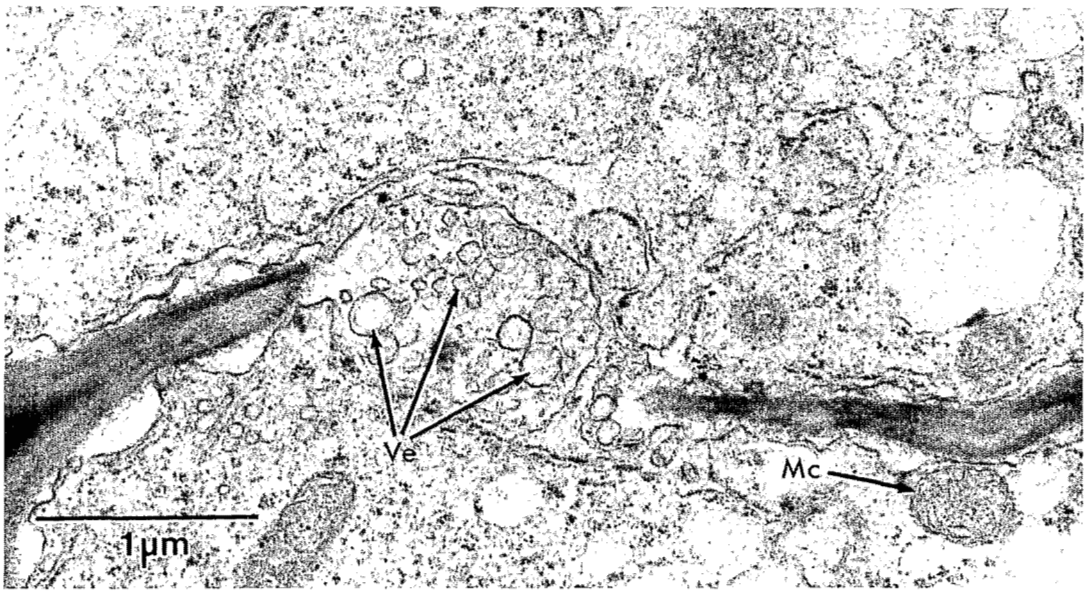

Fig. 8. Sections of Lee soybean roots, 3 days after inoculation with *Heterodera glycines* race 3. Polyhedral to spherical vesicles (Ve) accumulate at the termini of separated walls within a syncytium. Mc : mitochondria.

stylet orifice and inner labial receptors to form a feeding plug (Endo, 1978). This type of feeding plug was later described in other host-parasite interactions, including *Heterodera schachtii* in *Raphanus sativus* var. *oleiformis* and *Globodera pallida* and *G. rostochiensis* in potato roots (Wyss, Stender & Lehmann, 1984 and Rumpenhorst, 1984, respectively). The feeding plug apparently provides a guide for stylet movement during feeding and forms a seal after stylet retraction (Wyss, Zunke & Inst. Wiss. Film, 1986). Although the site of cell wall penetration may be only slightly damaged, host cell wall deposits are formed that produce an invagination of the cell wall at the point of stylet penetration. The cell wall build-up is not as extensive as that in reniform nematode infections (Rebois, Madden & Eldridge, 1975). The resultant wall deposit induced by *Rotylenchulus reniformis* was termed a peg, in which the stylet of an adult female was held in place during egg deposition. In contrast to *R. reniformis* infestions, feeding sites of cyst nematodes are more temporary as reported in observations of *H. schachtii* in rape roots where the stylet is retracted from the feeding site during repeated cycles of feeding (Wyss & Zunke, 1986).

Nematode secretions emanate near the tip of the stylet cone during feeding. Micrographs from interference-contrast light microscopy have shown the abundance of feeding tubes associated with cyst and root-knot nematodes (Rumpenhorst, 1984). In the absence of evidence for a direct contact between the feeding tube and the stylet, the bundles of feeding tubes are thought to be ducts for the movement of special plant cell secretions which are directed into a feeding ampulla. The nematode then feeds on the contents of the ampulla rather than directly on host cell cytoplasm. Thus syncytia and giant cells may function as gland cells from which nematodes ingest secreted products (Rumpenhorst, 1984). In *in vivo* observations of *H. schachtii* in radish roots, syncytial cytoplasm flowed past feeding tubes and host cell organelles were kept at the periphery of the cytoplasmic zone immediately surrounding the stylet (Wyss & Zunke, 1986).

Transmission EM observations of *H. schachtii* in susceptible roots of *Raphanus sativus* var. *oleiformis*, 3 days after nematode invasion, showed no signs of an ampulla, but revealed a cytoplasmic region adjacent to the stylet free of organelles and with fewer ribosomes and polyribosomes than in the surrounding cytoplasm (Wyss, Stender & Lehmann, 1984). A similar condition was observed in our study of *H. glycines* in the resistant cultivar, Bedford, within 18 h after inoculation. Mitochondria were scattered throughout the ISC but were less prominent in the immediate vicinity of the nematode secretion. Large numbers of mitochondria in the ISC and adjacent soybean cells is consistent with the reports of elevated oxido-reductive enzyme reaction sites in syncytia induced by the soybean cyst nematode in soybean roots (Endo & Veech, 1970). The term " feeding tube " has been used to describe the secretions of the reniform nematode, *Rotylenchulus reniformis*, during infections of cowpea and soybean roots (Rebois, Mad-

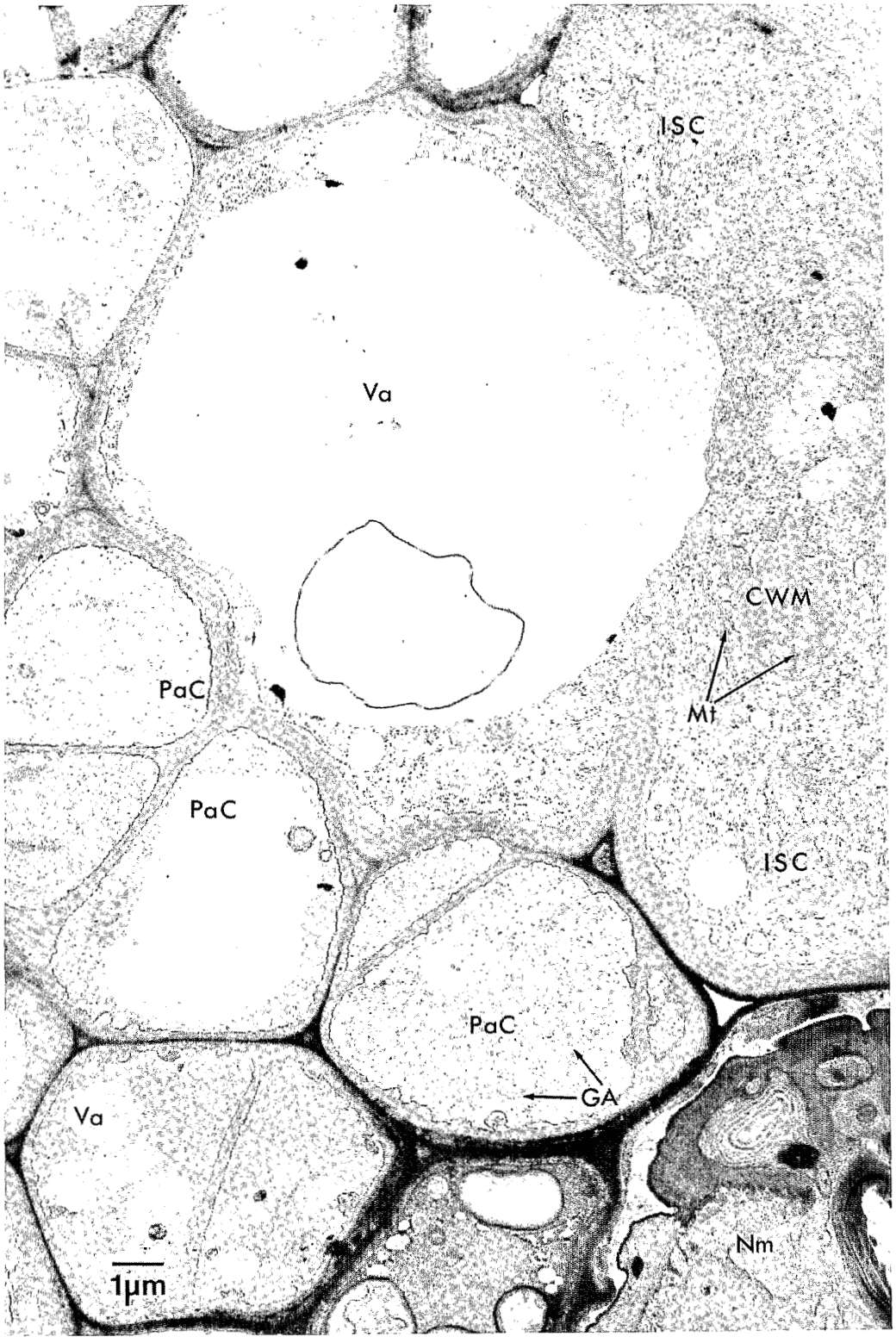

Fig. 9. The syncytium formed in Lee soybean root, 3 days after inoculation with *Heterodera glycines* race 3 shows a combination of susceptible host reactions. Cell wall dissolution is extensive. Cell adjacent to the initial syncytial cell (ISC) has a large vacuole but the breakdown of vacuoles (Va) is evident in other parts of the syncytium. The group of cells to lower left of the micrograph indicates hyperplastic activity in the presence of small parenchymatous cells (PaC). Signs of Golgi activity are similar to that observed in resistant line PI 88788 shown in Figure 16. CWM : cell wall material; GA : Golgi apparatus; Mt : microtubules; Nm : nematode.

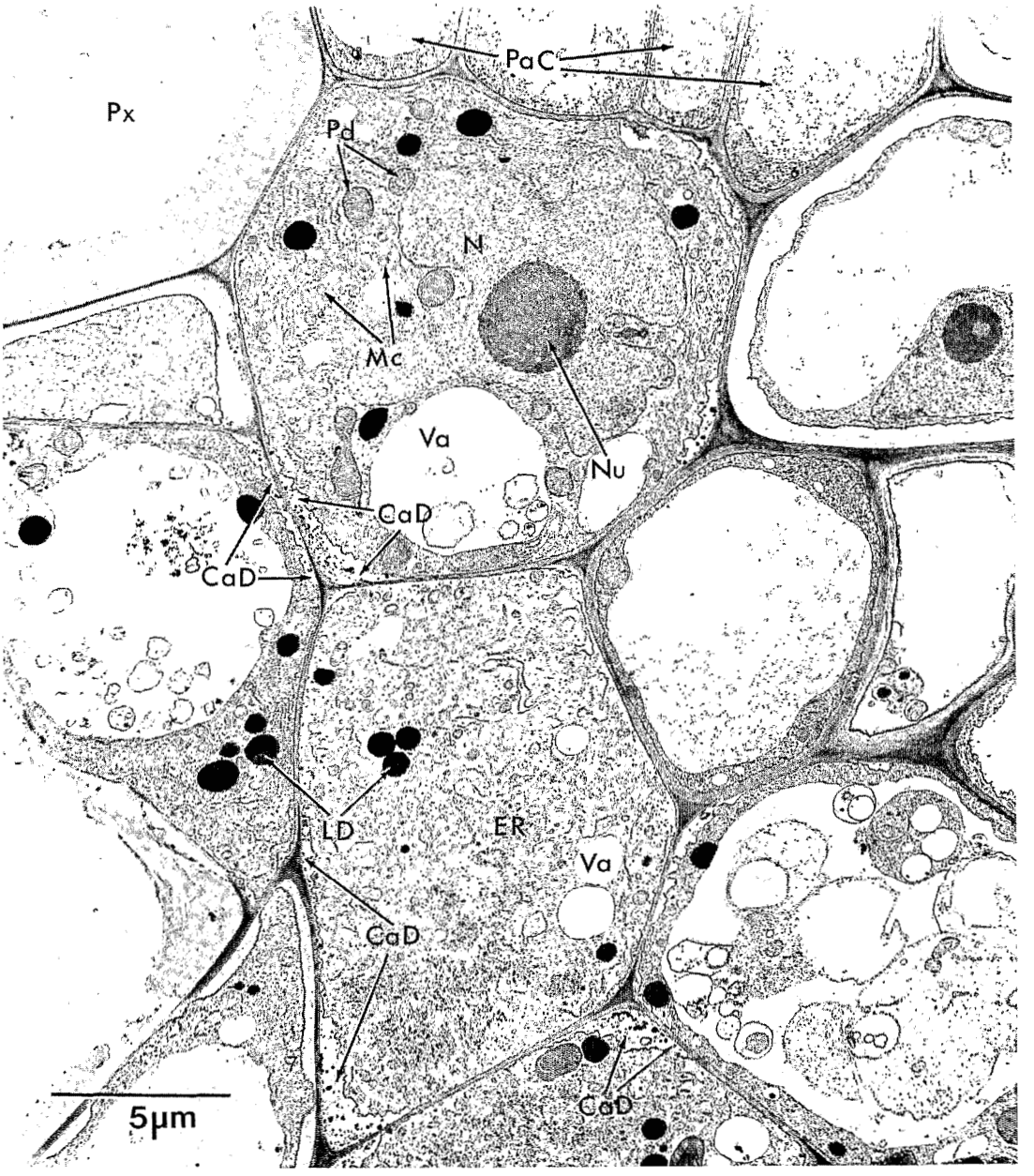

Fig. 10. Syncytium in Lee soybean root near protoxylem (Px) 4 days after inoculation with *Heterodera glycines* race 3. The nematode feeding site is at the lower part of the figure. Cellular changes within the syncytium consist of increases in cytoplasmic density with accumulations of endoplasmic reticulum (ER), mitochondria (Mc), lipid droplets (LD) or possible secretion granules, and plastids (Pd). Small to moderate-sized vacuoles (Va) are distributed throughout the syncytium. Callose depositions (CaD) occur on walls of adjacent cells forming the syncytium and near intercellular spaces. A hypertrophied cell with a nucleus (N) having a convoluted membrane lies adjacent to a protoxylem vessel and group of parenchyma cells (PaC). Nu : nucleolus.

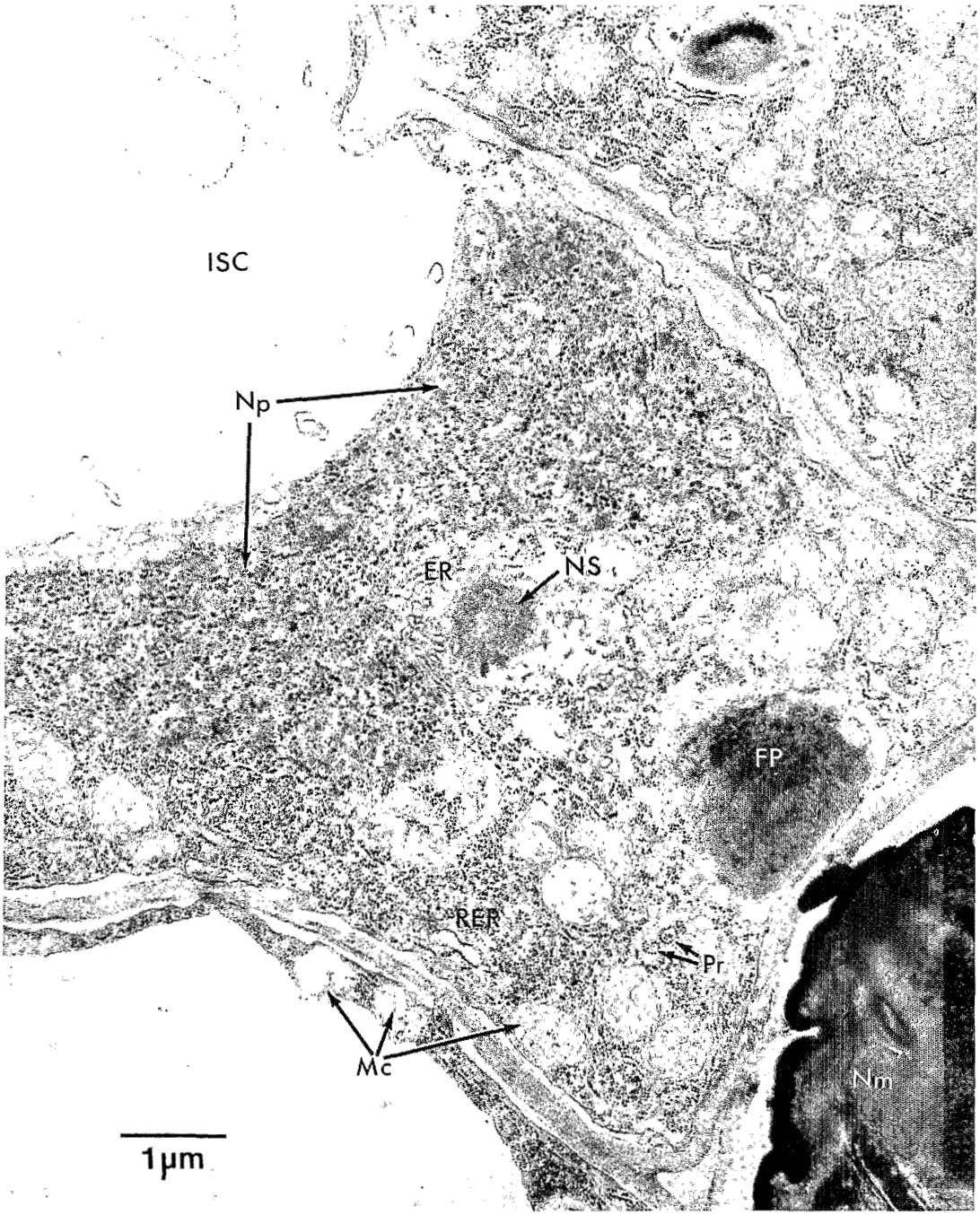

Fig. 11. Initial syncytial cell (ISC) and adjacent cell of resistant Bedford soybean root 18 h after inoculation with *Heterodera glycines* race 4. The electron-dense region near the nematode (Nm) is part of the feeding plug (FP). Appressed against a mass of what appears to be deteriorated nucleoplasm (Np) is a smaller electron opaque region consisting of nematode secretions (NS) surrounded by endoplasmic reticulum (ER). These ER tend to concentrate the secretory mass by forming a mass of folded smooth ER that in turn integrates with the surrounding rough endoplasmic reticulum (RER) that is widespread in the ISC. Other distinctive features of the host cytoplasm are numerous mitochondria (Mc) and polyribosomes (Pr).

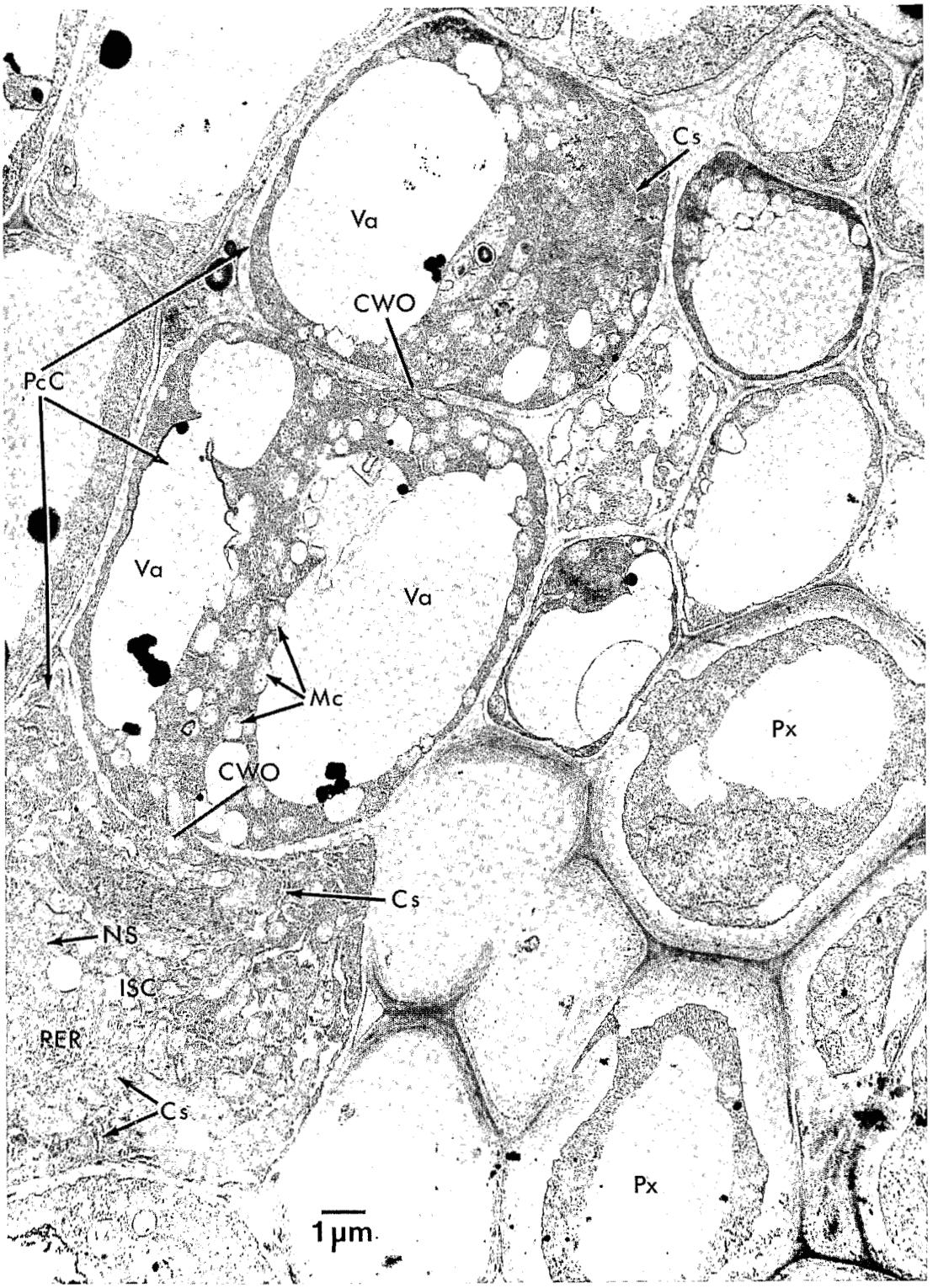

Fig. 12. Cross section of Bedford soybean root, 18 h after inoculation with *Heterodera glycines* race 4 shows cell wall dissolution and continuity of cytoplasm among adjacent pericyclic cells (PcC). The syncytium contains numerous vacuoles (Va) of variable size. The syncytial cytoplasm is more electron-opaque than adjacent non-syncytial cells. Rough endoplasmic reticulum (RER) with large cisternae (Cs) interspersed with numerous mitochondria (Mc) are common features of syncytial cytoplasm in the resistant cultivar. CWO : cell wall opening; ISC : initial syncytial cell; NS : nematode secretions; Px : protoxylem.

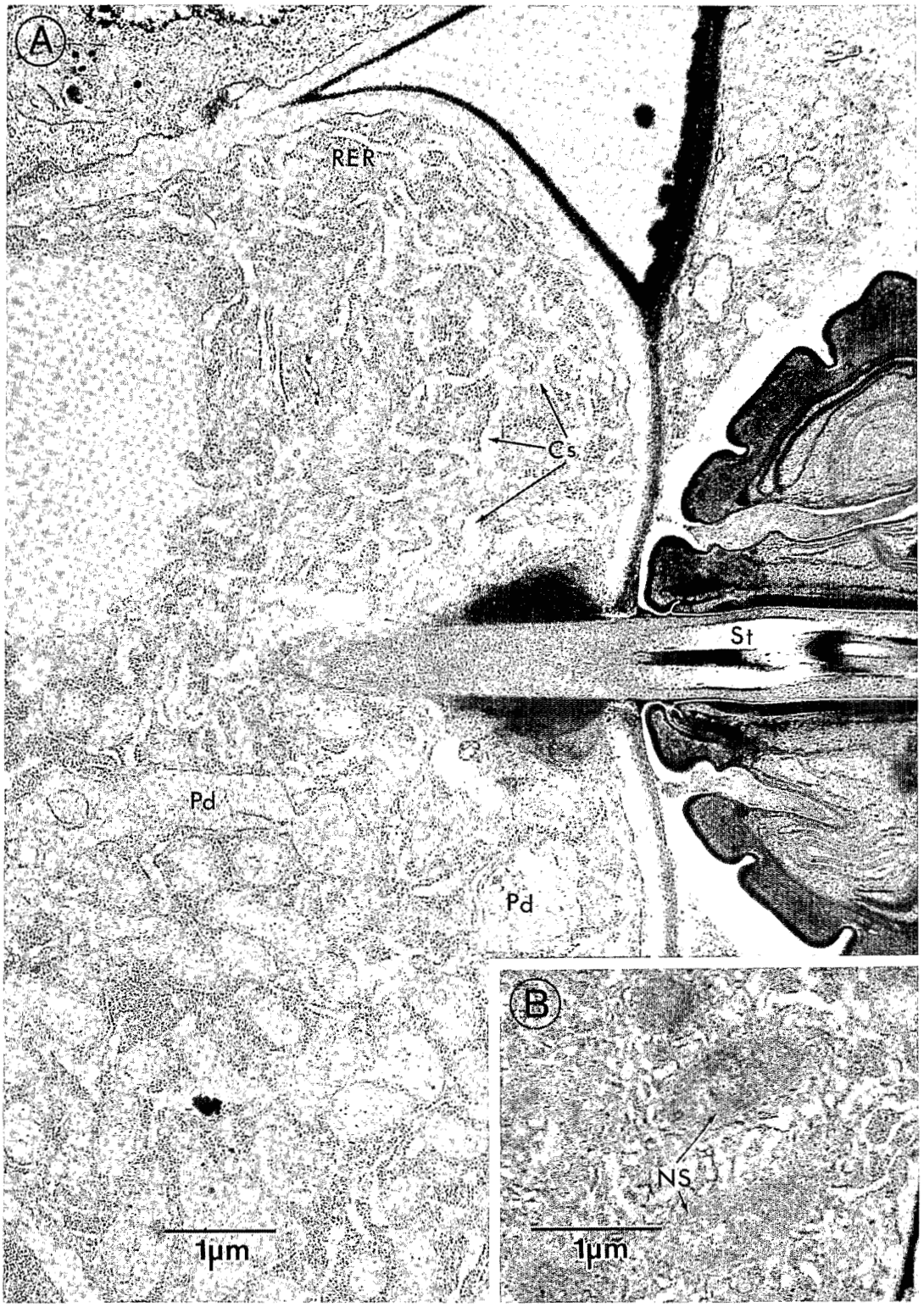

Fig. 13. Section of Bedford soybean root shown in Figure 12 but at a different level shows the stylet entry into the ISC. — A. Cisternae (Cs) and rough endoplasmic reticulum (RER) are extensive throughout the feeding site of the nematode. Although the cell to the right of the ISC has an enlarged nucleus (not shown) the primary host response is in the ISC and cells left of the nematode as shown in Figure 12. Pd : plastid. — B. Cross and tangential views of nematode secretions (NS) from one of serial section of the ISC in Figure 12. Secretions are located below site of stylet (St) of Figure 13 A.

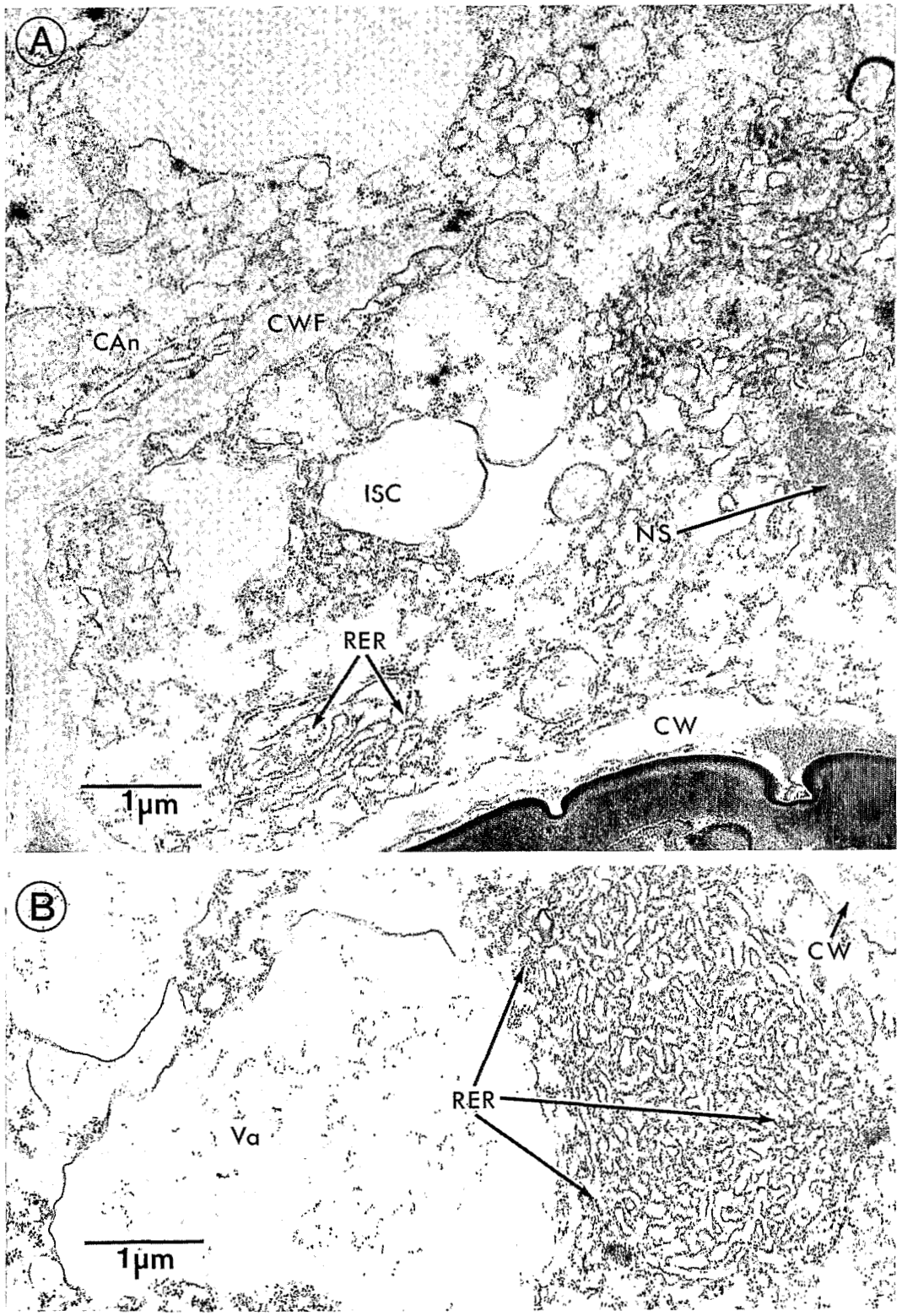

Fig. 14. Section through the initial syncytial cell (ISC) of resistant Pickett 71 soybean root, 2 days after inoculation with *Heterodera glycines* race 3. — A. Electron opaque regions dispersed among rough endoplasmic reticulum (RER) indicate site of nematode secretions (NS). The ISC is virtually integrated with the adjacent cell (CAn) with only a wall fragment (CWF) separating the two components of the syncytium. CW : cell wall. — B. Rough endoplasmic reticulum (RER) accumulations with moderate attachments of ribosomes way represent regions where nematode secretions had accumulated and later dispersed. CW : cell wall; Va : vacuole.

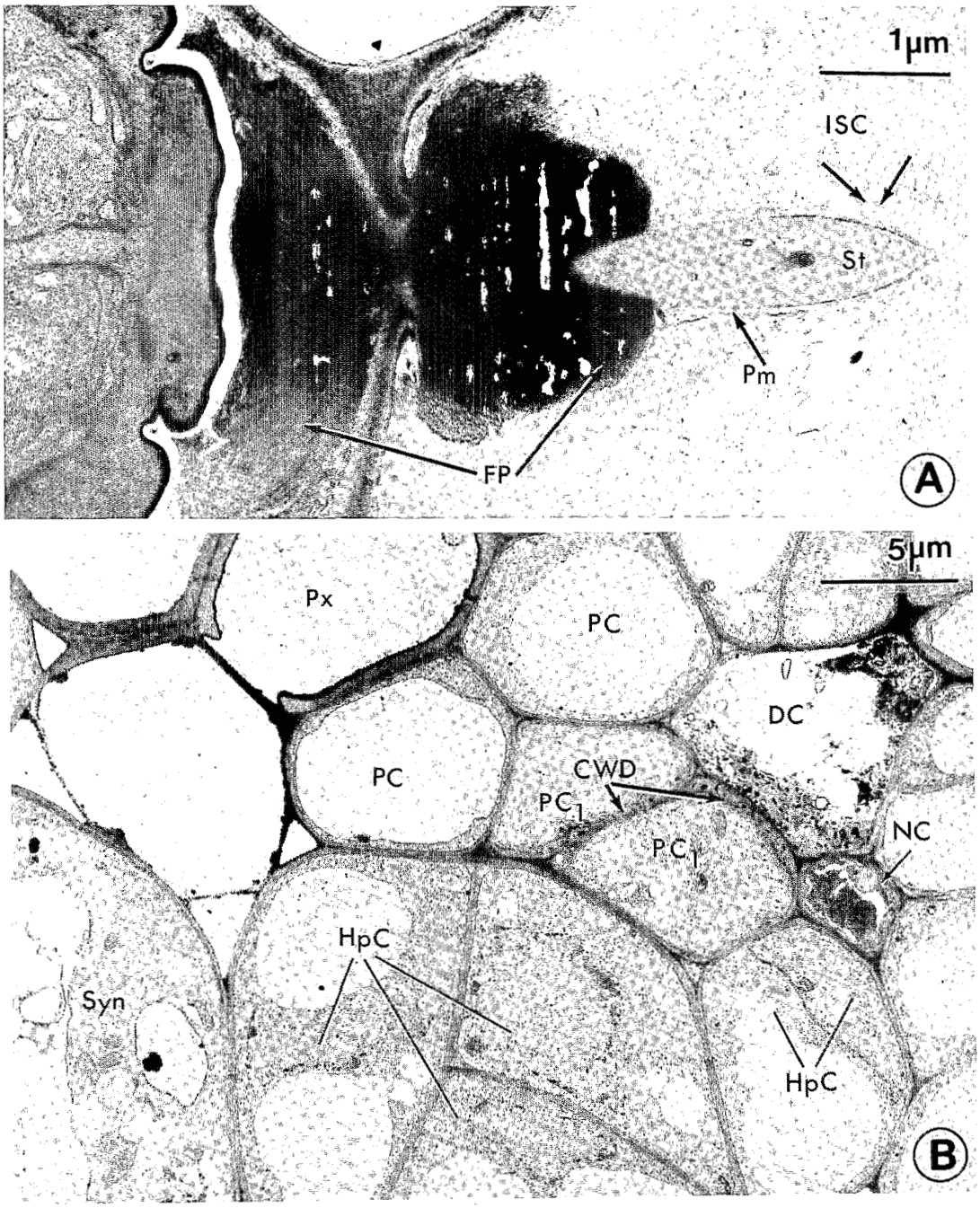

Fig. 15. A tangential section through the lip region and stylet (St) of *Heterodera glycines* race 4 at the feeding site of initial syncytial cell (ISC) of a resistant line PI 88788, 3 days after inoculation. — A. The electron-dense mass forms a feeding plug (FP) as is found in a susceptible host-parasite feeding site. The plasmalemma (Pm) appears to surround a major portion of the stylet tip except for a small area of low contrast membranes region at the upper right (arrow →). — B. The cluster of cells near the necrotic (NC) or deteriorating (DC) cells, distal to the nematode feeding site, are hyperplastic (HpC). The cells between the deteriorating cell and the clump of hyperplastic parenchymatous cells appear to be hyperactive (PC) as indicated by dense cytoplasm with Golgi and extensive cell wall depositions (CWD). PC : parenchymatous cell; Px : protoxylem; Syn : syncytium.

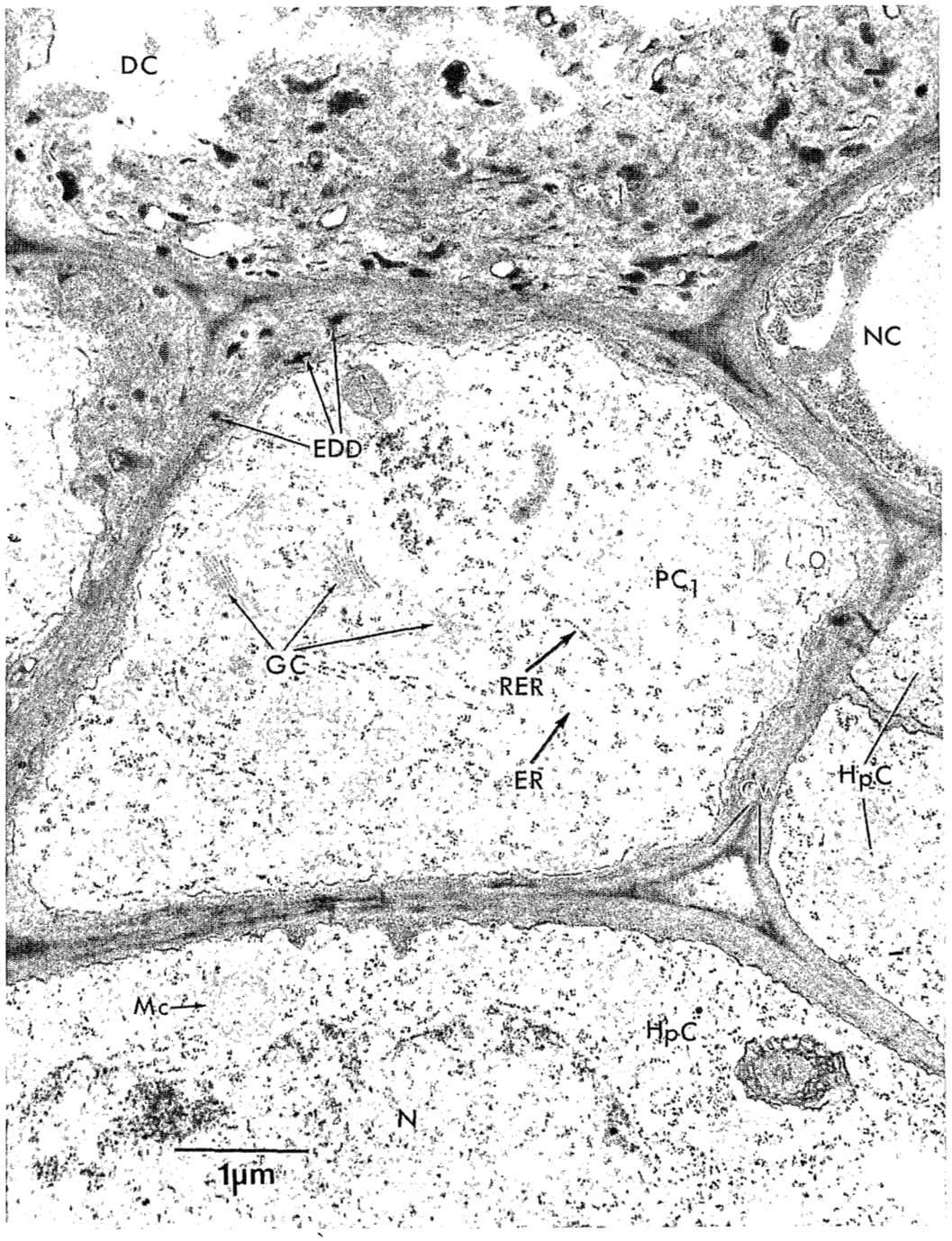

Fig. 16. A higher magnification of the hyperactive cell of Figure 15 B shows dense accumulations of smooth (ER) and rough ER (RER) and numerous sites of Golgi activity. Cell walls are thickened with electron-dense, strand-like, particulate deposits (EDD) shown in cross and tangential sections of an apparent hyperactive cell (PC) and adjacent cells. CW : cell wall; DC : deteriorating cell; GC : Golgi complex, HpC : hyperplastic cell; Mc : mitochondrion; N : nucleus; NC : necrotic cell.

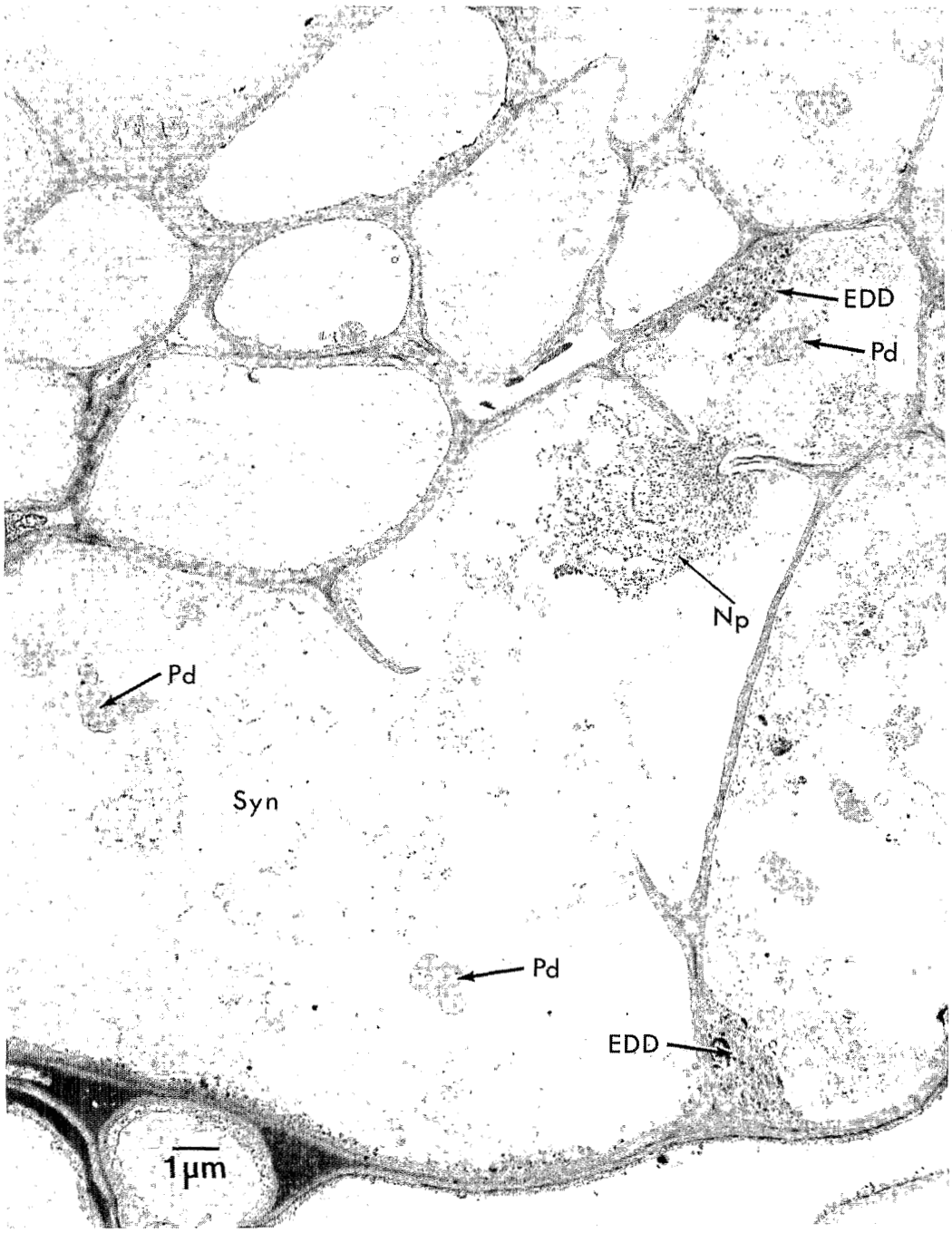

Fig. 17. A section of Pickett 71 soybean root, 4 days after inoculation with *Heterodera glycines* race 3. The almost empty central region of the once established syncytium represents a resistant reaction to infection by the soybean cyst nematode. The central region of the syncytium is almost devoid of organelles, except for residues of nucleoplasm (Np) at a wall opening in the syncytium (Syn). EDD : electron dense particulate deposits; Pd : plastid.

den & Eldridge, 1975; Razak & Evans, 1976; Rebois, 1980) and secretions produced by various other sedentary endoparasitic nematodes (Rumpenhorst, 1984). In this study, the cylindrical mass of secretions integrates with smooth ER that forms along its perimeter. Although the plasmalemma of the host was reported to surround the stylet tip of *H. glycines* and *H. schachtii* in soybean and radish roots, respectively, such a membrane did not extend outward at the stylet orifice of *H. glycines* to form a membrane-bound secretion (Endo, 1978; Wyss, Stender & Lehmann, 1984). The absence or low population of ribosomes on the ER immediately surrounding the secretions, as reported by Wyss, Stender and Lehmann (1984) and as shown in this report, may indicate a digestive or destructive effect of secretions on RER to cause a differential in the type of ER surrounding the secretions and the stylet cone. The presence of syncytial cytoplasm with elongated and intertwining cisternae within 18 h after inoculation of the resistant cultivar Bedford with SCN appears analogous to the report by Wyss, Stender and Lehmann (1984) that resistant *Raphanus* cultivars inoculated with *H. schachtii* contained RER with flattened cisternae. A similar host response was found 42 h after inoculation of resistant Peking soybean roots to SCN (Riggs, Kim & Gipson, 1973). Thus signs of host resistance apparently occur at a very early stage of infection. The dispersal of electron-dense material near the feeding site of a 2 day infection in a resistant cultivar (Fig. 14 A) may be involved in host resistance, however, in the same host, the presence of cell wall fragmentation and formation of small vacuoles are typical of susceptible host responses to infection (Endo, 1965; Riggs, Kim & Gipson, 1973; Kim, Riggs & Kim, 1987). The clumps of smooth ER in the syncytium may indicate regions where secretions were digested or dissipated. The modified morphology of cell walls, nuclei, and cytoplasmic components of a SCN-induced syncytium may be related to the dynamic flow of cytoplasm induced during nematode feeding, as demonstrated in film (Wyss, Zunke & Inst. Wiss. Film, 1986) and video tapes of host-parasite interactions (Wyss & Zunke, 1986).

The clusters of spherical to polyhedral shaped membranous vesicles enclosed by the plasmalemma at the edge of fragmented cell walls appear to be related to the early stages of cell wall breakdown (Fig. 8). The source and the nature of the contents of the membranous bodies are not clear, however, they resemble dissipated lysosome-like bodies. Furthermore, these membrane shells could be by-products of secretion granules packaged by the Golgi of the host. Direct evidence for the role of nematode secretions or host plant enzymes in cell wall digestion is lacking. Experiments using specific immunochemical labels may resolve the nature of the cell wall degrading substances.

Transmission EM studies of syncytial development in soybean roots at 3 and 4 days after inoculation with the SCN followed much the same pattern as described in previous observations (Endo, 1964; Endo, 1965; Gipson, Kim & Riggs, 1971; Riggs, Kim & Gipson, 1973; Kim, Riggs & Kim, 1987). In view of the video enhanced imaging of *Brassica napus* and the root responses to *H. schachtii* feeding (Wyss & Zunke, 1986), visualizing the direct influence of nematode secretions on cell wall dissolutions is not difficult. Although the host enzymes are considered to play the major role in cell wall dissolution during syncytial development (Jones & Dropkin, 1975), recent observations on feeding tubes or their products indicate a more direct influence of the nematode on host metabolism (Rumpenhorst, 1984; Wyss, Stender & Lehmann, 1984).

Although hypertrophy was an expected response of cells in the formation of a syncytium induced by the SCN, the hyperplasia adjacent to the syncytial boundary near the feeding site produced young cells that could provide tissues for expanded growth of the syncytium. Such activity near the protoxylem may be analogous to the stimulation of pericyclic cells in the formation of lateral root initials that arise in these regions of various plant species (Esau, 1953). The hyperplastic tissue observed in the resistant cultivar, PI 88788, infected by the SCN may relate to the necrotic cell response observed in Forrest soybeans 5 days after inoculation (Kim, Riggs & Kim, 1987). Necrosis was initiated adjacent to the lip region and extended through the tissue that surrounded the syncytium. Whether the hyperplasia observed at sites of the central positions of syncytia of Bedford 3 days after inoculation is a variation of a resistant host reaction is not clear. The cells that lie between the necrotic cells and syncytium, however, appear to be undergoing changes that may wall off or prevent further incorporation of host tissues. The extremely active cells at the perimeter of the hyperplastic tissue may give rise to new cells or more likely produce secretions that cause heavy cell wall deposits that could prevent further growth and lead to necrosis.

In observing changes in host response to nematode infection, the life cycle of the nematode must be considered. In syncytia induced by the soybean cyst nematode, expansion of the syncytium could be terminated by a resistant reaction within 18 h or more. Further, host responses may be dependent upon nematode secretion or food ingestion during the host-parasite interaction. Some changes in host cells, however, may occur without concomitant stimuli from the host and nematode, which may be the case during nematode molting at which time the nematode retracts its stylet and feeds again after stylet regeneration. Thus it appears that host cell changes leading to hypertrophy and hyperplasia related to syncytial development may be a stimulatory or regenerative process dependent on the nematode stage and compatibility of the host.

The recent advances in video-enhanced light microscopy coupled with electron-microscopy should provide

ways to further understand the processes involved in host-parasite interaction of cyst nematode infection and to explore means of nematode control by interrupting these infection processes.

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