

Feeding Plug Formation in Soybean Roots Infected with the Soybean Cyst Nematode

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ABSTRACT

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The ultrastructure of the early stage of syncytium development in soybean roots attacked by *Heterodera glycines* was examined to determine the interaction between the host syncytium and the nematode feeding apparatus. One day after inoculation, an electron-dense pluglike deposit was observed between the extended stylet of the nematode and the syncytial wall of the host. During the feeding process, the plug appeared to form a seal between the extended stylet and the cell wall. Four days after inoculation, the plug remained

in the opening of the cell wall after the stylet was withdrawn during the normal molting process. The plug apparently has continuity with secretions that appear to flow from the amphidial canals, the openings of the inner labial receptors, and the stylet vestibule. The plug consists of an electron-dense fibrillar mass that forms a structure that may be comprised of nematode secretions and cell wall deposits, both of which are limited by the plasmalemma of the syncytium.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, is one of several species that damage root tissues by forming syncytia or multinuclear cells that provide nutrition for the developing nematode. Previous studies (4, 5, 7) indicated that the multinucleate syncytia induced by the soybean cyst nematode form when the cytoplasm from contiguous cells intermixes following cell wall dissolution. In addition to cell wall destruction that occurs during the formation of syncytia, recent electron microscopic studies (9, 11) of syncytia induced by *Heterodera* and *Meloidogyne* species have revealed that the outer wall of the syncytium develops ingrowths which were termed "cell wall protuberances" or "ingrowths." Although these wall ingrowths occur as a pathological response to nematode infection, Jones and Northcote (11) compared them to the wall ingrowths of transfer cells that occur in higher plants (8, 10). Thus, the presence of protuberances indicated that giant cells may be a form of multinucleate transfer cells.

In a cinematic study, Wyss (19, 20, 21) described the feeding action of the ectoparasitic nematode, *Trichodorus similis* Seinhorst, on seedling cultures of *Nicotiana tabacum* L. and *Brassica rapa* L. var. *silvestris*. The activities involved in the nematode interaction with the host roots consisted of exploration, cell wall perforation with the stylet, salivation by the nematode, ingestion of host cytoplasm by the nematode, the withdrawal of the stylet from the feeding site, and the subsequent release of the nematode from a feeding tube that remained attached to the host cell.

In the tylenchoid ectoparasitic nematode, *Hemicycliophora arenaria* Raski, the parasite is attached to the feeding site of a citrus root by an adhesive plug that is formed in the anterior region of the nematode (14). A portion of the plug remains attached to the stylet when the nematode is separated from the feeding site. The authors were unable to determine whether the plug was formed from secretions by the nematode and/or from the plant. Kisiel et al. (12) described a similar adhesive plug that surrounded the stylet of *Hemicycliophora similis* Thorne during feeding on cranberry roots, and suggested that it was initiated by the nematode and not the plant.

The present study elucidates the morphology of the early stages of feeding by the soybean cyst nematode on the roots of susceptible and resistant soybean [*Glycine max* (L.) Merr.], and compares the dense plug surrounding the stylet with papillae that develop during fungal infections of plant cells.

MATERIALS AND METHODS

Larvae of *Heterodera glycines* were obtained from egg masses and cysts collected from roots of infected soybean plants that were grown in clay pots containing field soil infested with the soybean cyst nematode. Water suspensions of the larvae were pipetted onto the roots of susceptible and resistant soybean cultivars, Lee and Pickett, respectively. Inoculum was deposited on newly formed secondary roots that were then coated with fine-textured vermiculite, to retain the larvae in the vicinity of the roots. Next, groups of five seedlings were placed in 10-cm diameter clay pots containing moist vermiculite, and were covered with plastic film to retain moisture during

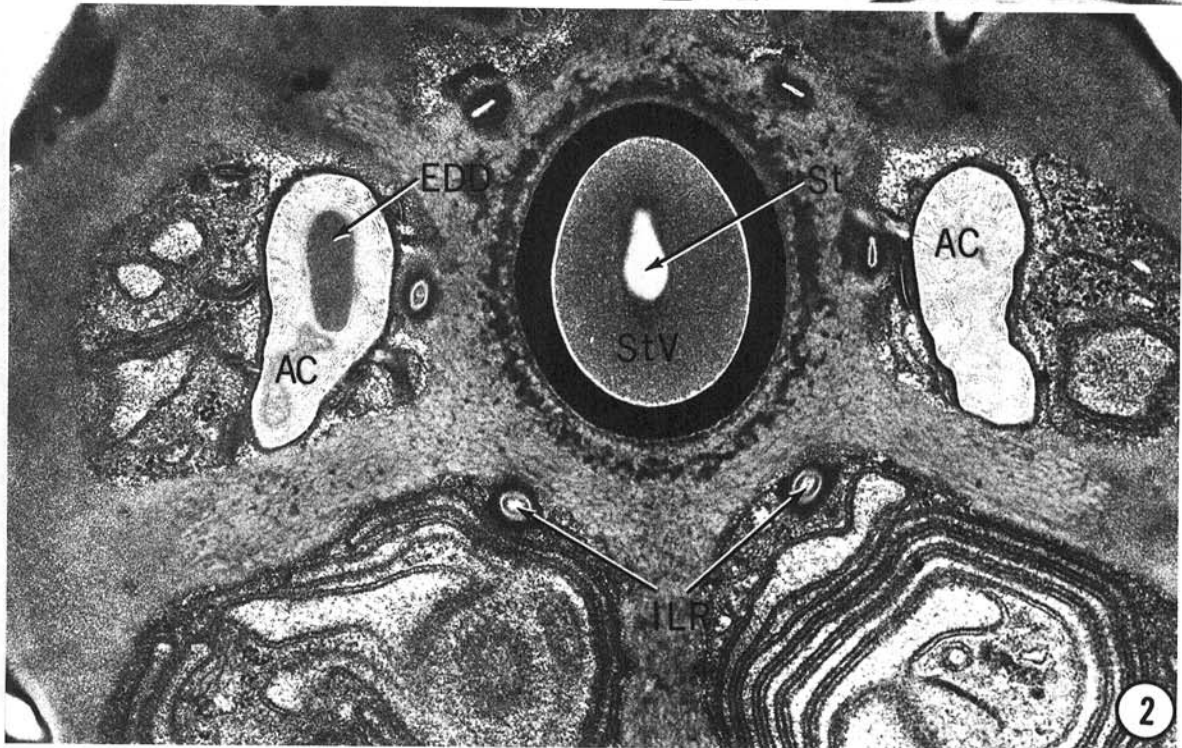
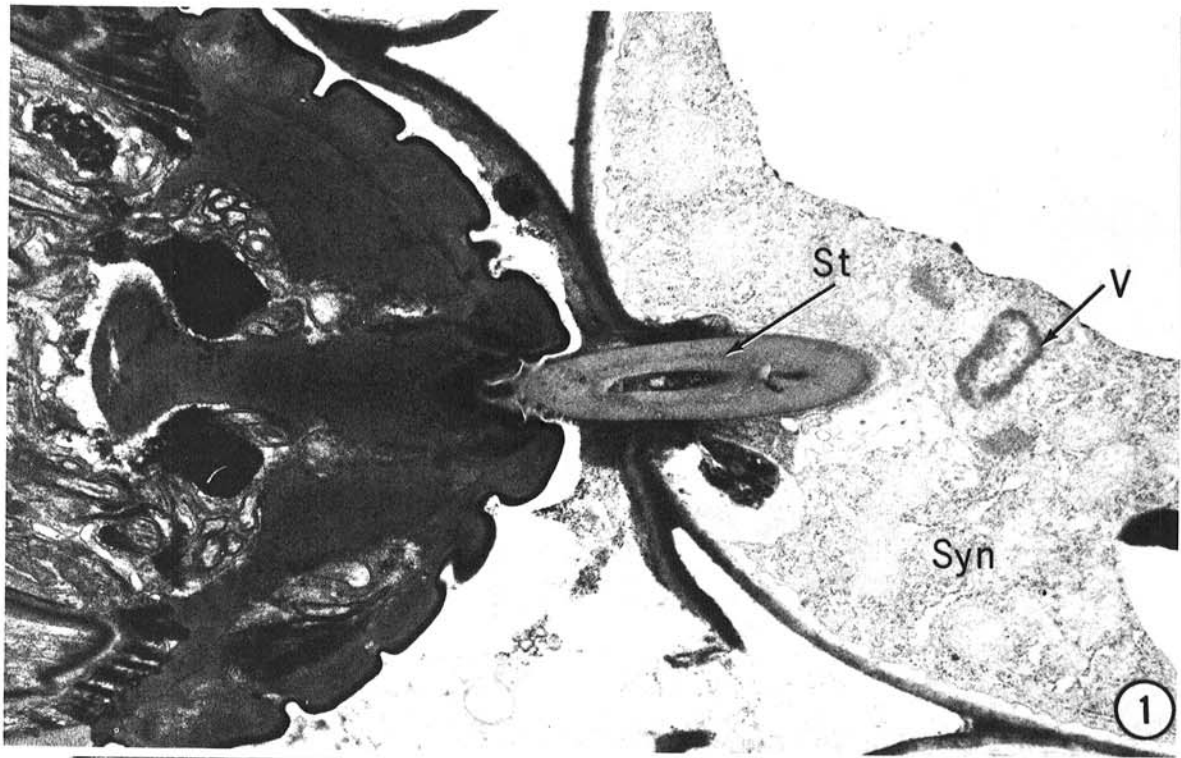


Fig. 1-2. 1) Feeding of *Heterodera glycines* on susceptible Lee soybean roots. An oblique section through a nematode stylet (St) 1 day after feeding commenced. The stylet has penetrated the cell wall and appears to have stimulated the beginning of a syncytium (Syn). Vesicle-shaped nematode secretions (V) appear in host cytoplasm (Syn) ($\times 14,400$). 2) Section of the head of a second-stage larva within a Lee soybean root illustrating possible sources of plug material. In a 2-day infection, the amphidial canal (AC), the stylet vestibule (StV), and the pores of the inner labial receptors (ILR) contain electron-dense to translucent material. Electron-dense deposits (EDD) occur in only one amphid at this level of sectioning. The tip of the stylet (St) is shown within the stylet vestibule ($\times 36,000$).



Fig. 3-4. Feeding of *Heterodera glycines* on soybean roots. The feeding site and amphidial gland regions of the nematode 1 day after feeding commenced on roots of the resistant soybean cultivar Pickett. 3) An electron-dense plug (P) occurs in the opening of the plant cell wall, which at this level of sectioning does not show the stylet ($\times 15,600$). 4) Another section of the nematode shown in Fig. 3. The stylet (St) appears in tangential view. Adjacent to the stylet are the basal regions of the amphidial gland cilia (AGC) and microvilli (Mv) of the neurosecretory components of the amphidial glands. Note the electron-dense deposits (EDD) that surround the lower region of the amphidial cilia ($\times 10,100$).

the inoculation period. After 24 hr, roots were washed and the plants were transferred to clean vermiculite. At 1, 3, 4, and 6 days after inoculation, roots were washed with tap water to remove adhering particles of vermiculite and were immersed in a drop of buffered 3% glutaraldehyde supported on a sheet of dental wax. While in this fixative, the infected regions of the secondary roots were cut into 2 to 4-mm segments and then transferred to vials. Fixation, rinsing, and postfixation in osmium tetroxide were carried out in 0.05 M phosphate buffer (pH 6.8). Fixation for 1.5 hr was followed by washing in six changes of buffer over a period of 1 hr. The tissue then was postfixated in 2% osmium tetroxide for 2 hr, dehydrated in an acetone series, and infiltrated with a low-viscosity embedding medium (17). Silver-gray sections of selected root segments were cut on a Sorvall MT-2 ultramicrotome with a diamond knife, and mounted on noncoated 194 × 48- μ m (75 × 300-mesh) copper grids. The sections were stained with 2% aqueous uranyl acetate (10 min), then with lead citrate (5 min). The thin-sections were viewed in a Philips 301 electron microscope operating at 60 kV with a 20- μ m objective aperture.

RESULTS

During stimulation of a syncytium by a second-stage larva of the soybean cyst nematode, a pluglike deposit and cell wall modification occur within 1 day after inoculation in susceptible (Fig. 1, 2) or resistant (Fig. 3, 4) soybean roots. The cell wall modification and electron-dense deposits surrounding the stylet are directly associated with stylet penetration and feeding by the nematode.

A cross section of the second-stage larva in root tissue reveals possible sources of plug material. The amphidial channels of larvae at 2 days after inoculation of Lee soybean roots (Fig. 2) show electron-dense deposits within a matrix of lighter material and electron-translucent particles. In the same section, granular materials also occur in the stylet vestibule and in the space surrounding the elongated cilia of inner labial receptors. Sections through a larva 3 days after inoculation of the susceptible cultivar show extremely electron-dense deposits within amphids of a larva with an extended stylet. Serial sections of these amphids revealed the presence of dark deposits in both amphidial canals. These deposits have continuity with the region surrounding the basal region of cilia and the microvilli of amphidial glands (Fig. 4).

In a 3-day infection of susceptible roots, a plug forms and extends into the lumen of one of several plant cells, the fine structure of which becomes altered (Fig. 5). The fine-structural modifications in these cells consist of numerous ribosomes that occur either as free particles in the cytoplasm or as polysomes (Ps) on the surface of vesiculated endoplasmic reticulum (Fig. 5). Cell walls between the stimulated cells and the adjacent tissue eventually undergo dissolution. As a result, a syncytium is formed as illustrated in the resistant cultivar Pickett (Fig. 6).

Initially the penetrated cell wall appears as a fragmented projection of material extending into the syncytium (Fig. 1). Within 3 days after inoculation, the cell wall adjacent to the nematode lip region increases in

thickness (Fig. 5). Electron-dense deposits on the syncytial wall surround the aperture made by the stylet (Fig. 5-8). These deposits form the base of an electron-dense structure that is bounded by the host plasmalemma. Electron-dense material, other than the deposits around the stylet, occurs near the terminus of the plug and stylet (Fig. 5). Nonmembrane-bound materials, which appear to be nematode secretions, occur near the stylet terminus and within the syncytial cytoplasm (Fig. 6). In oblique sections of 3-day infection sites of resistant plants, the electron-dense material of the plug surrounds the stylet and contacts the surface cuticle of the anterior portion of the nematode (Fig. 6-8). In addition, this material appears continuous with a homogeneous substance that lies in the canals of the amphids, the inner labial receptors, and the stylet vestibule (Fig. 8).

During the molting process, the plasmalemma surrounding the plug remains intact (Fig. 9). The plug is surrounded by a concave ring of cell wall material that projects into the cytoplasm of the syncytium. The plug-like material extends into the space that results when the nematode moves away from the feeding site during molting. Plug material in the plant and the contents of the amphidial channels appear continuous. An electron-dense deposit, which occurs between the end of the stylet and the stoma opening, indicates the presence of deposits that may have contributed to the initially formed plug. The morphology of the inner labial receptors and the amphidial channels is clearly delineated in the molting stage of this nematode. As shown by Roman and Hirschmann (16), the entire conical part of the stylet and surrounding hypodermal tissue are sloughed off during the molting process of tylenchoid nematodes.

The third-stage larva remains near the feeding site established by the second-stage larva and repenetration can occur adjacent to the original site of stylet penetration. Figure 10 illustrates an oblique section of the stylet that extends through the syncytium cell wall and into the host cytoplasm. Electron-dense plug material and electron-lucent particles, previously observed in relation to the second-stage larva, also were associated with stylet penetration by the third-stage larva. A papilla-like structure adjacent to the present feeding site probably resulted from feeding by the second-stage larva. Serial sections through the region revealed a perforation of a cell wall and the presence of plug material. The thickened syncytial wall surrounds the plug material, forming a seal between the syncytium contents and the nematode.

DISCUSSION

The cell wall ingrowths associated with nematode feeding and syncytium development were discussed by Riggs et al. (15) in an ultrastructural study of resistant soybean roots inoculated with the soybean cyst nematode. They observed irregular thickening of the inner walls of syncytia. In addition, the plasmalemma was invaginated, forming cytoplasmic pockets containing vesicles and tubules that appeared structurally similar to "boundary formations" (6) and paramural bodies (13) of noninfected plants.

The cell wall modifications observed in this study are associated with the nematode stylet and the electron-dense material that comprises a major part of the plug in

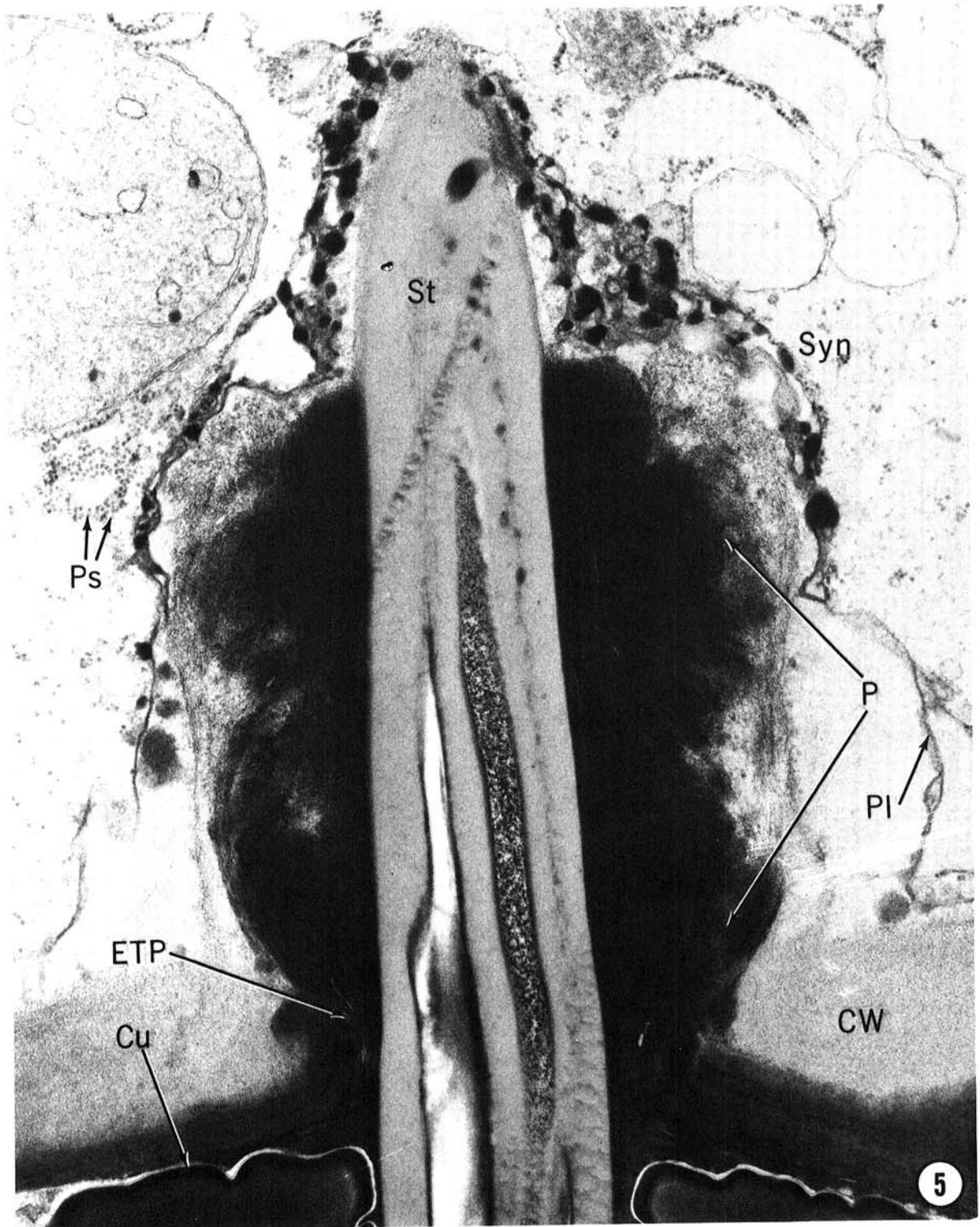


Fig. 5. Feeding of *Heterodera glycines* on soybean roots. The stylet (St) of the nematode is extended into the syncytium. The electron-dense plug (P) has a fibrillar substructure and extends through the cell wall (CW) opening into the space between the outer surface of the syncytium and the cuticular (Cu) surface of the nematode's lip region. Some of the electron-translucent particulate areas (ETP) in the plug appear similar to the electron-translucent areas in the secretions that appear in the anterior regions of the amphidial canal as shown in Fig. 2. The plasmalemma (Pl) of the cell encloses the plug. Electron-dense deposits adhere to the surface of the convoluted plasmalemma. The syncytial (Syn) cytoplasm contains clusters of polysomes (Ps) ($\times 42,000$).

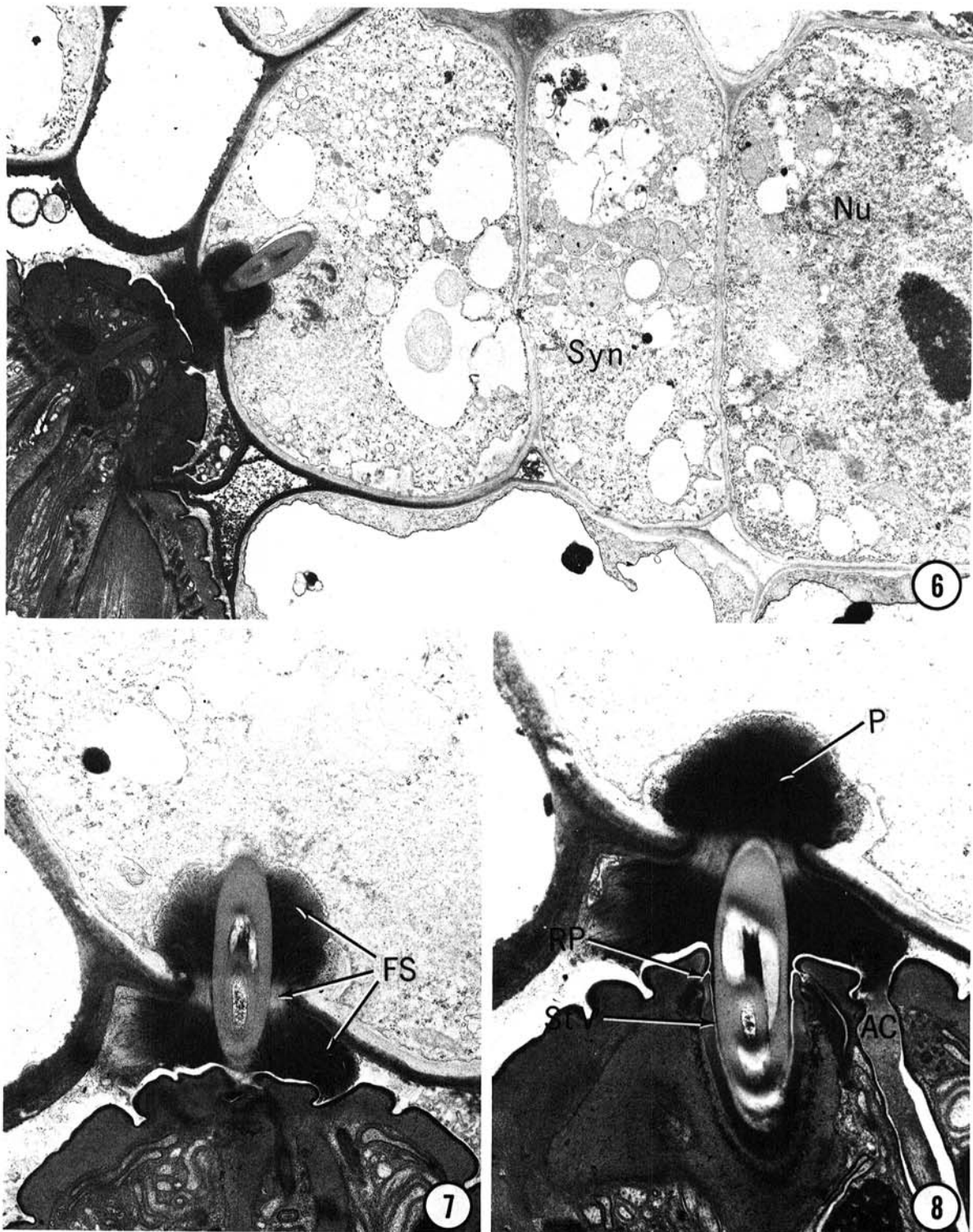


Fig. 6-8. Feeding of *Heterodera glycines* on soybean roots. An infective second-stage larva with stylet extended into a syncytium (Syn) of resistant soybean cultivar Pickett 3 days after feeding commenced. 6) The plug and stylet are illustrated in serial sections. The enlarged nematode indicates that feeding has occurred and the source of nutrition is the syncytium shown with its dense cytoplasmic content. The enlarged malformed nucleus (Nu) is characteristic of syncytial nuclei ($\times 6,000$). 7) The fibrillar substance (FS) of the plug (P) appears as parallel striae adjacent to the stylet where a median section is made through the cell wall aperture and the stylet ($\times 12,000$). 8) The plug (P) has continuity with the contents of the amphidial canal (AC) and is adjacent to the inner labial receptor pore (RP) and the entry to the stylet vestibule (StV) ($\times 15,300$).



Fig. 9. Feeding of *Heterodera glycines* on soybean roots 4 days after inoculation of the susceptible soybean cultivar, Lee. A larva is shown in a molting stage. The dark granular material beneath the cuticle (Cu) and surrounding the stylet tooth (St) will be sloughed off during the molting process. Note the plug (P) in the syncytium wall. Inner labial receptors (ILR), amphidial canal (AC) ($\times 12,000$).

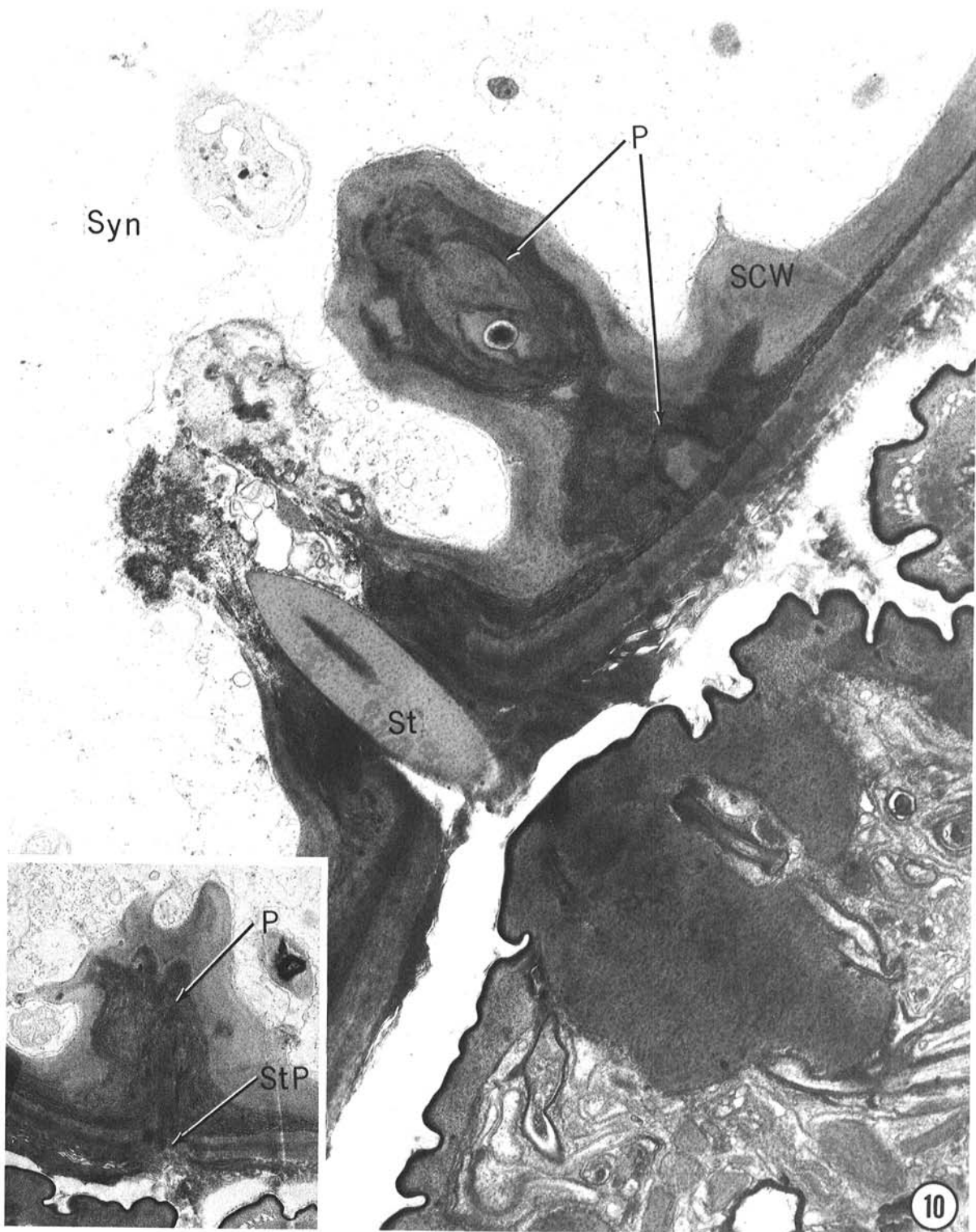


Fig. 10. Feeding of *Heterodera glycines* on soybean roots. Stylet penetration and feeding plug structures 6 days after feeding commenced. A third-stage larva is shown with its stylet (St) extended into a syncytium (Syn) of a susceptible host. Adjacent to the present feeding site is a plug (P) that apparently was formed by the same larva during the second larval stage. The thickened syncytial cell wall (SCW) surrounds the plug material, sealing the site of stylet penetration and separating the syncytial cytoplasm from the outside environment ($\times 21,000$). The site of stylet penetration (StP) by the second-stage larva is shown in the insert ($\times 12,000$).

the syncytium wall. The electron-dense material, through which the stylet is extended, appears to originate primarily from the nematode but some contribution may have come from the host cytoplasm.

The encystment and penetration of a fungus, *Plasmodiophora brassicae*, in the root hair of cabbage (1, 2) require the deposition of a fibrillar adhesive material that attaches a fungus zoospore cyst to the host cell. After the host wall is penetrated by a bullet-like structure which is termed a Stachel the fungus enters the host cell. The presence of the adhesive material and the papilla arising from the fungus spore penetration may be analogous to the early stages of nematode infections.

The amphidial gland and its component parts are possible sources of the electron-dense deposits that form the plug. Continuity of the plug material with the contents of amphidial canal may be related to neurosecretory role of the amphidial gland (18). Electron-dense material associated with the amphidial gland has continuity with the accumulations surrounding the bases of the cilia in the amphidial canal and the microvillous projections of the neurosecretory gland of the amphid. In contrast to the relatively light granular material shown among amphidial cilia of adult males of *Heterodera glycines* (3), extremely dense deposits occur near the bases of amphidial cilia of recently hatched larvae and the infective second-stage larvae in the host tissue. Further work will be necessary to correlate the amphidial gland secretions with the plug material.

The plug material also may arise from the inner labial receptors because dense material is readily observed within membranes that enclose the pairs of cilia in the receptors. These deposits could extend from the pores of the inner labial receptors.

The dense material that accumulates within the stylet vestibule and has continuity with the plug may have a passive role in the plug formation. Wyss (19, 20, 21) mentioned the role of the stoma in the deposition of a feeding tube by the ectoparasite, *Trichodorus similis*. This tube was considered essential to the feeding process because the stylet of *T. similis* lacks an orifice and the tube serves as a suction device that extends from the stoma lining through the plant cell wall and into the host cytoplasm.

Finally, because the stylet is closely associated with surrounding electron-dense material, secretions from the stylet orifice may contribute to the plug. Fine granular deposits that occur at the perimeter of the plug may have been secreted through the lumen of the stylet and the esophagus.

The dense plug may function as a seal at two critical stages. During stylet penetration and probing, which occur early in the feeding process, the plug could provide a seal for the plant cell. Increased metabolic activity and destruction of adjacent cell walls occur in the cell that is penetrated by the stylet. These anabolic and catabolic processes result in the initiation of a feeding reservoir, the syncytium (Fig. 5, 6). The plug and the syncytium are initiated at a critical stage in the development of the soybean cyst nematode. Previous studies (4, 5) have shown the interdependency of the syncytium and the developing soybean cyst nematode. A second function of the plug may be to seal the cell wall after stylet withdrawal, which occurs during the molting process.

Because feeding second-stage larvae of the soybean cyst nematode are immobile and initiate a single syncytium, the need to maintain this syncytium in a viable and intact state becomes critical for subsequent larval stages that resume feeding on the same cell.

Future studies on the chemical nature of the plug material may provide new insights into the mechanisms of host response to nematode development and possibly provide clues for prevention or degradation of plug deposits that may inhibit nematode development and prevent tissue damage of the host plant.

LITERATURE CITED

1. AIST, J. R. 1976. Papillae and related wound plugs of plant cells. *Annu. Rev. Phytopathol.* 14:145-163.
2. AIST, J. R., and P. H. WILLIAMS. 1971. The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. *Can. J. Bot.* 49:2023-2034.
3. BALDWIN, J. G., and H. HIRSCHMANN. 1975. Fine structure of cephalic sense organs in *Heterodera glycines* males. *J. Nematol.* 7:40-53.
4. ENDO, B. Y. 1964. Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. *Phytopathology* 54:79-88.
5. ENDO, B. Y. 1965. Histological responses of resistant and susceptible soybean varieties, and back cross progeny to entry and development of *Heterodera glycines*. *Phytopathology* 55:375-381.
6. ESAU, K., V. I. HEADLE, and R. H. GILL. 1966. Cytology of differentiating tracheary elements. II. Structures associated with cell surfaces. *Am. J. Bot.* 53:765-771.
7. GIPSON, I., K. S. KIM, and R. D. RIGGS. 1971. An ultrastructural study of syncytium development in soybean roots infected with *Heterodera glycines*. *Phytopathology* 61:347-353.
8. GUNNING, B. E. S., and J. S. PATE. 1969. "Transfer cells" - plant cells with wall ingrowths, specialized in relation to short distant transport of solutes—their occurrence, structure, and development. *Protoplasma* 68:107-133.
9. JONES, M. G. K., and V. H. DROPKIN. 1975. Scanning electron microscopy of syncytial transfer cells induced in roots by cyst-nematodes. *Physiol. Plant Pathol.* 7:259-263.
10. JONES, M. G. K., and B. E. S. GUNNING. 1976. Transfer cells and nematode induced giant cells in *Helianthemum*. *Protoplasma* 87:273-279.
11. JONES, M. G. K., and D. H. NORTHCOTE. 1972. Nematode-induced syncytium—a multinucleate transfer cell. *J. Cell Sci.* 10:789-809.
12. KISIEL, M., J. CASTILLO, and B. M. ZUCKERMAN. 1971. An adhesive plug associated with the feeding plug of *Hemicyclophora similis* on cranberry. *J. Nematol.* 3:296-298.
13. MARCHANT, R., and A. W. ROBARDS. 1968. Membrane systems associated with the plasmalemma of plant cells. *Ann. Bot. N.S.* 32:457-471.
14. MC ELROY, F. D., and S. D. VAN GUNDY. 1968. Observations on the feeding processes of *Hemicyclophora arenaria*. *Phytopathology* 58:1558-1565.
15. RIGGS, R. D., K. S. KIM, and I. GIPSON. 1973. Ultrastructural changes in Peking soybeans infected with *Heterodera glycines*. *Phytopathology* 63:76-84.
16. ROMAN, J., and H. HIRSCHMANN. 1969. Embryogenesis and postembryogenesis in species of *Pratylenchus* (Nematoda: Tylenchidae). *Proc.*

- Helminthol. Soc. Wash. 36:164-174.
17. SPURR, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26:31-43.
 18. WERGIN, W. P., and B. Y. ENDO. 1976. Ultrastructure of a neurosensory organ in a root-knot nematode. *J. Ultrastruct. Res.* 56:258-276.
 19. WYSS, U. 1972. *Trichodorus similis* (Nematoda). Saugen an Wurzeln von Sämlingen (Rübsen). Film E 1763 der Enc. Cin. Göttingen. 12 p.
 20. WYSS, U. 1974. *Trichodorus similis* (Nematoda). Reaktion der protoplasten von Wurzelhaaren (*Nicotiana tabacum*) auf den Saugvorgang. Film E 2025. Institut für den Wissenschaftlichen Film, Göttingen. 19 p.
 21. WYSS, U. 1975. Feeding of *Trichodorus*, *Longidorus*, and *Xiphinema*. Pages 203-221 in F. Lamberti, C. E. Taylor, and J. W. Seinhorst, eds. *Nematode vectors of plant viruses*. Plenum Press, London and New York.