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Ultrastructural Investigation of Clover Roots during Early Stages of Infection by the Root-Knot Nematode, *Meloidogyne incognita*

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With 11 Figures

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Summary

Migration of root-knot larvae (*Meloidogyne incognita*) into the primary root tissues of red clover (*Trifolium pratense*, cult. "Kenland") was accompanied by separation and subsequent compression of cells in front of and along the path of the penetrating nematode. The protoplasts of the parenchymatous cortical cells did not respond to the presence of the penetrating larvae. However, as the nematode approached the differentiating vascular tissue, the cytoplasmic density of the pericyclic and meristematic cells increased. This increased density was accompanied by an alteration in the morphological features of the nucleus. In addition to these changes, two different types of extracellular material were observed during penetration. A homogeneous substance appeared in and around the external opening of the amphid; and an electron dense material was found along the middle lamellae of the separating plant cells and between the cuticle of the nematode and the cell walls of the host.

1. Introduction

The formation of root-knot galls caused by root-knot nematodes, *Meloidogyne* spp., has been frequently examined (NEMEC 1910, KOSTOFF and KENDALL 1930, CHRISTIE 1936, LINFORD 1937, OWENS and NOVOTNY 1960, DROPKIN 1969, KRUSBERG 1963, BIRD 1961, HUANG and MAGGENTI 1969 a, 1969 b). These studies have emphasized the initiation and development of syncytia, which are induced by feeding of the nematode. The earlier stages of infection have received less attention (CHRISTIE 1936, ENDO and WERGIN 1971, PAULSON and WEBSTER 1972). CHRISTIE (1936) found that root-knot larvae entering tomato roots caused some cellular destruction during migration into the root terminals. However, he agreed with the earlier observations of NEMEC (1910) that the usual mode of migration in root tissue was

intercellular. In addition, CHRISTIE (1936) frequently observed cellular hypertrophy in the cortex adjacent to the nematode as well as some distance away. The activity of larvae in fresh pineapple roots was examined by LINFORD (1937). Probing by the stylet of the nematode was readily observed, but because of optical difficulties he was unable to determine whether the nematode actually pierced the cells. LINFORD concluded that the stylet was used primarily to facilitate intercellular migration of the larva.
The purpose of the present study is to describe and discuss the morphological and ultrastructural changes that occur during the initial stages of root-knot infection of clover roots.

2. Materials and Methods

Larvae of *Meloidogyne incognita* were obtained from egg masses collected from roots of infected tomato plants grown in a greenhouse soil bed. Water containing the larvae was pipetted onto the roots of 3 day old red clover (*Trifolium pratense* cult. "Kenland") seedlings. Finetextured vermiculite was used to retain the larvae in the vicinity of the root tips. The seedlings were placed in 4-inch clay pots containing moist vermiculite and were covered with plastic film to retain moisture during the inoculation period. After 24 hours, plants were washed and transferred to clean vermiculite. One and 2 days after infection, 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. In this fixative, the infected regions of the roots were excised 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 hours was followed by washing in six changes of buffer over a period of 1 hour. The tissue then was postfixed in 2% osmium tetroxide for 2 hours, dehydrated in an acetone series, and infiltrated with a low viscosity medium (SPURR 1969). Silver-gray sections of selected root segments were cut on a Sorvall MT-2[1] Ultramicrotome with a diamond knife and mounted on uncoated 75 × 300 mesh copper grids. The sections were stained with 2% aqueous uranyl acetate (10 minutes), then with lead citrate (5 minutes). Thin sections were viewed in a Hitachi HU-11 C electron microscope operating at 75 kV with a 30 μm objective aperture.

3. Results

One day after inoculation, penetration sites could be localized approximately 1 to 5 mm from the root tip. Along this region of the root, ultrastructural changes were observed in the cortical, meristematic, and pericyclic cells. Because these modifications occurred before the initiation of extensive feed-

[1] Mention of specific products or companies does not imply endorsement by the U.S.D.A. over similar products or companies.

Fig. 1. Longitudinal section through the anterior part of a nematode that lies in the cortical region of red clover root. Physical pressure exerted by the invading nematode apparently causes the cortical cells to separate along their middle lamellae (arrows). ×8,800

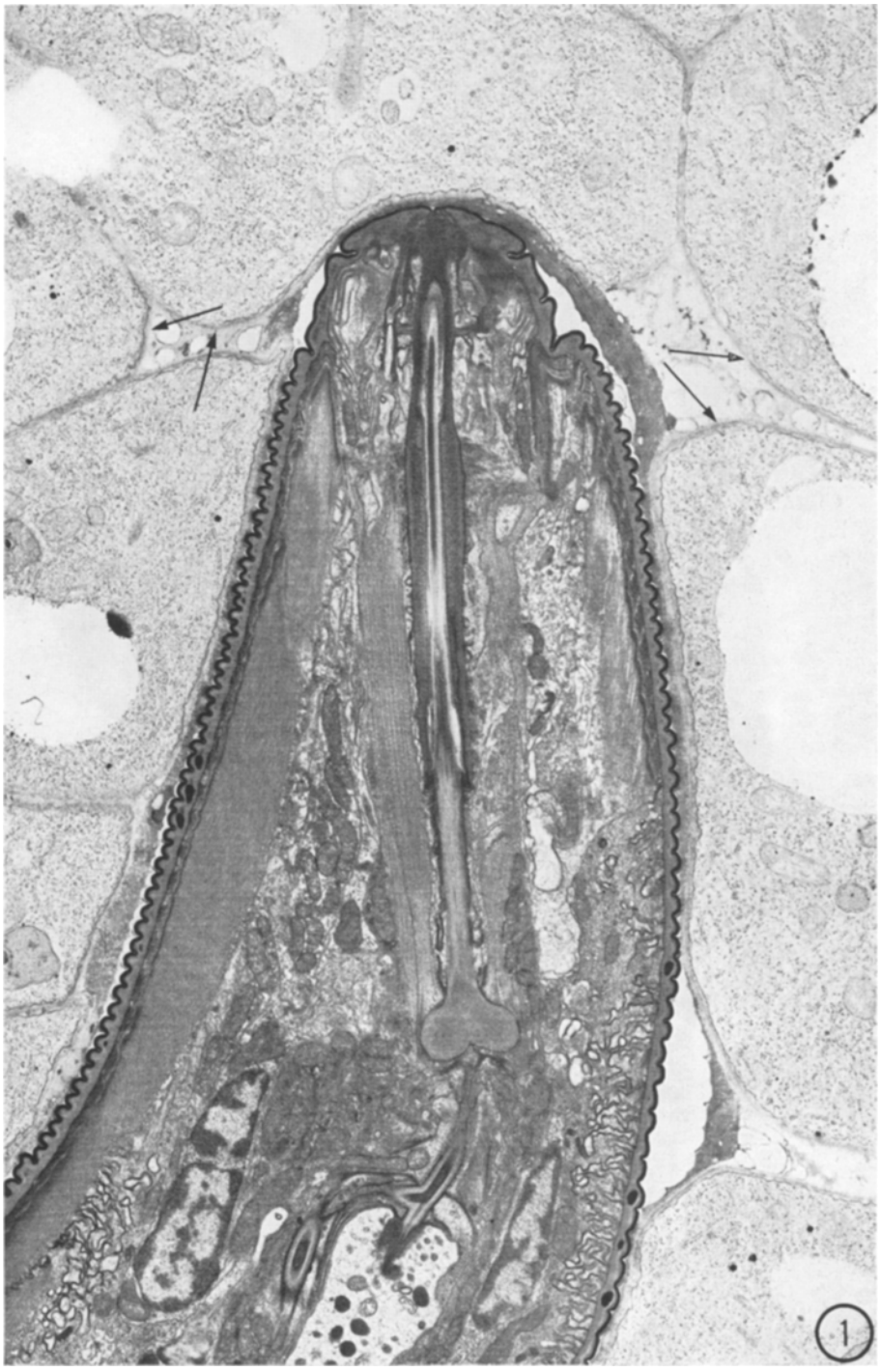


Fig. 1

ing, they probably represent responses that are associated with the movement and presence of the nematode, and in some cases, with the initiation of a syncytium.

3.1. Intercellular Penetration of the Cortex by the Invading Larvae

3.1.1. Separation of the Cortical Cells

During the early stages of invasion, the host cells are rarely ruptured; alternatively, the cortical cells are separated and displaced by the advancing larvae. At the lip region of the invading nematode, adjoining cortical cells separate along their middle lamellae (Fig. 1, arrows). This type of disjunction does not rupture the primary walls or damage the protoplasts of the affected cells. As penetration proceeds, the disjoined cells become distended and compressed along the body of the advancing larva (Figs. 2 and 3). Cortical cells of the root are generally isodiametrically shaped; however, the pressure associated with the penetration of the nematode causes the adjacent plant cells to flatten and to conform to the contour of the larva.

Except for the alteration in shape, no obvious ultrastructural changes are evident in the cortical cells during the early stages of penetration (Figs. 1 and 4). The types and relative numbers of organelles present in the cortical cells of roots do not significantly differ from those found in young meristematic cells.

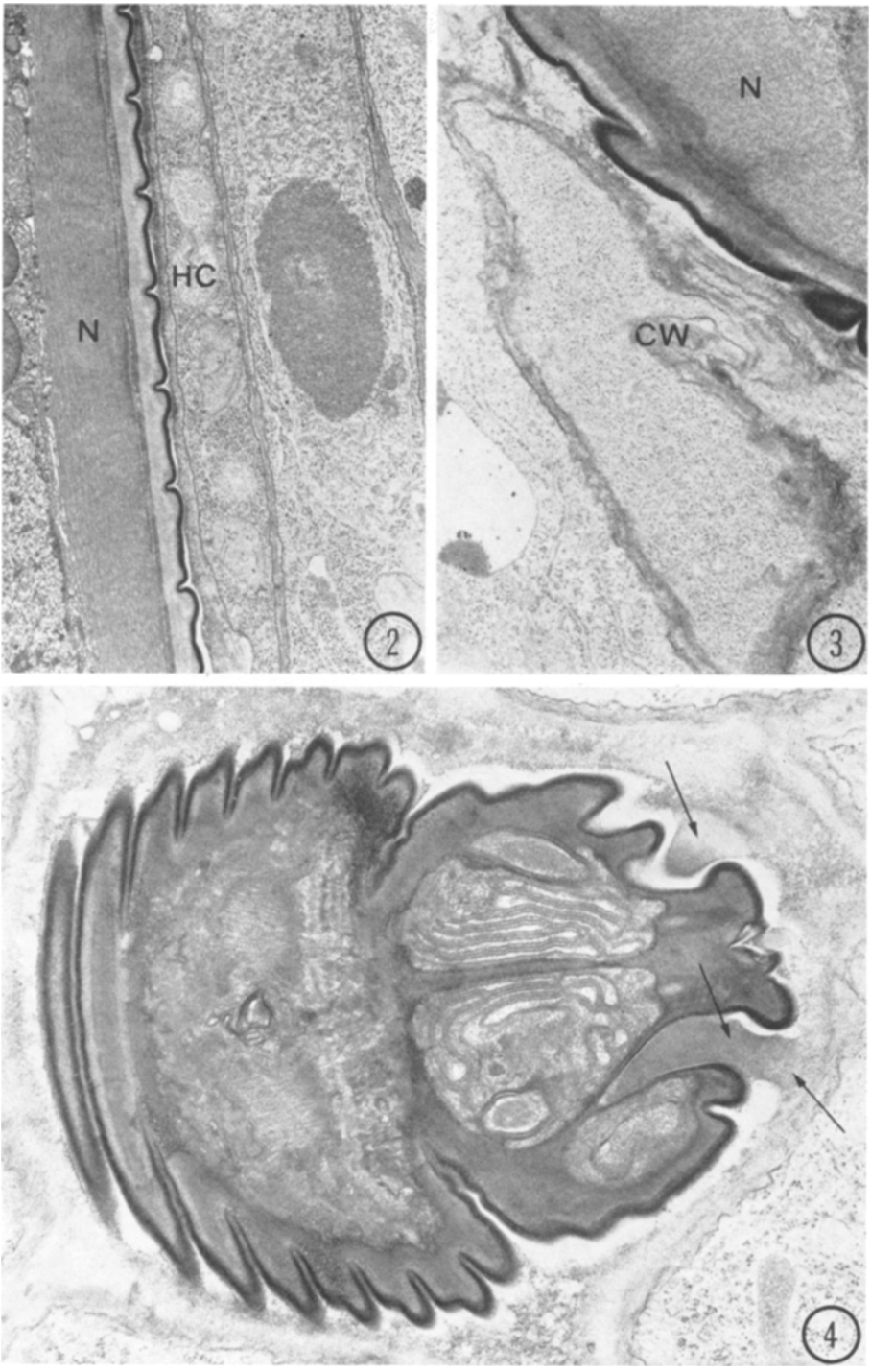
3.1.2. Appearance of the Nematode

An examination of penetration has failed to demonstrate the stylet extending into host cells. Furthermore, no damage to host cells can be attributed to the probing stylet.

A second characteristic of the invading nematode is the presence of a homogeneous electron-dense material in and around the amphid (Fig. 4, arrows). This material extends outward from the amphid to the outer layer of the primary wall of the host cell. Although the exact origin of this material cannot be ascertained, there is no indication that it results from cytoplasmic activity in the cortical cells of the plant.

Figs. 2 and 3. Parts of cortical cells that lie adjacent to a nematode (*N*) in red clover roots. During penetration of the root, host cells (*HC*) are flattened (Fig. 2) by the invading larvae, ×14,000, and cell walls (*CW*) are distorted (Fig. 3), ×19,500

Fig. 4. Tangential section through the anterior region of a nematode that is surrounded by cortical cells in a red clover root. A homogeneous electron opaque substance (arrows) can be observed in and around the opening of the amphid. ×20,500



Figs. 2–4

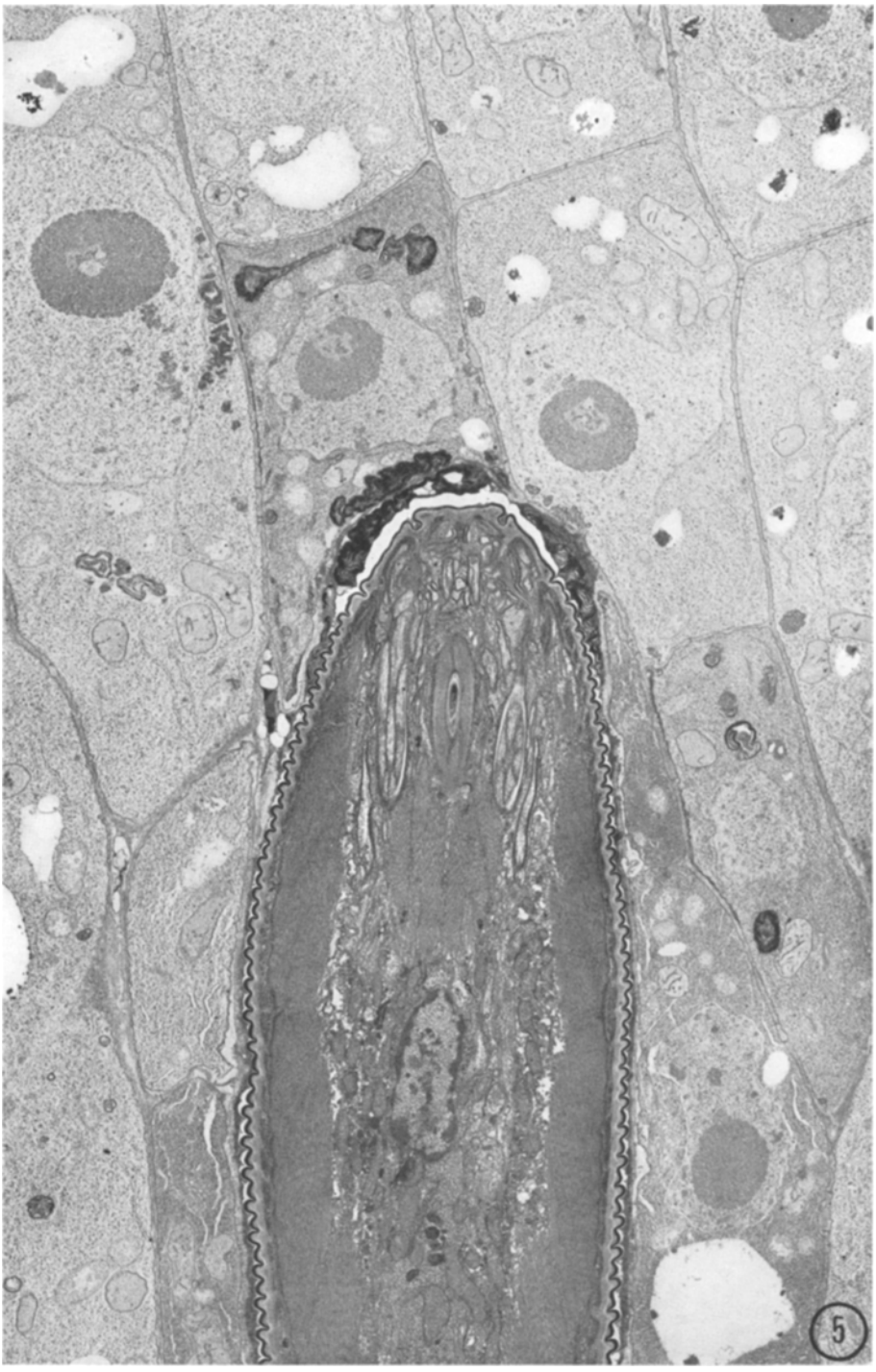


Fig. 5. A part of the meristematic region of red clover root containing a longitudinal section through a nematode. The plant cells that are adjacent to the larva are more prominently stained than the other unaffected host cells. ×5,800

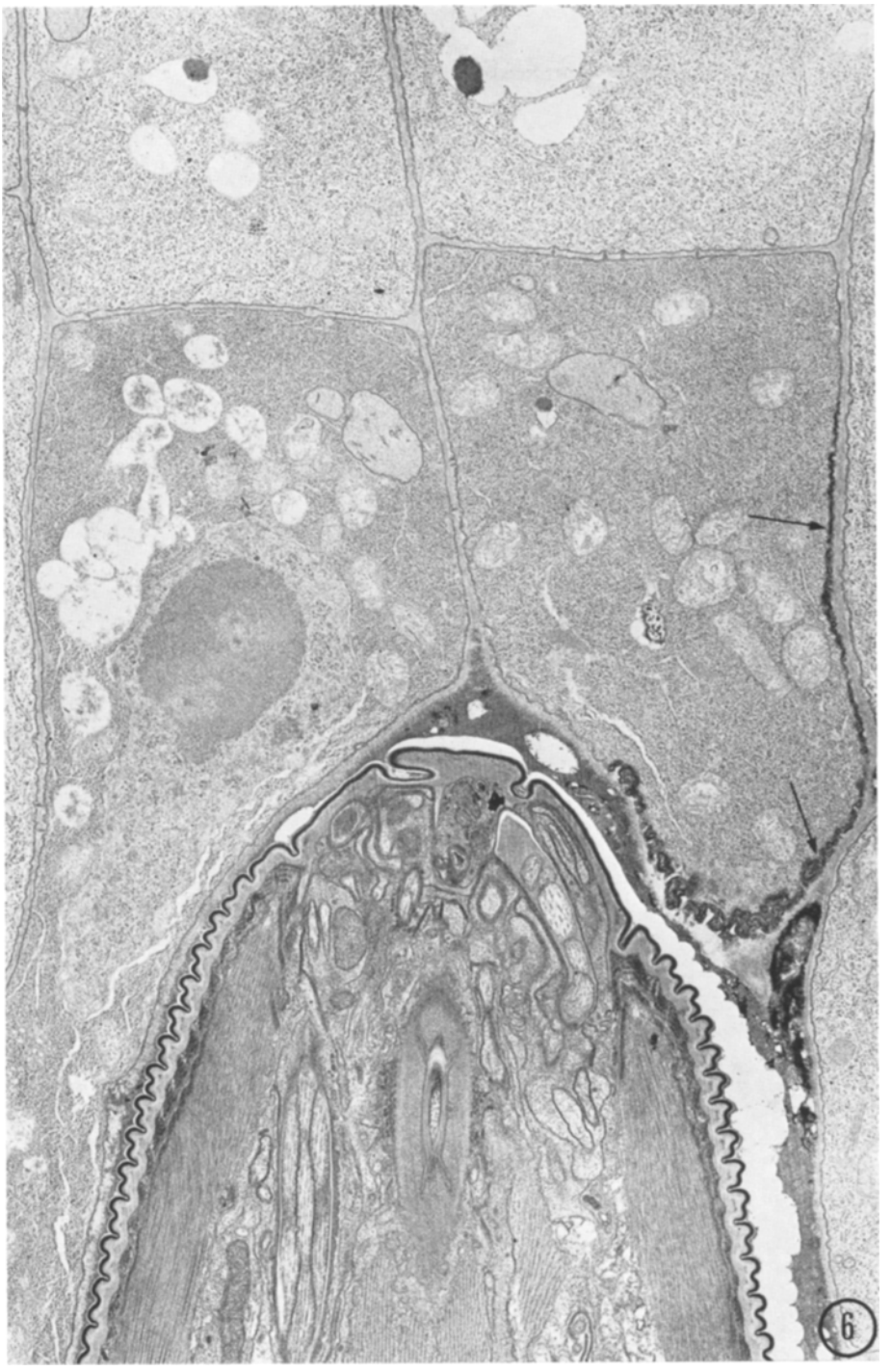


Fig. 6. Two stimulated red clover root cells at the anterior region of a larva. Penetration of the root by the nematode is often associated with distortion of host cells and their nuclei (cell at left) and with abnormalities of the plasmalemma (arrows, cell at right). ×11,000

3.2. Cytoplasmic Changes in the Meristematic and Pericyclic Cells

The protoplasts of the cortical cells, which were described above, are not visibly altered by the presence of the nematode. However, cytoplasmic changes occur in the meristematic (Figs. 5 and 6) and pericyclic (Fig. 7) cells that are contacted by the lip regions of penetrating larvae. As a result of these changes, the affected or stimulated cells stain more prominently and are easily distinguished from unaffected cells.

An increase in the number of ribosomes and the appearance of a granular matrix are largely responsible for the prominent staining in stimulated cells. The ribosome population increases to more than twice the number that is found in the unaffected cortical cells (Figs. 8 and 9). These new ribosomes are not bound to membranes of the endoplasmic reticulum, nor are they associated in obvious polysome configurations. Alternatively, they lie freely dispersed throughout the matrix of the cytoplasm. Coincident with an increase in the number of ribosomes is the appearance of a fine granular, cytoplasmic matrix (Figs. 8 and 9). Although the granular material that comprises this matrix is also in the cisternae of the endoplasmic reticulum and in the vesicles from the dictyosomes (arrows, Fig. 8), its specific origin could not be determined.

The cytoplasmic changes that occur in cells affected by the nematode are accompanied by morphological alterations in the nucleus. Pressure from the invading larva distort the shape of the cells as well as their nuclei (Figs. 6 and 9). Nucleoplasm in these deformed nuclei becomes more electron dense and loses the 150 Å ribosome-like particles that are common in nuclei of normal cells (Fig. 9). Nucleoplasm of deformed nuclei frequently have larger particles (450 Å) (Fig. 9, arrows). Finally, parts of the nuclear envelope dilate (Figs. 9 and 10); however, no discernible contents are observed in these swollen areas. Alterations in the structures of membranes also occur in the endoplasmic reticulum and in the plasmalemma. Cisternae of the endoplasmic reticulum become dilated and appear similar to the swollen areas of the nuclear envelope. However, unlike the nuclear envelope, the dilated segments of the endoplasmic reticulum frequently contain the finely granular contents previously described for the cytoplasmic matrix (Fig. 8). Certain areas of the membrane of the plasmalemma become highly convoluted or interfolded (Fig. 11). Although these abnormal configurations generally appear along cell walls that lie adjacent to a larva, occasionally they also can

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Fig. 7. Longitudinal section through the median area of a nematode near the vascular region of red clover root. The nematode lies between the large cortical cells (left) and a more darkly stained file of pericyclic cells (right). Parts of two young protophloem elements can be observed in the upper right. ×6,200

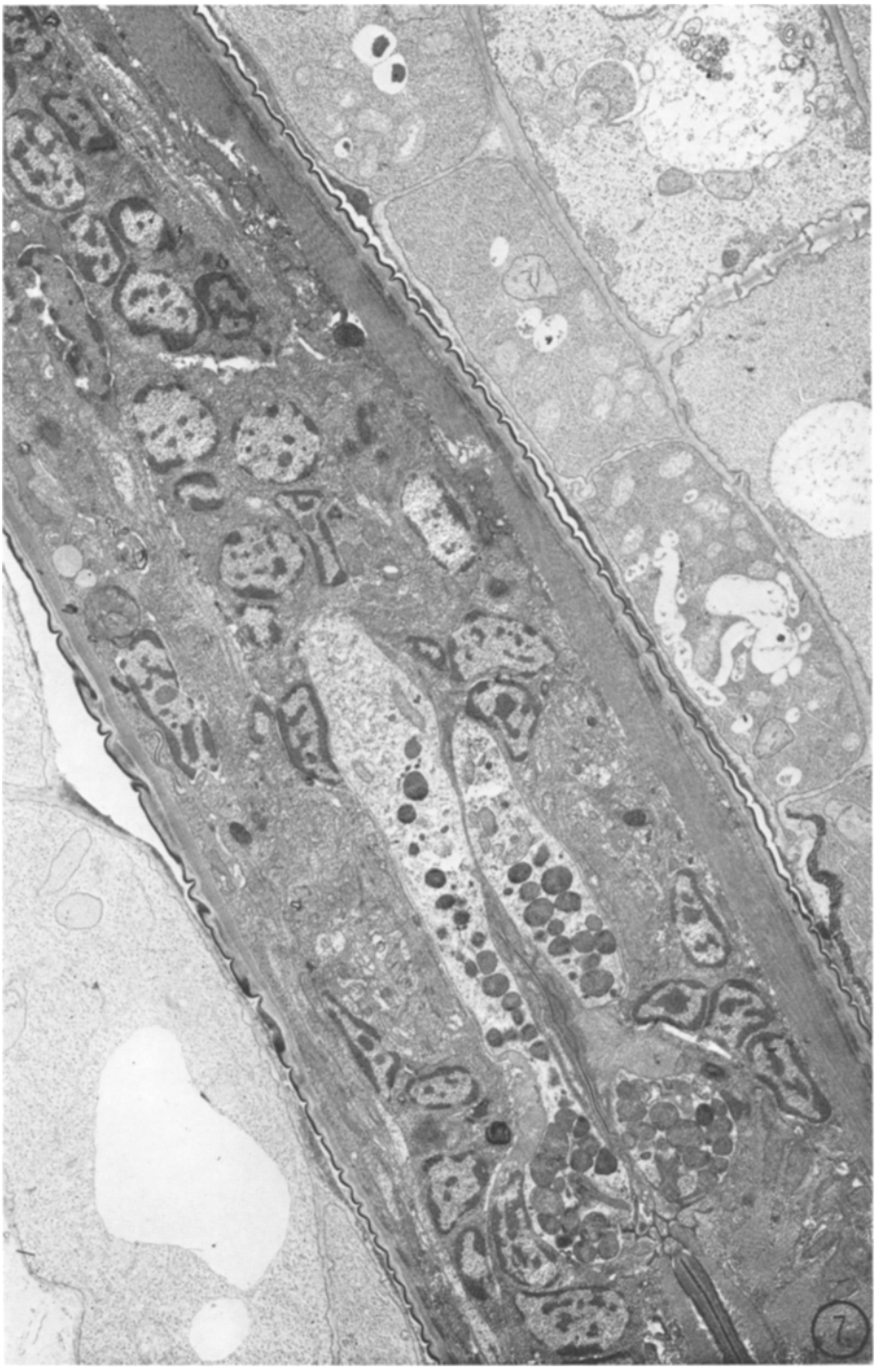


Fig. 7

be found along other walls of the same cell (Fig. 6, arrows). The fine structure of the nematode does not change as the nematode penetrates the cortical cells and encounters young undifferentiated cells or the vascular tissue. However, an electron dense layer of material is often observed external to a nematode as it lies amid stimulated cells (Fig. 10, arrows). This material is more electron dense than the homogeneous substance that was previously described in and around the amphid of the nematode (Fig. 4, arrows). It lies in the middle lamellae of cells near the lip region (Fig. 10) and also can be found laterally along the nematode between its cuticle and the opposing cell wall of the host (Figs. 6 and 7). Although this material is closely associated with the walls of stimulated cells of the host, its origin was not determined.

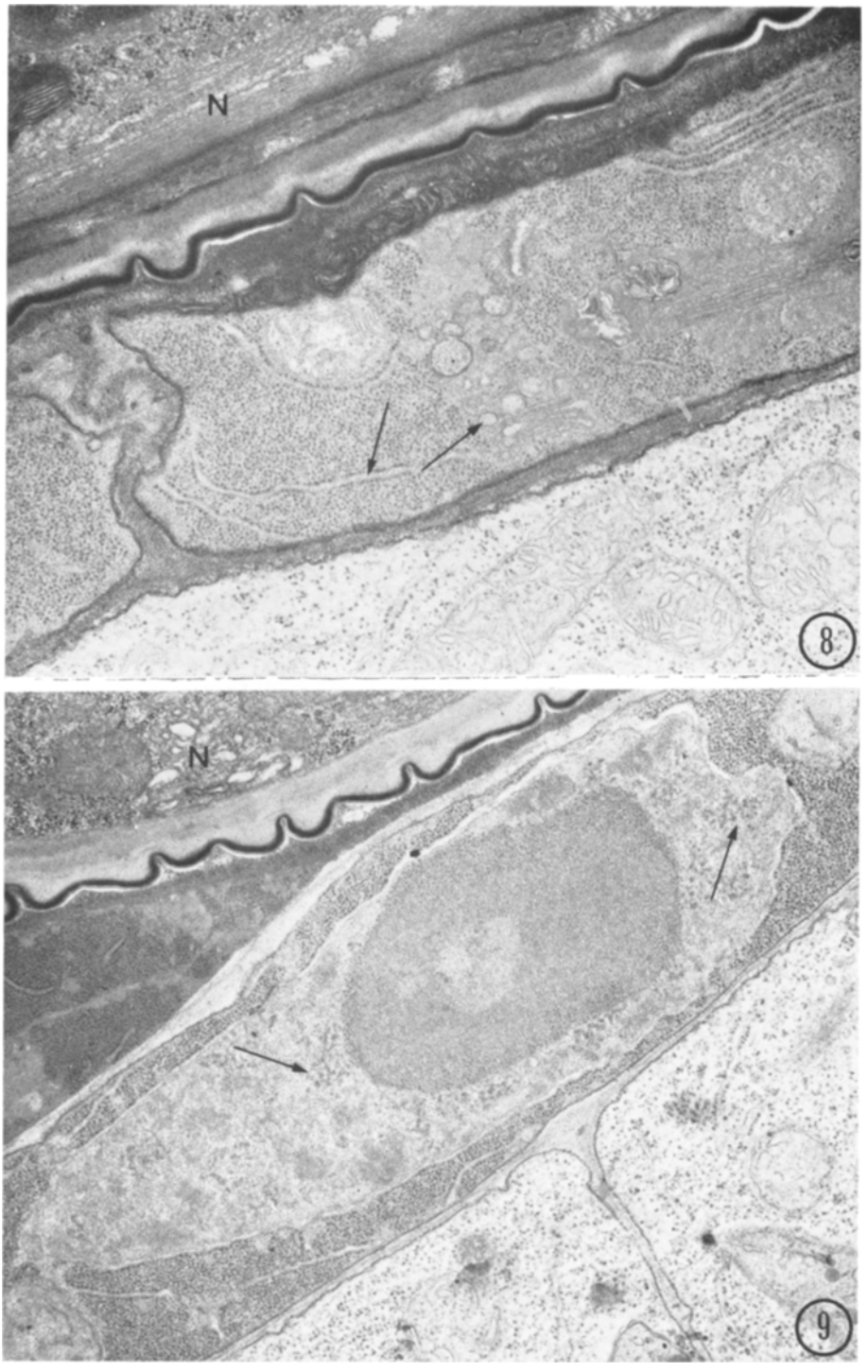
4. Discussion

This ultrastructural study illustrates the interaction between larvae of the root-knot nematode and cells along the path of the nematode during penetration and migration through clover roots. Previous histochemical studies of this species of nematode in soybean roots indicated that cells along the path of invading larvae respond to infection by having higher levels of enzyme activity than cells farther from the site of the nematode (ENDO and VEECH 1969). The potential for this increase in enzyme activity in clover roots is indicated by the marked increase in ribosomal numbers in clover root cells adjacent to the nematode. This increased metabolic activity may be caused by the pressure exerted by the nematode body as it moves between cells or by the probing action of the stylet.

The report on stylet probing by LINFORD (1937) and recent observations (ENDO and VEECH 1969) related to a histochemical study of soybean roots indicate that second-stage larvae probe between or through cells during tissue penetration. Thus, possibly the probings of cells could stimulate metabolic activity that is reflected by an increase in the ribosomes. Although no larvae were observed with stylets either extended into cells or projected much beyond the lip region of the nematode (Fig. 4), possibly the retracted position of the stylet may result from the larval reaction to fixation or from other manipulations of root tissues before or during fixation.

Fig. 8. Part of red clover plant cell adjacent to a nematode (*N*). The cytoplasm of the host cell, which has been stimulated by the larva, is dense with ribosomes and has a granular cytoplasmic matrix. The material that forms this matrix can be observed in the cisternae of the endoplasmic reticulum (left arrow) and in the vesicles from the dictyosomes (right arrow). ×29,000

Fig. 9. An elongate nucleus in a young red clover plant cell that has responded to the presence of a nematode (*N*). The nucleus is enlarged and the nucleoplasm has become electron opaque. In addition, the nucleoplasm contains groups of 450 Å particles (arrows) instead of the 150 Å ribosomelike particles that are characteristically found in nuclei of normal cells. ×25,000



Figs. 8 and 9

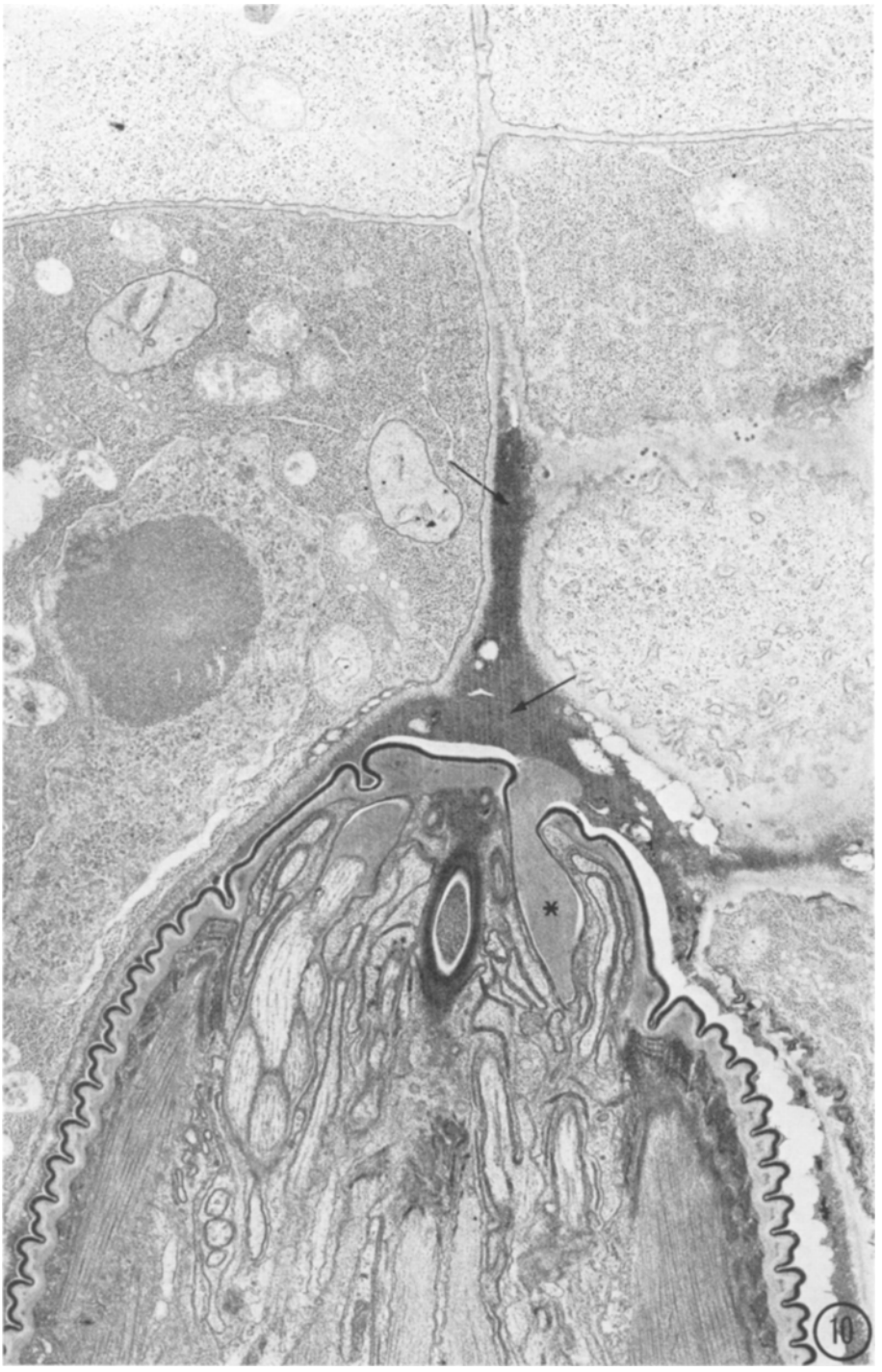


Fig. 10. Longitudinal section through the anterior part of a nematode that lies in the meristematic region of a red clover root. An electron dense layer of material (arrows) can be observed between cells at the lip region. This material stains more darkly than the substance (*) that is in the amphidial opening of the nematode. ×15,000

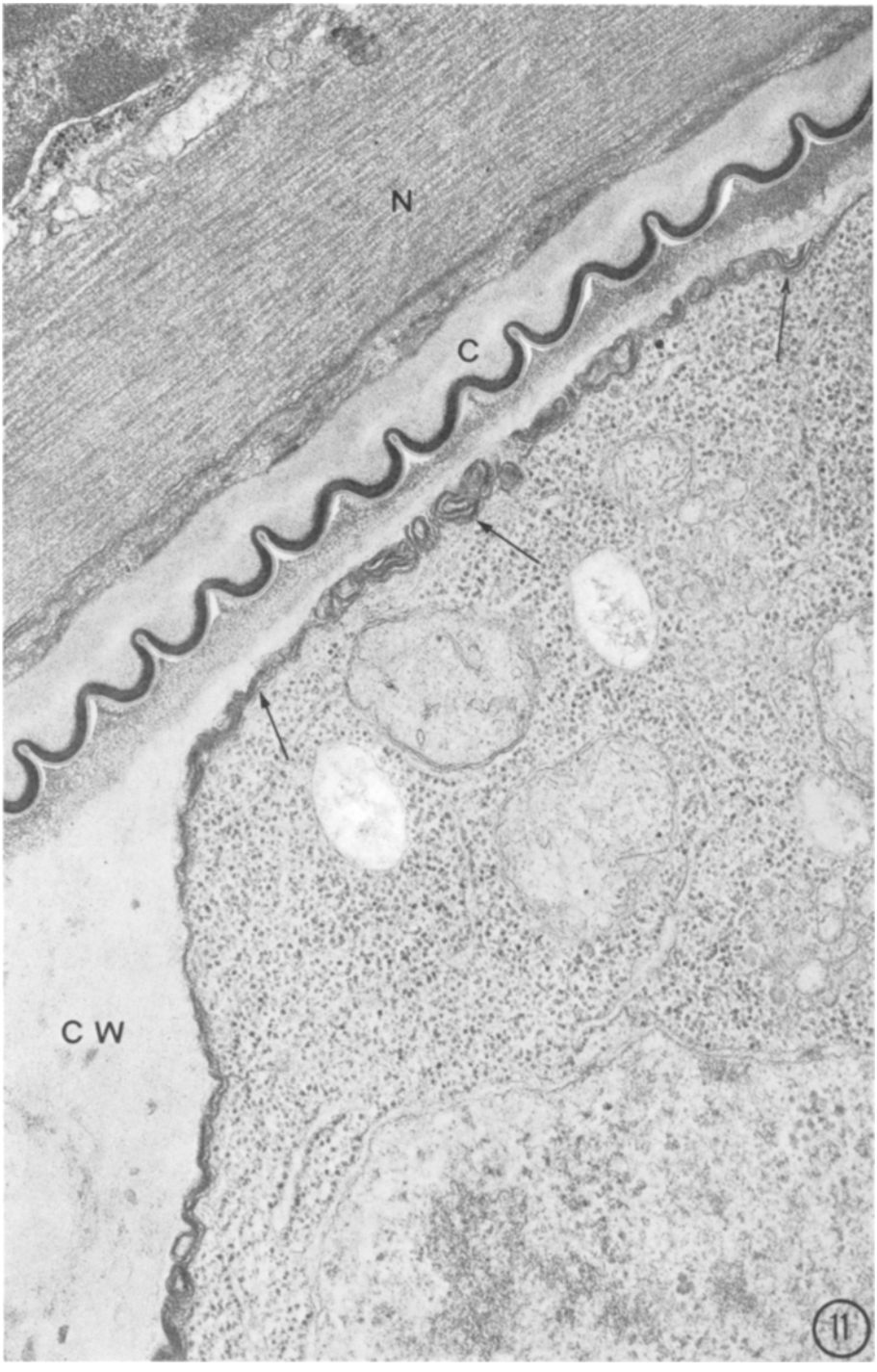


Fig. 11. Parts of young red clover plant cell and an adjacent nematode (*N*). The plasmalemma of the plant cell has become highly convoluted (arrows). *C* = cuticle of the nematode; *CW* = cell wall of the host cell. ×34,000

The electron dense materials (Figs. 6 and 10) that were observed along the middle lamella of separating cells and between the nematode and the host cells may be secreted by the nematode or plant. If this material is secreted by the nematode, it may be associated with material that alters or destroys middle lamellar structure and facilitates intercellular penetration by larvae. However, if the materials are of plant cell origin, the material may result from probing activity of the nematode, from damage caused by the separation of cells during larval migration, or from toxic substances related to defense reactions. A less electron dense homogeneous substance extends from the internal parts of the amphids to the exterior environment of the nematode (Fig. 10). This substance extending from the amphid region of the nematode is well defined from the more electron dense material adjacent to the plant cell in front of the nematode. The substance accumulating near the amphid openings might be distinguishable in terms of chemical composition. Previous studies by Bird (1966) demonstrated high esterase activity in the amphidial region of the root-knot nematode, *M. javanica*. However, specific sites of origin of these secretions are not available.

Amphids are thought to be chemoreceptors (Bird 1971). The function and mechanism by which amphids and the material extending from these structures are related to the sensory mechanism is not clear. Cilia within the amphids were clearly visible (Figs. 6 and 10); however, no structure other than the homogeneous substance was found between the cilia and the external environment during these early stages of infection.

These observations on the ultrastructure of host-parasite interaction provide further evidence of the extensive changes that take place during the early stages of infection. Future studies using cytochemical techniques at the ultrastructural level should contribute further to our understanding of the nature and function of these host-parasite interactions.

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