Cytology and Histology

Ultrastructural Changes Induced by *Scutellonema brachyurum* in Potato Roots

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ABSTRACT

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Within 12 hr after *Scutellonema brachyurum* began to feed on potato roots, dark brown lesions were initiated, which eventually extended several hundred micrometers axially in the root. Lesions involved cells not directly penetrated by *S. brachyurum*, which suggested a chemical as well as mechanical injury to tissue. Feeding was always intracellular and was generally restricted to epidermal and cortical cells. Ultrastructural alterations of potato root tissue occurred only in cells directly penetrated by *S. brachyurum*. An extensive membranous network surrounded the stylet in penetrated cells and appeared to originate from the plasmalemma, which invaginated around the stylet during penetration. Dense spherical inclusion bodies, which were interspersed in the membranous network, resembled material in the stylet orifice and buccal cavity of the nematode. Where the stylet penetrated the cell wall, a feeding plug formed that appeared to be continuous with material in the buccal cavity. Longitudinally arranged "macrotubules" of various diameters (20–40 nm, averaging 30 nm) occurred in groups of up to 25 in penetrated potato root cells. A residual matrix containing small, tightly packed spherical bodies was observed in root tissue parasitized for longer than 48 hr. It is believed that the residual matrix was the final stage of cytoplasmic degeneration. Bacteria were found only in cells disrupted by the nematode.

Additional key words: scanning electron microscopy.

Scutellonema brachyurum (Steiner) Andrassy is commonly found in soils used to grow tropical crops and ornamentals. *S. brachyurum* attacks Guinea yam (*Dioscorea rotundata*) in Puerto Rico (2), and in Nigeria it is considered a major problem on yams (*Dioscorea* spp.) (3,4). Other species of *Scutellonema* are associated with cotton in the southeastern United States (15,16,35). While injury to crops and gross symptomology have been described (1,2,15,16), histopathological studies have emphasized general feeding behavior (20) and the characteristics of heavily diseased or moribund tissue (3). Feeding of *Scutellonema* has been characterized as truly ectoparasitic or superficially endoparasitic (15,16). *S. brachyurum* also has been reported to mature and reproduce within root tissue (16).

Several investigations have dealt with the histopathology of other members of the Hoplolaimidae, including *Hoplolaimus* (18,19,26,29) and *Helicotylenchus* (21,23). These studies have examined more critically the morphological changes in parasitized roots. Lesions appear red or brown and often extend axially several hundred micrometers from the site of penetration (26,29). Surface depressions and flecked cell contents are common around feeding sites that usually support only a single nematode (18,21,26). These ectoparasites generally move through cells, not between them, creating large cavities within cortical tissue. Feeding has rarely been observed to extend into the endodermis or stele of the root.

Ultrastructural pathology of nematode-parasitized plants generally has been restricted to sedentary endoparasites (7,8,25) and ectoparasites in the order Dorylaimida (36). At the time this report was written, only two investigations (13,19) of the ultrastructural pathology at feeding sites of ectoparasitic hoplolaimids had been published. Nothing was known about the fine structure of cellular modifications induced in host plants by *Scutellonema* spp.

The purpose of this study was ultrastructural examination of host responses during initial penetration by *S. brachyurum* and description of the sequence of cellular events that occur in parasitized potato roots.

MATERIALS AND METHODS

True seeds of potato (*Solanum tuberosum* L.) were obtained in 1980 from R. E. Webb, Chief, Vegetable Laboratory, Beltsville Agricultural Research Center, USDA, Beltsville, MD. Seeds of

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tomato (*Lycopersicon esculentum* L., 'Globemaster Hybrid 65'), obtained in 1979 from the Burpee Seed Co., were used for all greenhouse experiments.

Observation chambers. A water agar system was used for direct observation of all aspects of feeding behavior and lesion development. Observation chambers were constructed by cutting a 4 × 5-cm rectangular hole in the bottom of plastic 9-cm-diameter petri dishes and covering the hole with a 4.5 × 6-cm glass coverslip held in place with epoxy cement. Observation chambers were surface sterilized by treatment with 1.0% NaOCl for 30 min at 23 C followed by two rinses in sterile distilled water (SDW). Growth medium consisted of 86 ml of water, 14 ml of full-strength Hoagland's solution, and 1.25 g of Bacto agar. The full-strength Hoagland's solution was filter sterilized through a 0.22-μm filter and added to liquid water agar cooled to 50 C. Prior to solidification, 15 ml of this medium was poured into surface-sterilized observation chambers.

Potato seeds were soaked for 24 hr in SDW, surface sterilized for 30 min in 1% aqueous chlorhexidine gluconate, and washed three times in SDW. Seeds were aseptically germinated on the growing medium in petri dishes maintained at 21 C and with a 12-hr light cycle (20,000 lux). After 6 days, six seedlings were transferred to each observation chamber. Each seedling was inserted about 2–3 mm into the agar medium and allowed to grow for 6–10 days prior to inoculation. During this time rootlets reached the bottom of the chamber and grew laterally along the glass coverslip for several centimeters.

Adult females and immature juveniles of *S. brachyurum* were obtained either from oleander (*Nerium oleander*) or from glasshouse-reared tomato seedlings that had been inoculated 3 wk after germination with populations of juvenile and adult female *S. brachyurum*. Nematodes were extracted by sugar flotation (12) and treated with 0.5% aqueous chlorhexidine gluconate for 30 min at room temperature (~23 C). After being washed three times in SDW, they were placed for 24 hr on a double layer of facial tissue suspended 1 cm deep in distilled water on a stainless steel screen, and sealed in a plastic refrigerator container. Nematodes that migrated through the facial tissue were collected on a 38-μm (400-mesh) sieve and 50 *S. brachyurum* (either adult females extracted from oleander or juveniles extracted from tomato 2 mo after inoculation) were handpicked to a dish of SDW from which they were pipetted in a volume of 1 ml to the observation chambers, each containing six established potato seedlings. After inoculation, except for observation periods, all chambers were kept in total darkness for the duration of the experiment. Observations on feeding behavior were made through stereo dissecting and compound light microscopes during a 120-hr period.

Preparation of parasitized roots for transmission electron microscopy. All fixation and buffer washes were conducted at 4 C. Observation chambers containing infected seedlings were chilled for 2 hr at 4 C and then flooded with chilled 3% glutaraldehyde in 0.20 M phosphate buffer, pH 6.8. This fixative was allowed to permeate the contents of the observation chambers for 3 hr before removing cubes of agar (4 mm on a side) that contained segments of infected roots. Agar-encapsulated root segments were fixed an additional 15 hr in fresh glutaraldehyde solution, washed six times in 0.20 M phosphate buffer, pH 6.8, during 4 hr, then postfixed for 16–18 hr in 1% osmium tetroxide in 0.20 M phosphate buffer, pH 6.8. After three additional washings with chilled SDW within 1 hr, root sections were slowly brought to room temperature. Some of the roots were stained in the agar block with 2% aqueous uranyl acetate for 1 hr at 55 C followed by three poststaining washes in SDW. Root tissue was dehydrated in an acetone series (10% increments) with three changes in 100% anhydrous acetone preceding infiltration overnight with Spurr's "B" embedding medium (30). Blocks were cured for 18–24 hr at 70 C.

Silver-gold sections were cut with a Dupont diamond knife mounted in either an LKB Ultratome III or Sorvall MT2-B ultramicrotome. Sections were collected on naked 74-μm (200-mesh) copper grids and stained. Thin sections of root tissue that had been stained in the agar block were further stained with Reynold's lead citrate (22) for 10 min at room temperature. Tissue sections stained after cutting were treated with 2% aqueous uranyl acetate for 20 min at 70 C, then poststained with Reynold's lead citrate for 10 min at room temperature. Sections were viewed and photographed on a Hitachi H-500 or JEOL 100 CX transmission

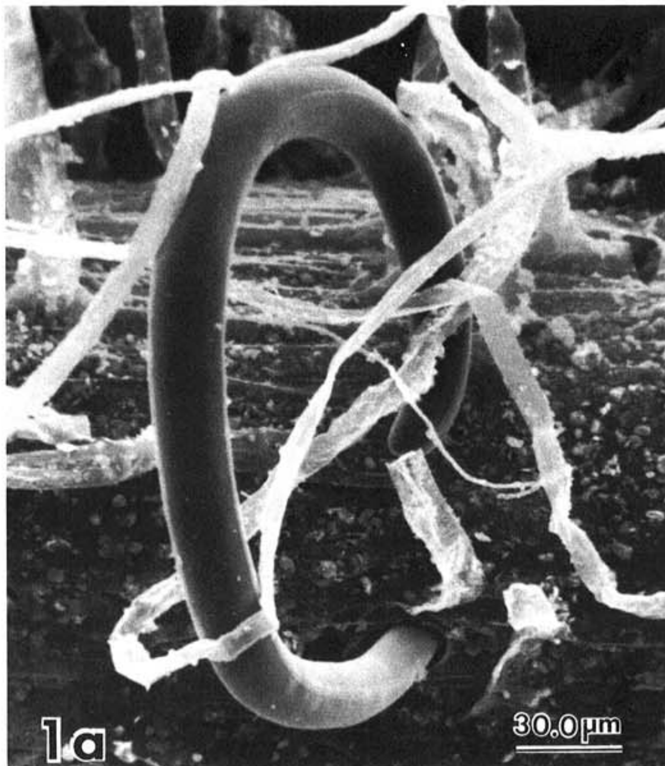
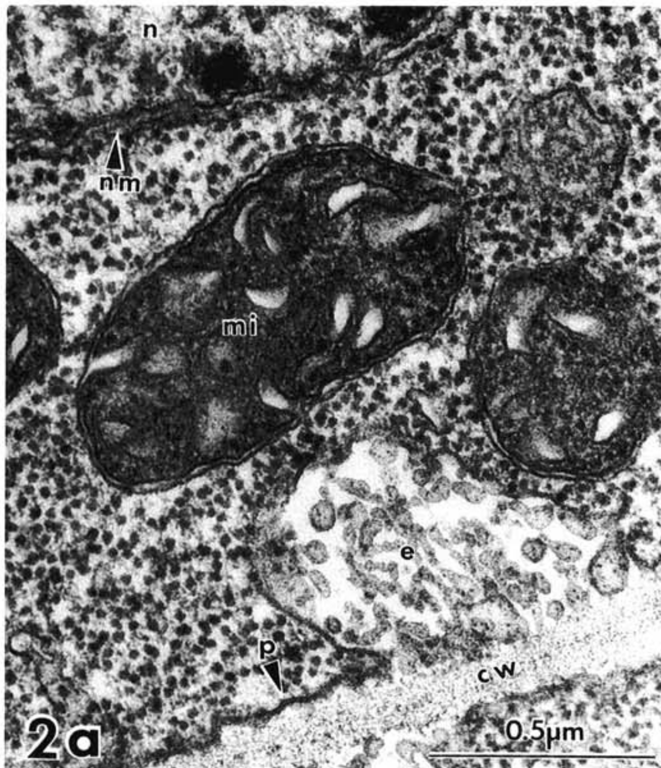
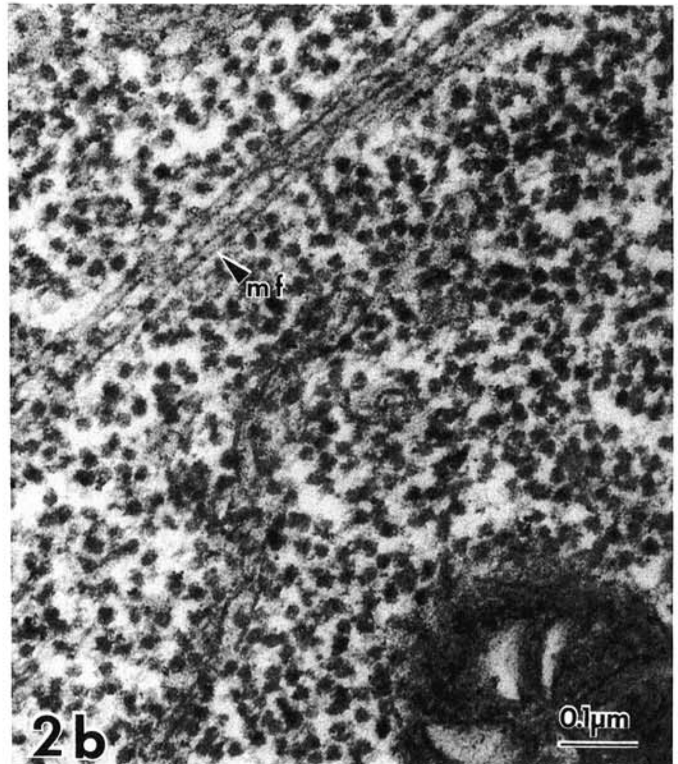


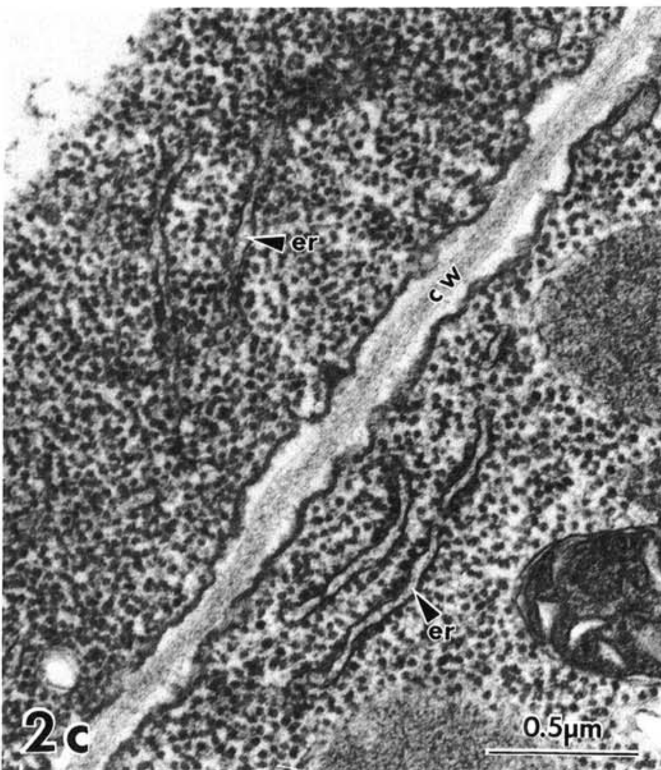
Fig. 1. Scanning electron micrographs of *Scutellonema brachyurum* penetrating potato roots. Nematodes generally entered roots only partially (**1a**), but occasionally were observed entirely within roots (**1b**).

electron microscope. Observations were made on sections from seven different roots.

Preparation of parasitized roots for scanning electron microscopy. Six 1-wk-old seedlings were transferred to Plexiglas chambers (5 × 6 × 1 cm) containing 30 cc of steam-sterilized 0.84-mm (20-mesh) silica sand. Seedlings were allowed to grow to a height of 6 cm for approximately two more weeks before inoculation. Plexiglas chambers were placed beneath a 1-L glass beaker and exposed to a 12-hr light cycle (20,000 lux) at 21 C. Nematodes, reared on tomato, were extracted and surface sterilized







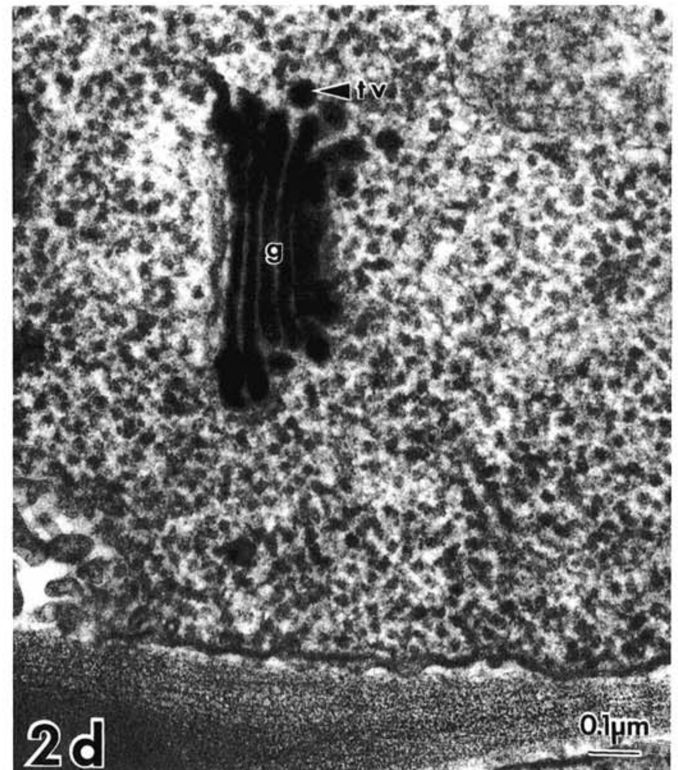


Fig. 2. Transmission electron micrographs of control potato root tissue. Organelles were regularly shaped and contained intact membranes with easily recognizable substructure. **2a,** The plasmalemma (p) was closely applied to the cell wall (cw) except where excretory vesicles (e) were formed. Mitochondria (mi) were oval with well-defined membranes and cristae. The nucleus (n) was surrounded by a nuclear membrane (nm). **2b,** Rarely observed microfilaments (mf) were always straight and uniform in diameter (16–18 nm). **2c,** Endoplasmic reticulum (er) was common throughout control sections. **2d,** Golgi bodies (g) exhibited a well-formed stack with numerous transitory vesicles (tv).

in the manner described earlier. Inoculum consisted of suspensions containing ~400 juveniles of *S. brachyurum* and just a few saphrophytic nematodes per milliliter. One milliliter of inoculum suspension was added to each of three chambers and an equal volume of distilled water was added to a fourth chamber as a control. Two milliliters of quarter-strength Hoagland's solution, the sole water and nutritive source, was applied at the rate of 2 ml every 48 hr until harvest of roots 3 wk after inoculation.

Chambers were chilled for 2 hr at 4 C and sand particles adhering to the roots were removed with a gentle stream of chilled distilled

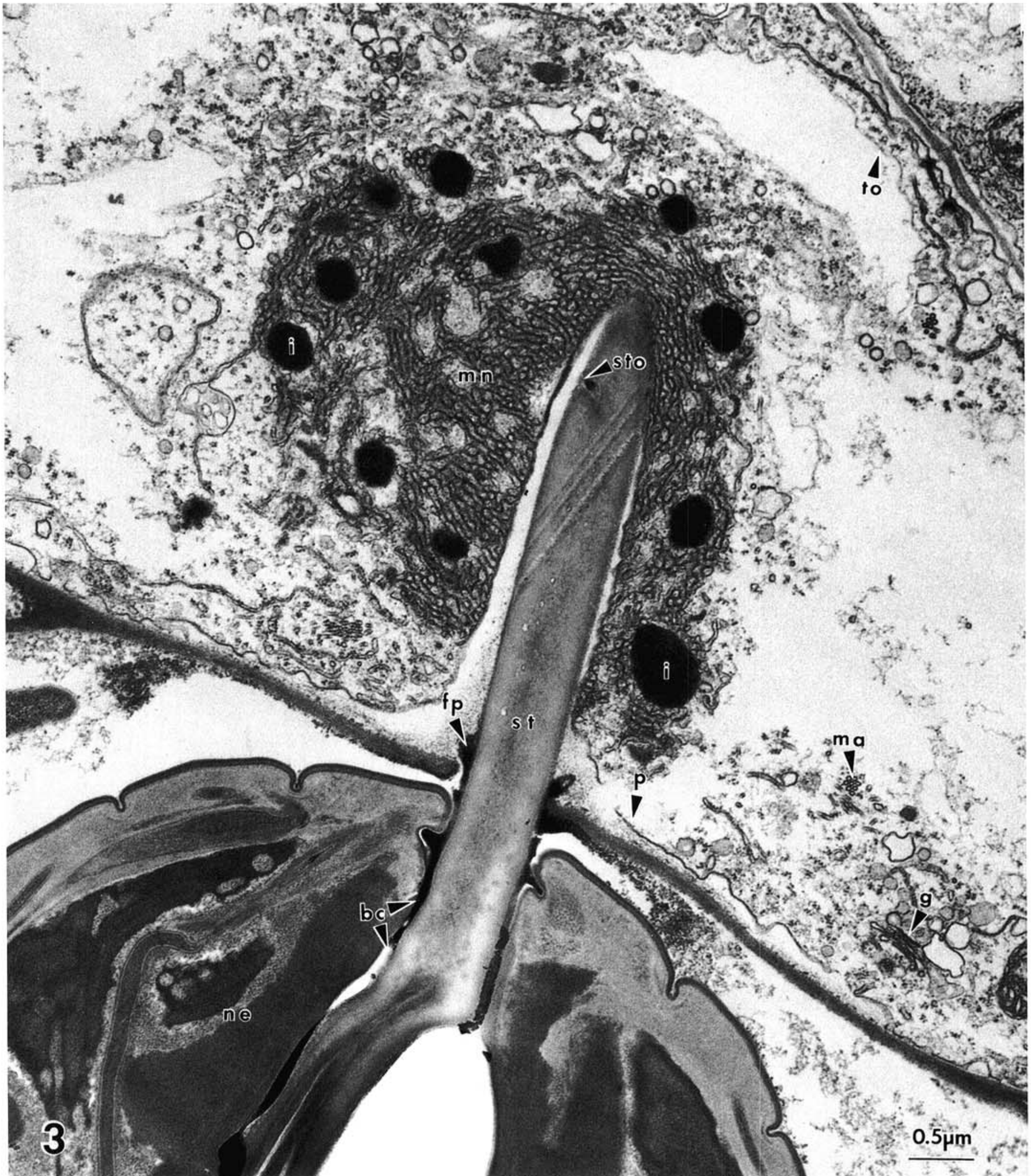


Fig. 3. Cross section through penetrated root tissue with stylet of *Scutellonema brachyurum* inserted into a cortical cell. A feeding plug (fp) formed where the nematode's (ne) stylet (st) penetrated the cell wall. The material forming the feeding plug seemed to emanate from the buccal cavity (bc). Associated with the stylet was a membranous network (mn) containing spherical inclusion bodies (i) that had the same ultrastructure and electron density as material found in the stylet's orifice (sto). Macrotubules (ma) occurred throughout the penetrated cell. Tonoplast (to) and plasmalemma (p) were discontinuous and Golgi bodies (g) had irregular membranes.

water. Washed roots were immediately placed in water chilled to 4 C and 7- to 8-mm segments of root tissue were cut and immersed in fixative at 4 C.

Fixation, buffer washes, and acetone dehydration was the same as for roots prepared for transmission electron microscopy. After fixation and dehydration, root segments were critical-point dried, mounted on double-stick tape, coated with 15 nm of gold-palladium (60:40) in a Polaron E5100 sputter coater, and observed and photographed with an ISI DS-130 scanning electron microscope.

RESULTS

Feeding and symptomology. Juvenile *S. brachyurum* reared on tomato were more active than adult females from populations on oleander. Juveniles began feeding as early as 12 hr after introduction to the agar medium, while adult females were never observed to initiate feeding before 24 hr. Juveniles, from tomato, fed throughout the agar system on individual roots and root tips. Adult females preferred roots lying adjacent to other roots, and rarely fed on individual roots or root tips. When more than one nematode attacked two adjacent roots, all nematodes initially preferred only one of the roots. Migration to the adjacent root followed after considerable cell damage was sustained by the first root.

Adult females and juveniles assumed coiled positions during initial feeding and penetration. After 24–36 hr of feeding, and after penetration to a depth of one or two cells, nematodes uncoiled and took on a variety of body configurations. Typically, nematodes entered roots individually by puncturing one or two epidermal cells, usually in or near areas of dense root hairs, and expanded the penetration hole by physical pressure as they moved inward (Fig. 1). Feeding generally was restricted to epidermal and cortical cells, with penetration always occurring intracellularly. Stylet thrusts and pumping of the metacorpus were intermittent. Following stylet thrusts, a rest period of several hours preceded metacorpal activity, which lasted no longer than 1 hr. This sequence was repeated several times before further penetration was observed. Up to four cells were penetrated within 48 hr and as many as six to eight cells were penetrated within 96 hr. Feeding ceased and nematodes migrated from the feeding site between 96 and 120 hr after feeding began.

Nematodes were never observed within roots in the agar system, but in the sand system nematodes were observed coiled entirely within a root or with only their tails exposed (Fig. 1b). No eggs were observed within root tissue in either system, but they were commonly seen in the agar system near roots that supported actively feeding adult females.

Light-tan to dark-brown lesions developed within 24–48 hr after feeding began at that site. Discoloration of cells began near the head of the nematode within 12–24 hr and spread to adjacent cells within 48 hr, even if those cells were not penetrated by the nematode or its stylet. Lesions extended several hundred micrometers axially from the site of penetration. Lesion development and extension ceased when nematode feeding stopped and roots were vacated.

Ultrastructure. Cells in lesions that were not penetrated directly but showed visual discoloration 48–72 hr after feeding began were similar to control cells (Fig. 2a–d) and lacked obvious ultrastructural alterations typically observed in penetrated cells. Cell organelles in this part of the lesion appeared normal with intact membranes and no changes in shape or size. Cortical cells immediately adjacent to penetrated and parasitized cells likewise failed to show obvious ultrastructural alterations. Only cells that were breached by the stylet were demonstrably modified. Most striking of these modifications was a membranous network that formed around, and was associated only with, the stylet's path of entry into cortical cells (Fig. 3). The network was oriented in the plane of the stylet in sections directly through the long axis of the stylet (Fig. 3), but cross sections of roots cut above or below the path of stylet entry revealed the network in cross-sectional view (Fig. 4). The membranes were composed of a double layer of material that was highly variable in shape and width, and had the appearance of an interwoven membrane system.

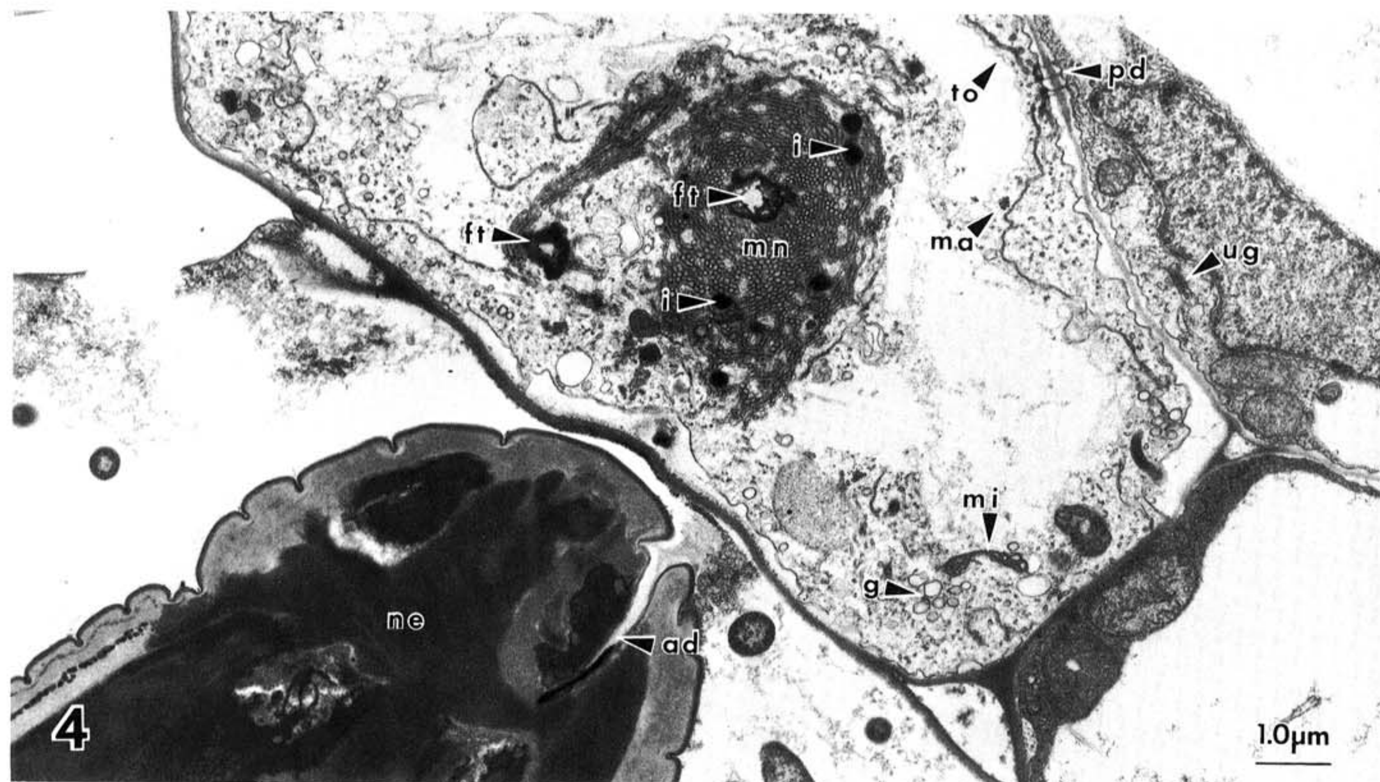


Fig. 4. Parasagittal cross section above the path of the stylet of *Scutellonema brachyurum* in potato root tissue. Transitory vesicles formed from the Golgi bodies (g). Amphidial ducts (ad) contained material that resembled inclusion bodies (i). (to = Tonoplast, ug = normal Golgi bodies, mi = mitochondria, ne = nematode, ma = macrotubules, mn = membranous network, pd = plasmodesmata, and ft = feeding tube.)

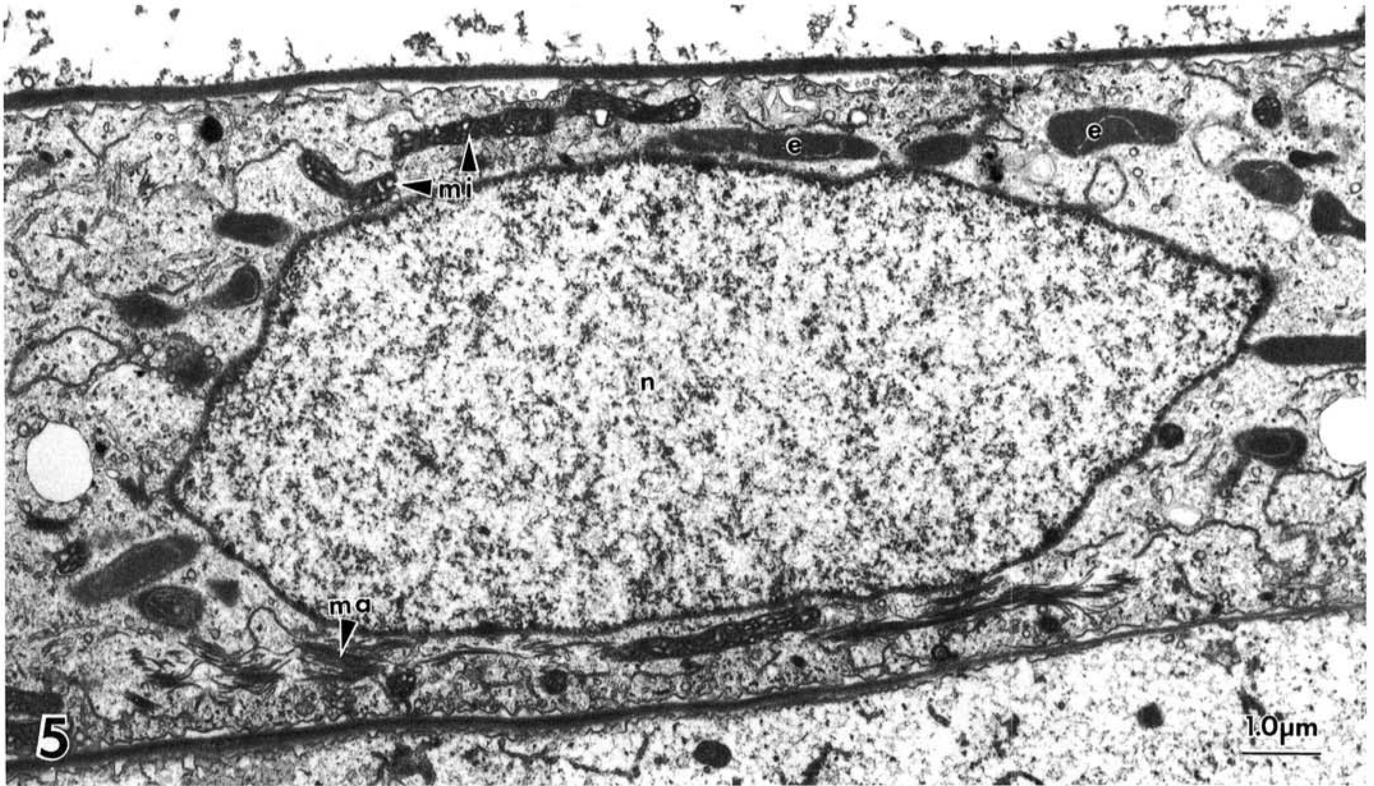
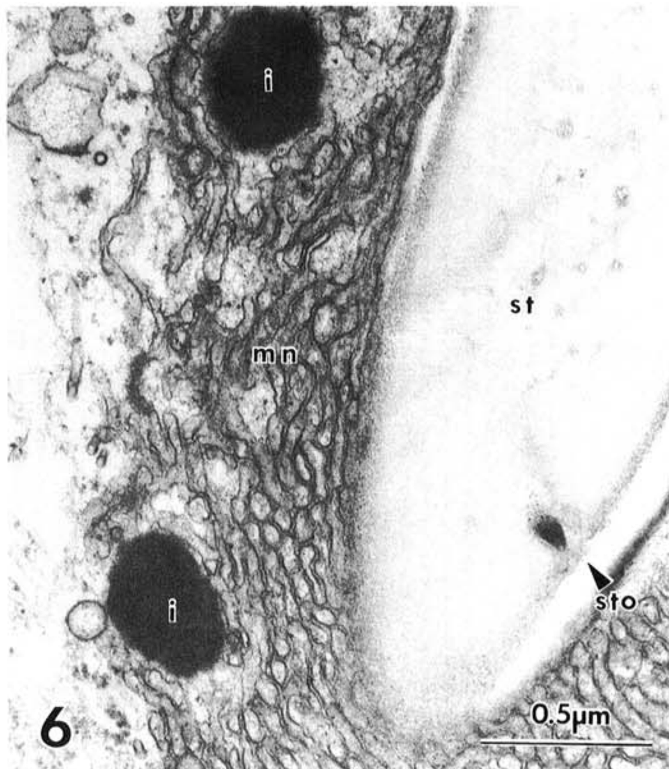
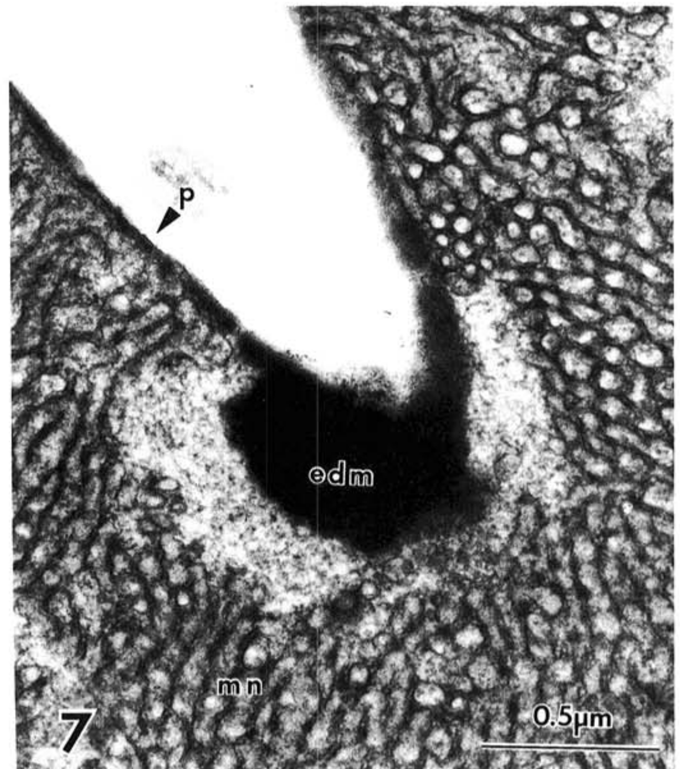


Fig. 5. Longitudinal thin section through a cortical cell of a potato root parasitized by *Scutellonema brachyurum* for 36 hr. Elongate vesicular bodies (e) differed greatly from the inclusion bodies observed in the membranous network near the path of stylet entry. (mi = Mitochondria, ma = macrofilaments, and n = nucleus.)

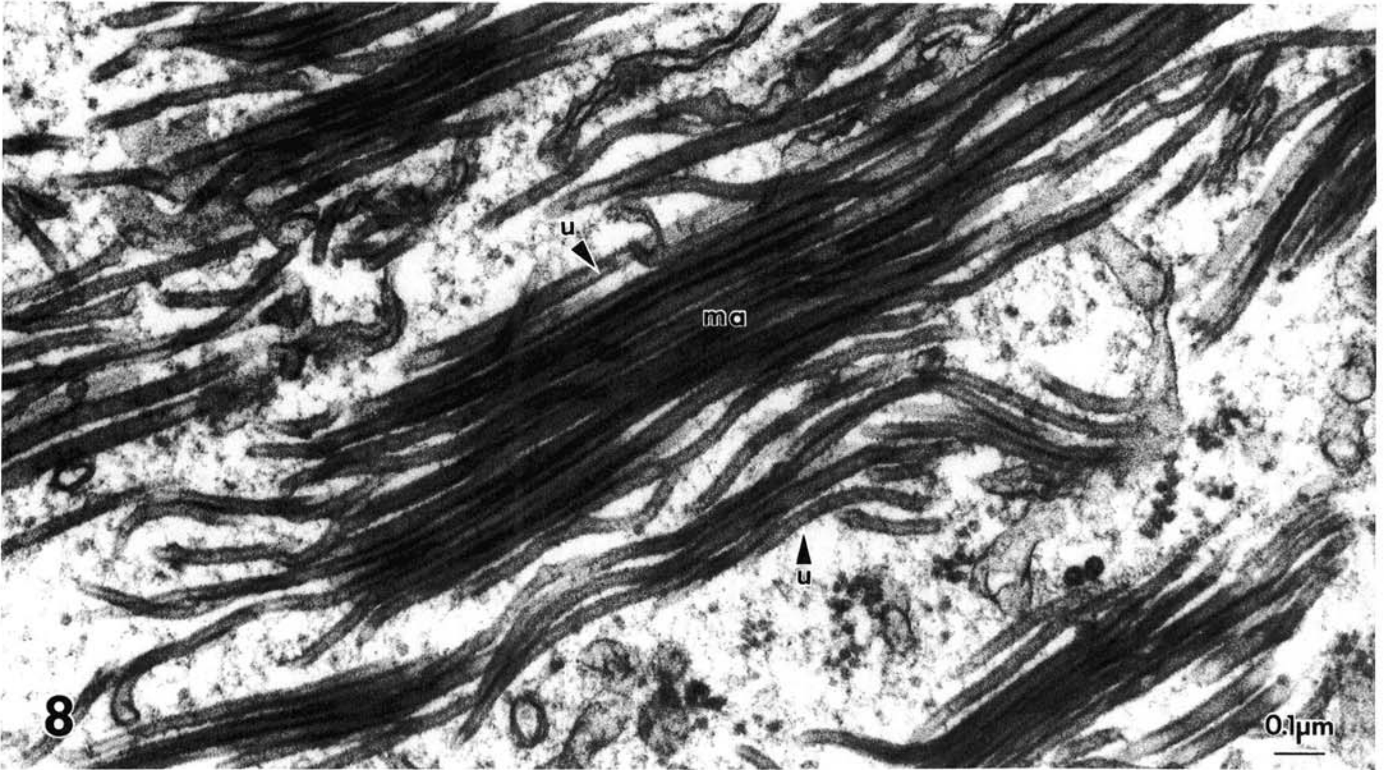


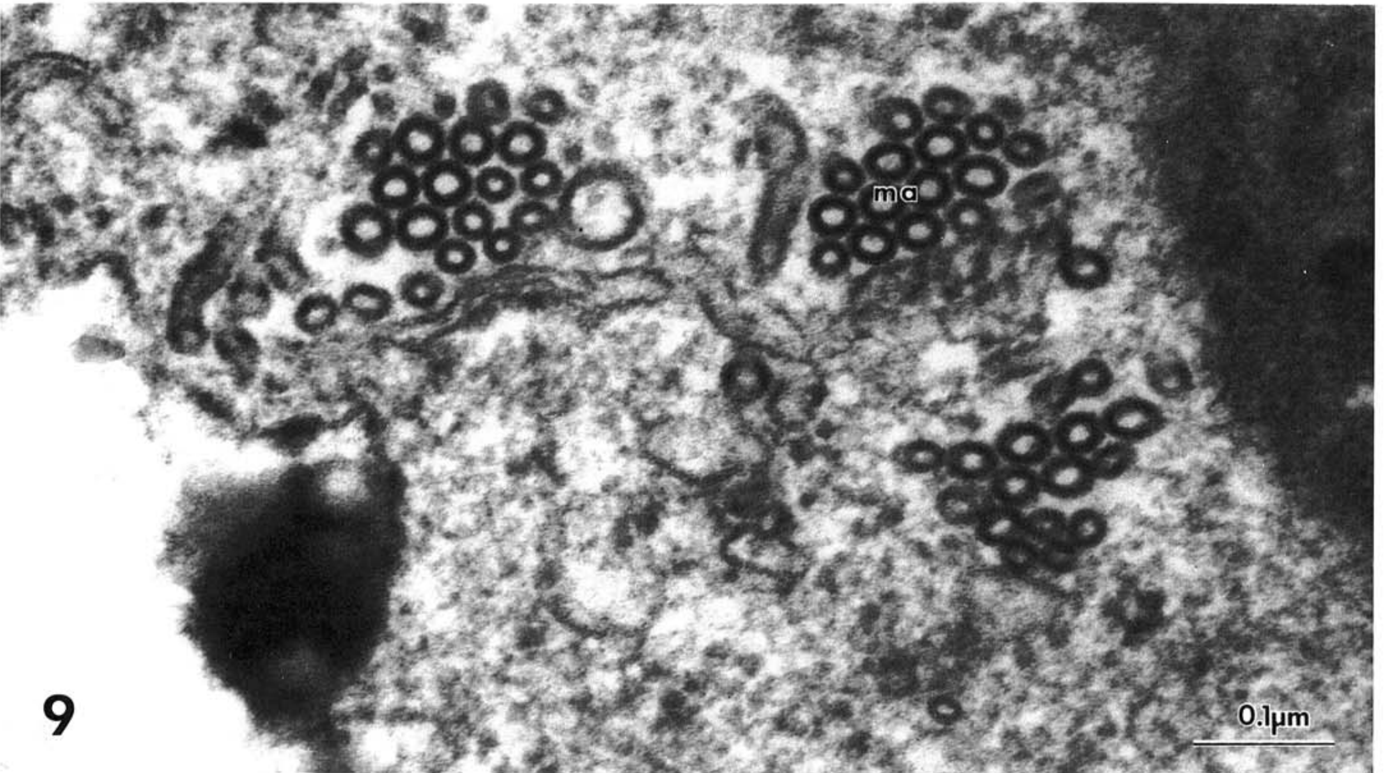


Figs. 6–7. Parasagittal thin sections through the path of stylet entry into potato roots parasitized (48 hr) by *Scutellonema brachyurum*. **6,** Material found in the stylet orifice (sto) was similar in density and ultrastructure to the inclusion bodies (i) found in the membranous network (mn). **7,** Electron-dense material (edm) in root cell cytoplasm following withdrawal of the stylet. (p = Plasmalemma, and mn = membranous network.)

Associated with the area of stylet entry and usually contained within the membranous network were dense inclusion bodies (Figs. 3 and 4). These bodies appeared roughly circular in both longitudinal and cross sections and showed little internal substructure except for a densely packed grainy matrix. They differed from elongate vesicular bodies found throughout altered cells (Fig. 5) in shape, internal substructure, and lack of an external membrane. Material with an appearance like that found in inclusion bodies was present in the stylet orifice (Fig. 6) and cell cytoplasm directly adjacent to an area where a stylet was previously inserted (Fig. 7). Also found in cross sections of the membranous network were structures (Fig. 4) that closely resembled the feeding tubes reported in cells parasitized by *Rotylenchulus reniformis* (24,25). Longitudinal sections of the network failed to reveal these structures.

As the stylet penetrated the cell wall, the plasmalemma

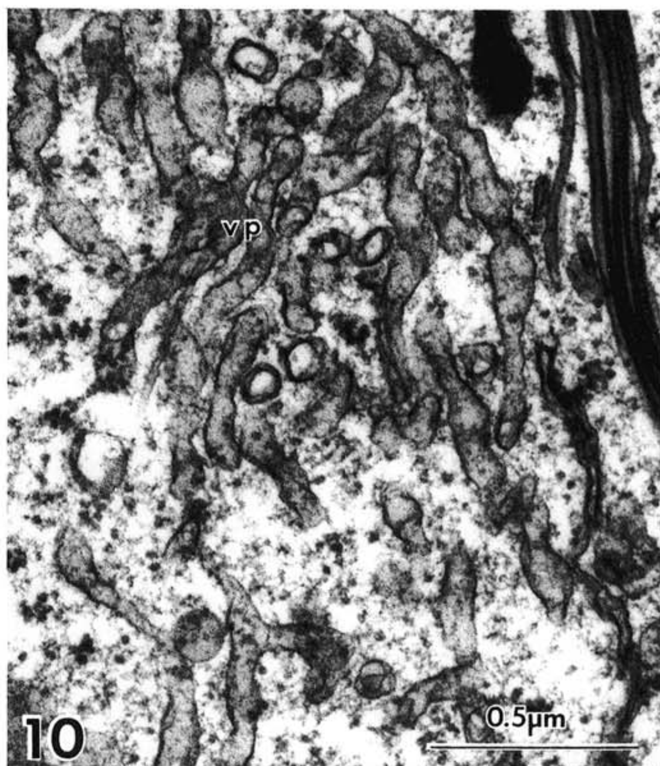


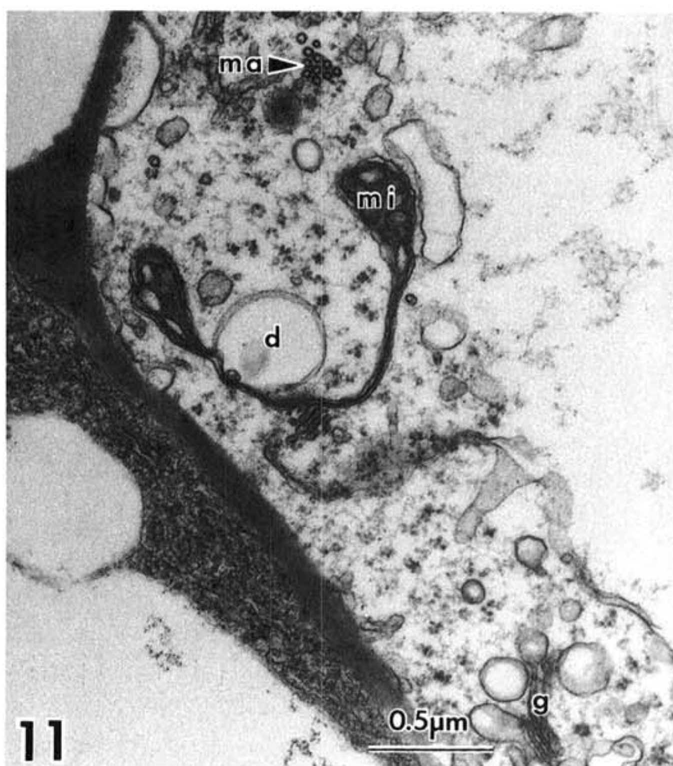


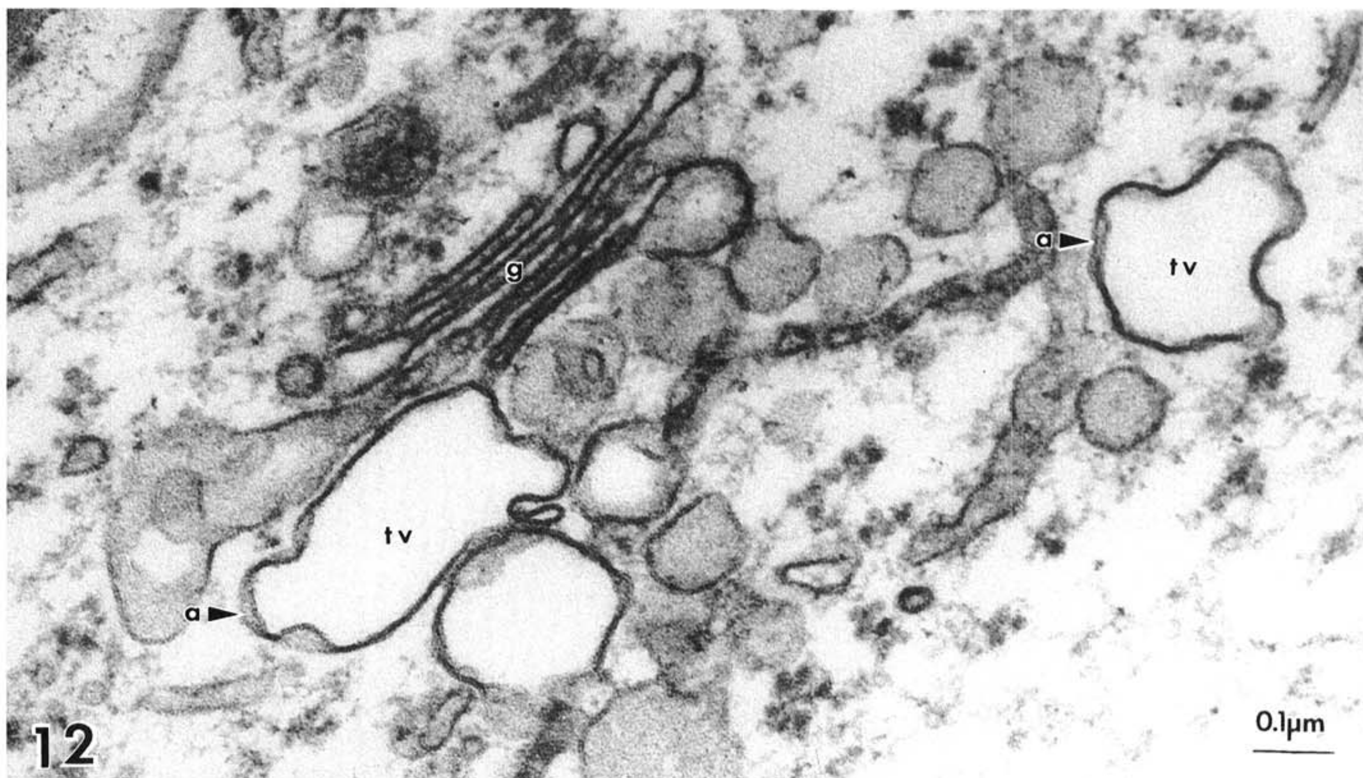
Figs. 8–9. Details of macrotubules formed in potato root cells parasitized by *Scutellonema brachyurum*. **8,** Macrotubules (ma) in longitudinal sections were uniform along their lengths and bound by a trilaminar unit membrane (u). **9,** Cross section through clusters of macrotubules (ma).

invaginated around the stylet (Fig. 3), forming a channel that maintained its integrity even if the stylet was withdrawn (Fig. 7). The plasmalemma was disrupted throughout the cell and was observed to separate from the cell wall. Evidence of endocytosis and exocytosis seen in controls and unaffected cortical cells (Fig. 2a) was not observed in penetrated cells.

Material of unknown origin formed a feeding plug during penetration of plant cell walls by the nematode's stylet (Fig. 3). Plug material surrounded the stylet and lined the buccal cavity of the nematode (Fig. 3). Plug material appeared to seal the gap between the stylet and the cell wall, and often filled invaginations of the buccal cavity (Fig. 3). Similar material was found deep within the amphidial duct (Fig. 4), but no material directly traceable from the amphids to the feeding plug was observed.







Figs. 10–12. Alterations of membranes in cells of potato root tissue parasitized by *Scutellonema brachyurum*. **10,** Membrane-bound vesicular network. Vesicular pouches or sacs (vp) were irregular in shape and diameter. **11,** Mitochondria (mi) were distorted or collapsed with degenerate membrane systems (d). (ma = Macrotubules, g = Golgi bodies.) **12,** Transformation of Golgi bodies (g) into large, irregular transitory vesicles (tv) with altered membranes (a).

Common to all recently penetrated cells (24–48 hr) were complexes of tubular structures (Figs. 3, 5, 8, and 9). Tubes varied in diameter (20–40 nm) (Fig. 9) and most were oriented longitudinally in the cell (Figs. 5 and 8). Groups of tubes were found not only near the area of stylet entry, but throughout the parasitized cell. Tubes were uniform in diameter along the length of individual tubes, and in cross and longitudinal sections their walls appeared to be a trilaminar unit membrane (Figs. 8 and 9) similar in thickness (8–10 nm) to unit membranes of controls (Fig. 2a). Groups of 20 or 25 tubes were closely packed, but lacked interconnections. Distinct from these tubular arrangements and from the membranous network surrounding the path of stylet entry was a third system of vesiclelike pouches or sacs distributed randomly throughout the parasitized cells (Fig. 10). The diameter of the sacs and the thickness of membranes forming the sacs were variable.

Other organelles were altered to various degrees in penetrated cells. Golgi bodies gradually were transformed into large transitory vesicles that separated from the Golgi stack and formed a loose group of variably shaped and sized vesicles (Figs. 4 and 12). Mitochondria were distorted (Fig. 11) and often extremely elongate (Fig. 5). Their outer membrane sometimes either separated and formed pouchlike vesicles (Fig. 11) or the regularity of the intermembrane space was lost. The central vacuole of mature cortical cells was lost as cytoplasmic material proliferated and tonoplast degenerated (Fig. 4). The tonoplast, which was barely visible in control and unaffected cells, became swollen and disintegrated, allowing the cytoplasm to mix with vacuolar material (Figs. 3 and 4).

Nuclear membranes were poorly defined in penetrated cells (Fig. 5) and nucleoli were not observed in nuclei. Associated with the loss of the nucleolus was a reduction in identifiable rough endoplasmic reticulum and of cellular or membrane-bound ribosomes.

About 40–50 μm from the path of stylet entry, the tonoplast appeared to be less affected and the central vacuole had a higher degree of integrity (Fig. 13). Mitochondria were less distorted, but tubular structures were common and Golgi bodies were observed to have degenerated like those near the path of stylet entry.

Roots parasitized for 72–96 hr and subsequently vacated by the nematodes for 24–48 hr showed large cortical cavities (Fig. 14), but little evidence of the cellular alterations noted earlier. Parasitized cells contained a dense unit membrane-bound matrix composed of numerous electron-dense spherical bodies (Figs. 14 and 15). The matrix occurred in cortical cells 48 hr (Fig. 15) after feeding began, but was more extensively developed at 72–96 hr (Fig. 14). It was never observed in cells actively supporting nematode feeding (Figs. 4, 5, and 13). Bacteria were present only in cells that were breached and/or disrupted by nematode feeding (Fig. 14). Evidence of enzymatic breakdown of cell wall material was not observed in parasitized or ruptured tissue.

DISCUSSION

Feeding behavior and extension of lesions axially along the root, beyond cells directly fed upon by *S. brachyurum*, was consistent with reports of feeding by related species (18,19,23,26) and suggested a chemical as well as a mechanical mechanism of injury to root tissue. Ultrastructural alterations were never observed in cells not penetrated by the nematode's stylet, including those directly adjacent to feeding cells. Obvious darkening of these areas, therefore, may result from physiological and/or biochemical cell alterations that were not manifested ultrastructurally, were lost in preparation of tissue, or were not revealed by standard stains for electron microscopy.

The membranous network surrounding the stylet of *S. brachyurum* was interpreted as a proliferation of the plasmalemma induced by nematode secretions or as a host reaction to the physical presence of the nematode's stylet. Similar membrane proliferations, termed paramural bodies or plasmalemmasomes, have been observed in viroid-plant interactions (27) and virus-mouse interactions (17), but have not been reported for nematode-host interactions except for *R. reniformis* and cotton in which the

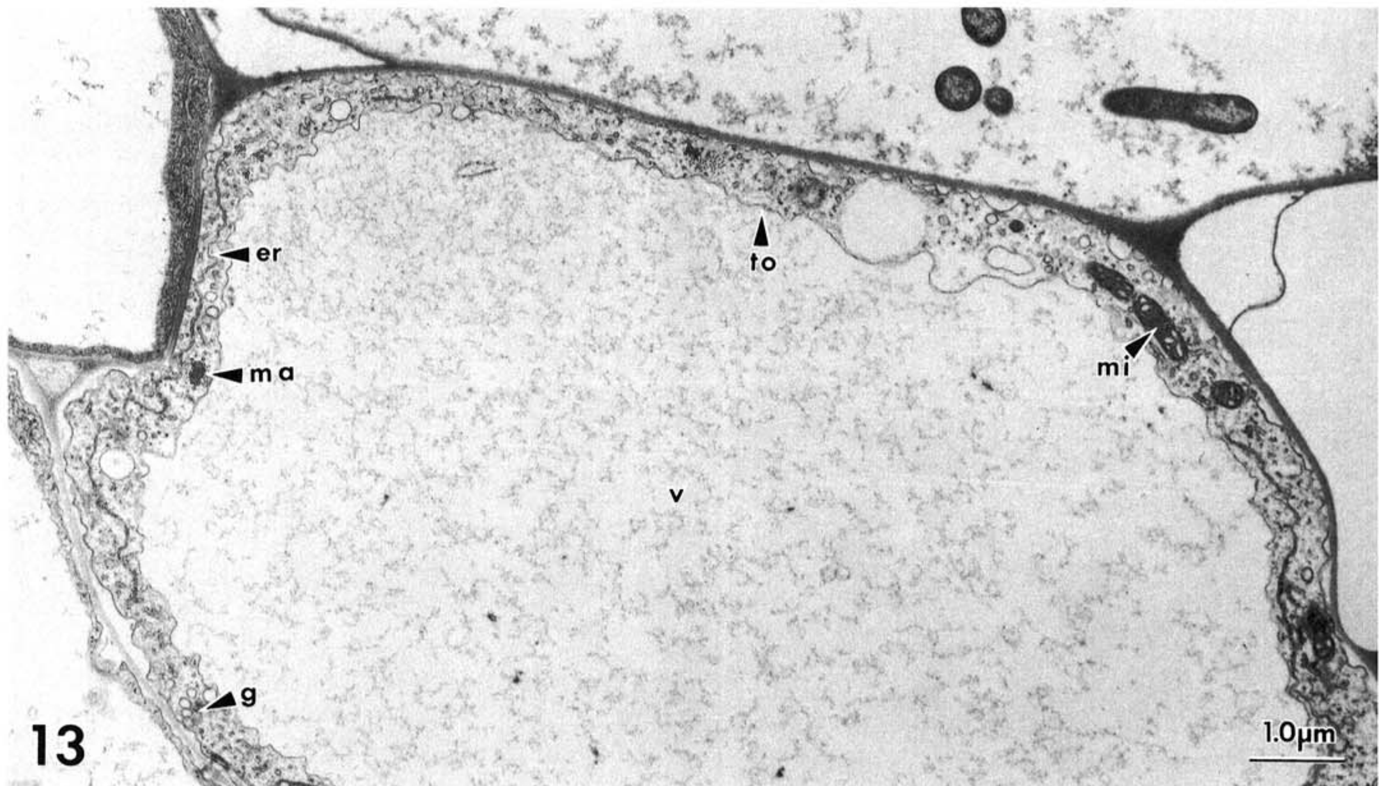
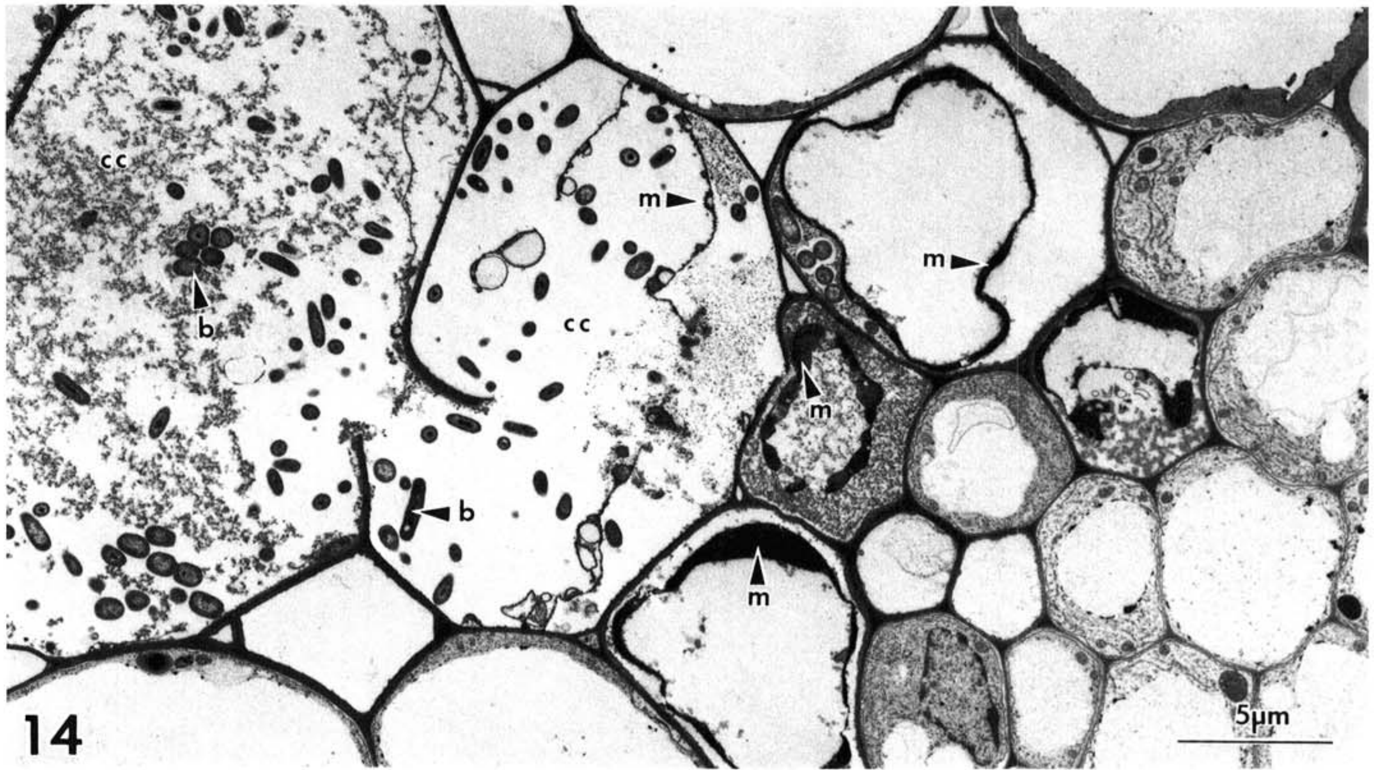


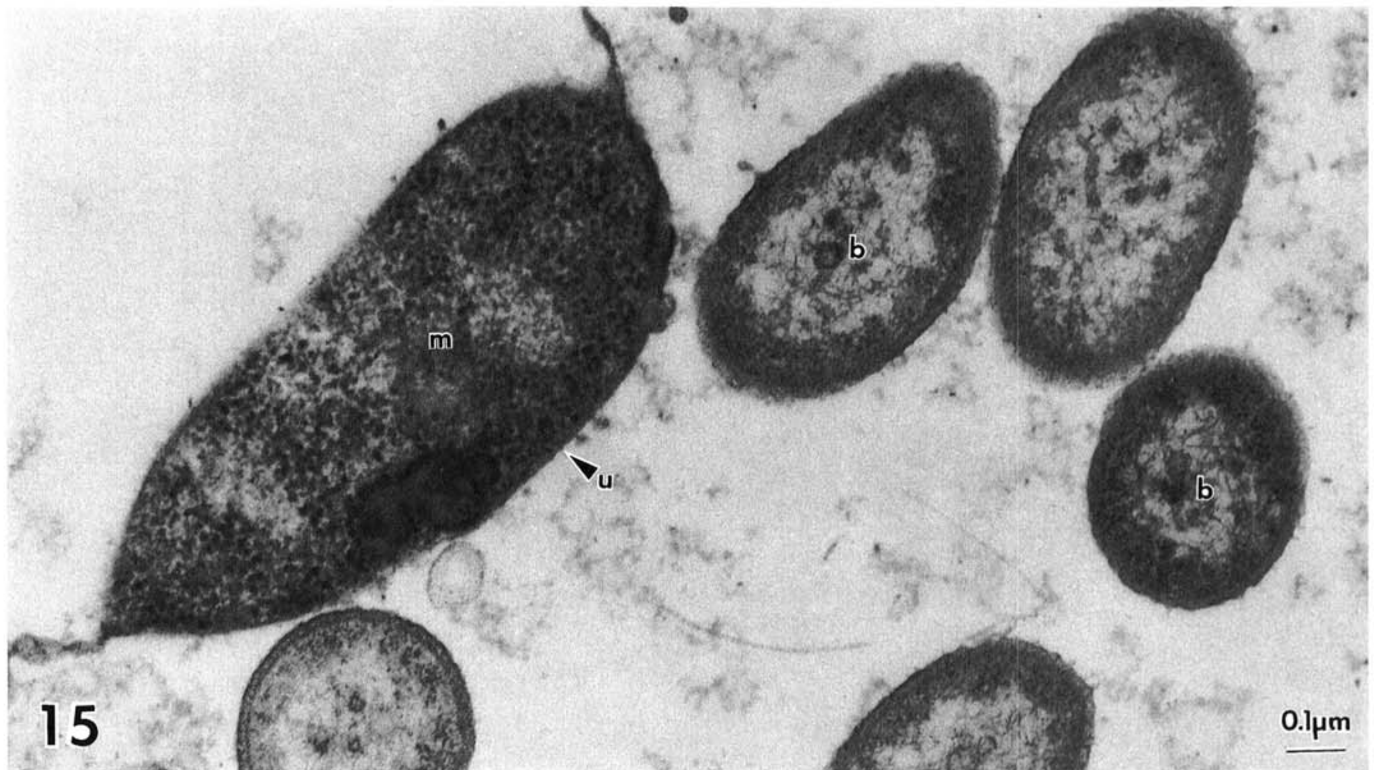
Fig. 13. Thin section through proximal end of potato root cell penetrated by *Scutellonema brachyurum*. Stylet penetration occurred approximately 40–50 μm distal to the section. Golgi bodies (g) and macrotubules (ma) were similar to those found near the path of stylet entry, but mitochondria (mi) tonoplast (to), central vacuole (v), and endoplasmic reticulum (er) were intact and ultrastructurally similar to those of the controls.

development of paramural bodies is less extensive (24).

If there is a direct relationship between nucleic acids and membrane proliferations in viroid and virus-host systems (10,11,17,34), then it seems reasonable to expect that a similar mechanism might account for the proliferation of the plasmalemma observed in potato root cells parasitized by *S. brachyurum*. However, aggregations of endoplasmic reticulum are characteristic of normally differentiating sieve elements of *Gossypium hirsutum* (32) and, in this genus, are associated with the nuclear envelope and microtubules as the nucleus degenerates (33).

Rebois et al (25) found dense spherical inclusion bodies in cells of susceptible soybeans parasitized by *R. reniformis* and surmised





Figs. 14–15. Feeding sites of *Scutellonema brachyurum* in potato root tissue from which the nematode had withdrawn. **14,** Cortical cavities (cc) contained numerous bacteria (b) and a matrix (m) interpreted as residual coagulated cytoplasm. The residual matrix was found only in cortical cavities or in cells with definite cell wall disruption. **15,** Spherical bodies of the residual matrix (m) were similar to those observed in older (72–96 hr) feeding sites. (u = Unit membrane, b = bacteria.)

that they were of nematode origin. Similar bodies noted in this study (Figs. 3, 4, and 6) resembled material associated with the stylet orifice of *S. brachyurum*, but their origin and composition was not readily apparent.

Another similarity to cells parasitized by *R. reniformis* was seen in the structures that Rebois et al (25) and Rebois (24) termed "feeding tubes" (Fig. 4) and described as secretions from the nematode's stylet. While we have no evidence to support this contention, or to suggest a possible function of these structures, their occurrence at the feeding site of at least two, and possibly three (13), members of the Hoplolaimidae indicates that they form an important part of the host/parasite relationship within this large and diverse family.

Other cell alterations induced by *S. brachyurum* indicated a general senescence of cell cytoplasm. Degeneration of mitochondria, rough endoplasmic reticulum, Golgi bodies, tonoplast, and central vacuole is consistent with the effects of aging in senescing wheat and barley leaves (9,28). The separation and contraction of the plasmalemma observed in this study also would be expected in senescing tissue. Organelle breakdown is possibly enhanced and accelerated by nematode penetration, but these alterations are not unique to the *S. brachyurum*-potato interaction.

Formation of tubular groups, henceforth termed "macrotubules," in potato cells penetrated by *S. brachyurum* is one alteration not generally anticipated in senescing cells. The size and structure of macrotubules observed in this study differed markedly from either microtubules or microfilaments typically occurring in cells: they were larger and more variable (20–40 nm) than microtubules (25 nm), microfilaments (4–6 nm), or the 10-nm filaments typically found in cells (6). Cross-sectional views of true microtubules revealed a substructure of 13 protofilaments arranged helically (6), whereas macrotubules lacked any discernible substructural protofilaments and their walls appeared to be a trilaminar unit membrane. A comparison of the macrotubules in parasitized potato cells and microfilaments in control cells (Fig. 2b) showed few similarities. Although both were linear and regular in diameter, microfilament walls were not a unit membrane and their diameters (17 nm) were smaller. Large tubular structures (46 nm in diameter) have been induced to form in the protozoan *Sticholonche zanclia* under low-temperature conditions (5). These tubular structures were derived from the disassembly of tubulin in microtubules, but were ultrastructurally distinct from the macrotubules found in potato root cells parasitized by *S. brachyurum*.

Large tubules (56–66 nm) also were found in the leaf glands of *Phaseolus vulgaris* and these tubules, in contrast to microtubules, remained intact after treatment at 4 C (31). Root tissues parasitized by *S. brachyurum* were routinely subjected to chilling prior to fixation in order to immobilize the nematodes. Macrotubules, however, were apparent only in cells penetrated by the nematode's stylet. It seems unlikely, therefore, that the macrotubules described here resulted from low-temperature treatment. Furthermore, the macrotubules found in the leaf glands of *P. vulgaris* were confluent with the membranes of the endoplasmic reticulum from which they appeared to arise. Similar connections were not observed in parasitized potato root cells. Thus, on the basis of size, ultrastructure, and origin, macrotubules associated with the feeding of *S. brachyurum* differ significantly from those described previously. Speculation on their function would be unwarranted without further investigation.

The feeding plug formed by *S. brachyurum* was not as large as those formed by other nematodes (7,14,36). The material forming the plug appeared to originate from the buccal cavity. However, possible origin in the amphidial duct or gland could not be eliminated. The origin of feeding plug material in other nematodes was thought to be the stylet orifice, the inner labial receptors, and/or the amphidial canals (7,36).

The part of this study designed to establish sequential events in early penetration and pathogenesis of root cells showed that initial penetration (24–48 hr) resulted in ultrastructural modifications that progressively decreased in intensity with increasing distance from the path of stylet entry. Golgi body breakdown progressed from increased production of transitory vesicles to a total breakdown of the stack, which resulted in loose collections of vesicles with distorted and irregular unit membranes. Mitochondria elongated and slowly lost the integrity of their intermembranal space. Eventually, the structure of internal cristae was lost and the outer membrane burst. Endoplasmic reticulum and ribosomal complexes were reduced in most cells penetrated by *S. brachyurum*. Macrotubules were formed early and occurred throughout the parasitized cell.

In contrast, cells that had been parasitized 72–96 hr and vacated by *S. brachyurum* an additional 24–48 hr contained a residue of material we believed to be coagulated cytoplasm. This material was also observed in cortical cells destroyed by nematode passage, and in pericycle and endodermal cells with disrupted cell walls. Based upon these observations, it was concluded that cells containing the coagulated matrix were in the final stages of degeneration. However, too few cells were examined to reconstruct the sequence of events leading to the death of the parasitized cell.

The parasitized cell in Figs. 3 and 5 was the third and innermost cell penetrated by a juvenile *S. brachyurum* within 48 hr after feeding began. Assuming that the same sequence of ultrastructural alterations occurred in all three parasitized cells, a very rapid response by host cells to penetration to *S. brachyurum* would be necessary, with modifications and ingestion of cytoplasm requiring 12–14 hr per cell. Further sectioning of infected material and a more precise method of determining the initiation of feeding on individual cells would be necessary to firmly establish this assumption.

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