Host-Parasite Interaction of Resistant Sugarbeet and Heterodera schachtii[1]

M. H. Yu and A. E. Steele[2]

Abstract: The host-parasite relationships between *Heterodera schachtii* Schm. and the nematode-resistant diploid *Beta vulgaris* L. line '51501' were examined via serial sections of secondary rootlets. Second-stage larvae penetrated sugarbeet roots and migrated up to 1.95 mm before establishing permanent feeding sites. Most sedentary larvae were oriented parallel to the root axis or in various diagonal or folded positions in the cortex. Nematodes adopted no definite orientation with regard to the root apex. Nematode feeding stimulated formation of multinucleate syncytia in host tissues. Syncytia were 0.3–1.1 mm in length, up to 90 μm × 150 μm in cross section. Root diameters were enlarged close to feeding sites. Usually nematodes deteriorated concomitant with necrosis of syncytia, and dead nematodes frequently appeared macerated or flattened and deformed. Most nematodes did not develop to maturity in the resistant host tissues. Cavities left by collapse of syncytia were filled by growth of parenchymatous tissue. *Key words: Beta vulgaris* L., cyst, histopathology, necrosis, nematode resistance, syncytium.

Sugarbeet (*Beta vulgaris* L.) is the principal host for the sugarbeet cyst nematode, *Heterodera schachtii* Schm. Selection for a true-breeding genotype of *B. vulgaris* resistant to *H. schachtii* within sugarbeet cultivars has been unsuccessful (1,2,8). However, the three wild species—*B. procumbens* Chr. Sm., *B. webbiana* Moq., and *B. patellaris* Moq.—in the section Patellares are highly resistant to *H. schachtii* (7). *B. procumbens* has shown the highest degree of resistance (6). Nematode resistance has been transferred from *B. procumbens* into sugarbeet genome by interspecific hybridization (14).

Previous studies have described syncytial development in roots of sugarbeet susceptible to the cyst nematode (9,15). Little information is available, however, on the histopathology of *H. schachtii* infection in the resistance cultivars of sugarbeet. In this study we describe histopathology and nematode development in a resistant diploid sugarbeet infected with *H. schachtii*.

MATERIALS AND METHODS

Sugarbeet nematodes used in this study were collected from a *H. schachtii*-infested field near Chualar, California, and were increased for inoculum on sugarbeets in greenhouse pot cultures. Mature brown cysts were selected and treated with a hatching solution (17) in 20-cm pans and maintained at 27 C in an incubator. Most larvae used for inoculum hatched within 5 d.

The nematode-resistant diploid sugarbeet line '51501' is a progeny of *B. vulgaris* × *B. procumbens* hybrids (18). Seeds were germinated in steam-sterilized sand and seedlings transplanted at the two-leaf stage to aluminum foil cylinders (6 × 17.5 cm) containing soil with 40 *H. schachtii* cysts (estimated to have 4,000 larvae hatched). Forty-five days after transplanting the seedlings, the external surfaces of roots were examined for the presence of white females. Plants with fewer than five females were replanted and inoculated two additional times each with 2,500 larvae. Six weeks after the third inoculation those plants (approximately 5 months old) still not supporting five females were selected as resistant plants.

The secondary roots were removed from the tap roots and the selected resistant plants transplanted to 450-g styrofoam cups containing two parts sterilized clay-loam soil and one part sand mixture. Plants were maintained in an incubator at 27 C for a 16-hour photoperiod. Three days after transplanting, 2,000 active larvae were pipetted to the soil immediately around each sugarbeet plant. Preliminary studies showed that few *H. schachtii* larvae penetrate secondary roots within 1–3 d after inoculation. To obtain numbers of infected roots sufficient for histological examination, we exposed roots to larvae for 4 d. The plants were then

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removed from the cups and the roots thoroughly washed to remove soil. The plants were transplanted into clean containers filled with sterilized soil for further development of the nematodes.

Segments from infected secondary roots were excised for histological examination at 4–5-d intervals up to 31 d after inoculation. Tissues were killed, fixed, and stored in Nawaschin type Craft III fixative (13) at room temperatures. Root segments were embedded in Paraplast+ embedding medium, and 10–12-μm longitudinal and transverse sections were cut with a rotary microtome. Serial sections were mounted on slides, stained with a modified hematoxylin-safranin-fast green staining schedule, and examined microscopically. Results of these examinations were compared with the histopathology of susceptible sugarbeet described in previous studies (15,16).

RESULTS

Entry and development of H. schachtii in resistant host tissues: Examination of whole, unstained roots revealed longitudinal to ovoid necrotic lesions at larval penetration sites. Often lesions were located on slightly swollen areas of the roots. Within 4 d of inoculation, numerous larvae had penetrated into the sugarbeet roots and migrated into the cortex. Some larvae quickly established a position for feeding while others migrated a short distance in the cortex leaving tunnels of broken cells. Invasion courts containing nematodes ranged up to 1.95 mm in length. In several cases, invasion courts extended for some distance from the nematode feeding sites. Some root cavities did not contain nematodes.

Nematode penetration and migration within the roots was intracellular. Most larvae had completely penetrated the root tissue, others only partially. Larvae frequently traversed a root tip adjacent to the root cap and positioned themselves less than 0.5 mm from the root tip. Broken cells of the invasion tracts as near as 0.6 mm to the nematode were often necrotic.

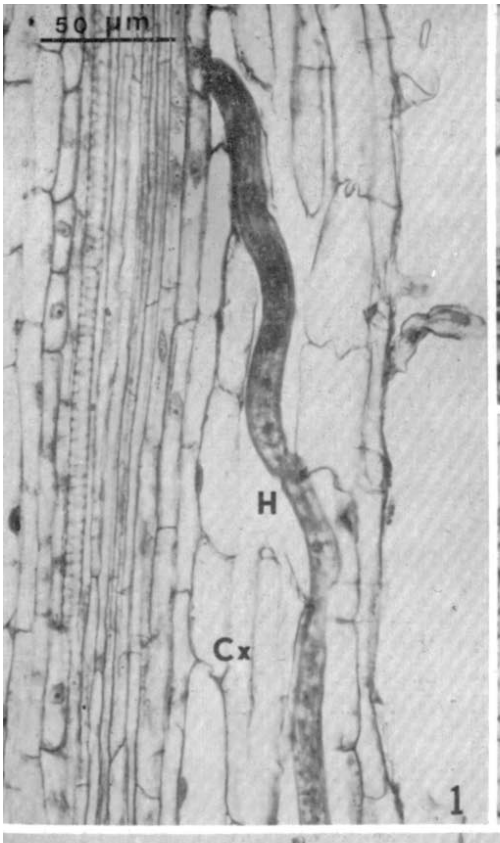
Upon reaching their feeding positions most larvae became oriented parallel to the root axis, but some were in diagonal positions within the cortex (Fig. 1). Some larvae were positioned entirely within endodermal cell layers, while tails of other larvae were partially extended three or four cell tiers into the cortex or were exposed on the root surface. The head regions of feeding nematodes were within or close to the endodermal cells and were oriented toward the vascular system (Figs. 2, 3).

Larvae frequently adopted folded positions within the cortex, and two or more sections of a single larva were observed in the same plane (Figs. 4, 5). Thus attempts to determine the number of nematodes in a particular root area required the examination of serial sections.

Ten days after inoculation, male larvae were observed in root sections with their bodies coiled within the old larval cuticle (Figs. 6, 7). Fourth- and early fifth-stage males were coiled into two or three folds within the third-stage cuticle. Several male larvae appeared to have penetrated only shallowly in the cortex. Males were not found in tissues after 20 d.

The first indication of larval deterioration was observed 10 d after inoculation. Deteriorated nematodes usually appeared macerated and devoid of recognizable organs or contained deformed organs. In transverse sections, dead larvae commonly showed cuticular indentations or infoldings (Figs. 8–10). After 20 d or longer, the sclerotized cephalic framework of the nematode frequently retained its original shape. The remaining anterior portion of the nematode contained little or no internal contents and was partially flattened, whereas the posterior third of the nematode occasionally contained obscure, disorganized, or deformed reproductive systems. The length of measurable larval remains ranged between 340 and 470 μm. Judging from cuticular markings that were detectable, several female nematodes developed to the fourth larval stage.

Histopathological reaction of resistant sugarbeets: Within 4 d of infection, phase illumination showed crystalline cytoplasmic granules in cells fed upon by nematodes (Fig. 11). Thereafter, the affected cells and nuclei enlarged, cytoplasm increased in density, and cytoplasmic granules became more hyperchromatic (Figs. 5, 12, 13). Syncytia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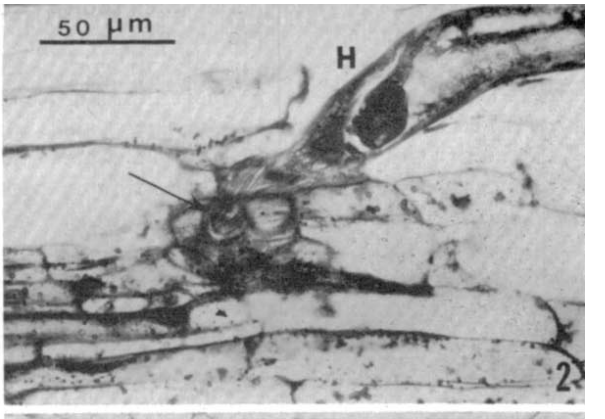
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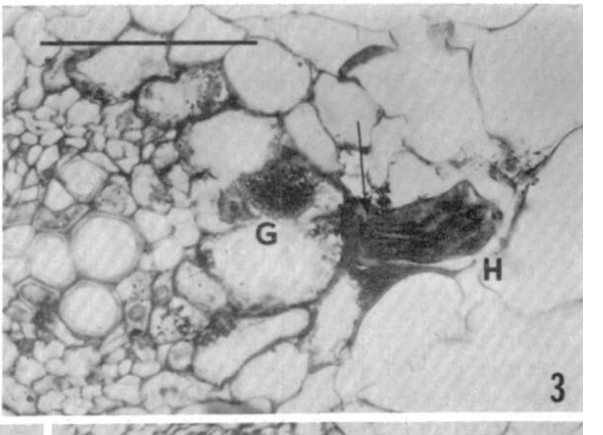
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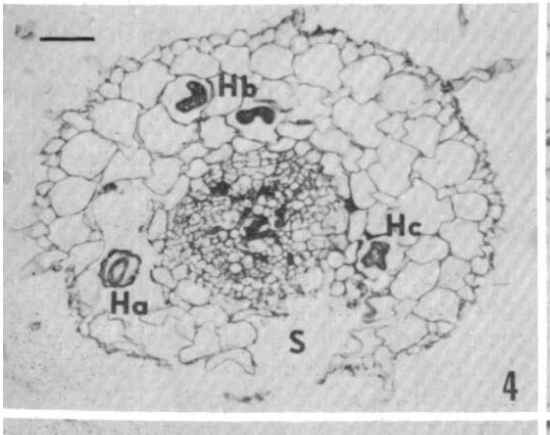
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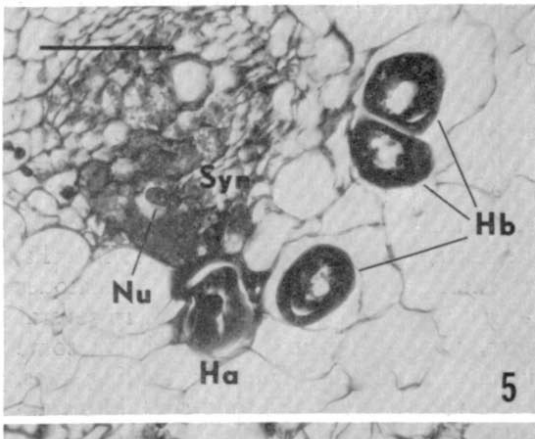
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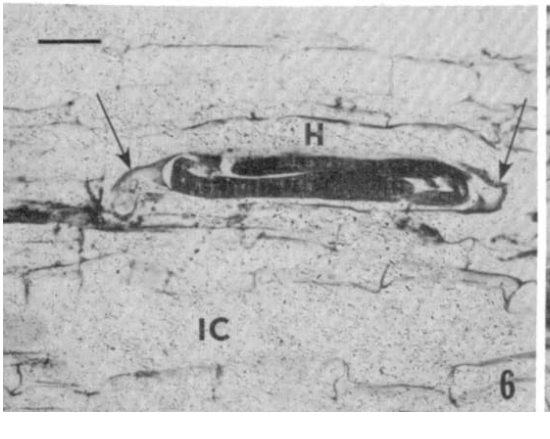
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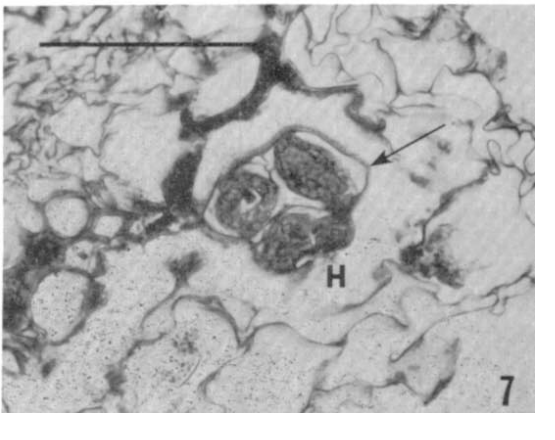
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typically developed within the stele and incorporated cells of the pericycle, protophloem, and interfascicular parenchyma. Occasionally syncytia were initiated at the centripetal boundary of cortical cells and extended into the stele.

Each syncytium extended from the initial cell to neighboring cells near the outer layers of the vascular cylinder. The developing syncytium spread longitudinally in both directions, by gradual dissolution of cell walls and coalescence of cytoplasm, and merged as one continuous multinucleate cytoplasmic unit (Figs. 12, 13). Differences in the size and staining of syncytial nuclei were observed. Syncytial cytoplasm eventually became turbid and heavily stained. The nuclei frequently showed different reticular structures (Fig. 13). Syncytia usually attained maximum size adjacent to the nematode feeding points about 10 d after inoculation. The maximum width of syncytia near feeding sites was as large as 90 μm at the edge of pericycle and 150 μm from the pericycle to the center of the stele. After 14 d, syncytia ranged from 0.3 to 1.1 mm in length. Cell walls close to nematodes and vascular elements were often diffuse and prominently stained (Figs. 5, 7–10, 12–14).

The shapes of syncytia were longitudinal and resembled an asymmetrical spindle, broader at one end and gradually tapered to the other. In a root segment parasitized by a single nematode, the syncytium displaced xylem elements in a limited sector of the root. The presence of discontinuous cell walls indicated that syncytial complexes were formed by the coalescence of cytoplasm from adjacent cells. In multiple infections, adjacent syncytia resulted in extensive vascular damage. Single infections in the same root region frequently induced syncytia that formed discrete units.

The enlarged multiple syncytia had a secondary effect of enlarging roots near the feeding sites of the nematodes. Less frequently, localized enlargement of roots was caused by repeated division of pericyle cells. Swellings extended 60–90 μm beyond the 300-μm mean root diameter.

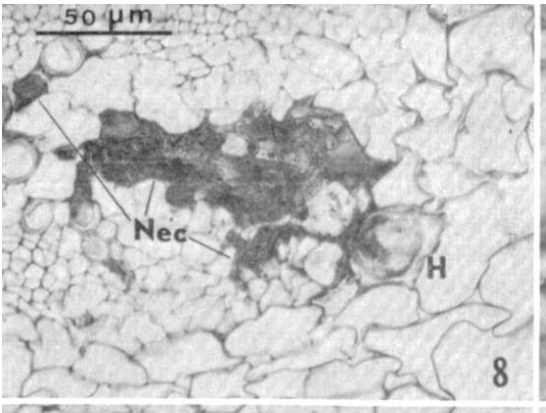
Deterioration of syncytia was observed within 10 d after inoculation. By this time, some necrotic syncytia had separated from vascular tissues leaving large cavities devoid of plant cells (Figs. 13–14). Thereafter necrosis became progressively more severe, resulting in total collapse of the syncytium (Fig. 15), thereby leaving extensive cavities in the roots. Necrotic syncytia in samples taken 25 d after inoculation were usually, but not always, associated with deteriorated larvae. After 30 d, many larvae had degenerated to the point that they were difficult to identify as nematodes. Rejuvenated parenchymatous tissues invaded spaces left by the receding syncytial walls (Fig. 16).

DISCUSSION

Second-stage larvae that entered the roots of resistant sugarbeets showed great variability in establishment of feeding positions. Some larvae wandered within the cortex changing direction at acute angles thereby causing extensive injury in the invaded tissues. Rarely, larvae only partially penetrated the root before becoming sedentary. Steele (16) reported that some larvae frequently do not completely enter roots but remain attached with posteriors external to the root systems.

The terminal feeding position and orientation were not the same for all larvae. For example, in a 2.5-mm root segment where five nematodes were arranged tandemly in the cortex of a root, at least one was oriented in a reverse direction. This was in agreement with previous research by

Figs. 1–7. 1) Diagonal penetration of *Heterodera schachtii* (H) inside the cortex (Cx) of sugarbeet root, 4 d after inoculation. 2,3) Bending of the head region (arrows) of *H. schachtii* toward the root vascular elements, shown in longitudinal and transverse sections, 10 d. Note the cluster of cytoplasmic granules (G) in Fig. 3. 4,5) Multiple infection of sugarbeet root by *H. schachtii* (Ha, Hb, and Hc); one nematode (Hb) in folded position showing two and three separate sections, respectively, 10 d. Note the sloughing (S) of cortical tissues, a normal event in sugarbeet (4), and hyperchromatic cytoplasm and nucleus (Nu) in the initial cell of syncytium (Syn), about 20 μm from the anterior tip of a nematode (Ha) in Fig. 5. 6,7) Male larva coiled within cast cuticle (arrows), shown in longitudinal and transverse sections, 14 d. Note the invasion court (IC) of nematode in Fig. 6, and the cast cuticle surrounding the coiled male larvae in Fig. 7 indicating that the fourth molt has occurred. Scale bar = 50 μm.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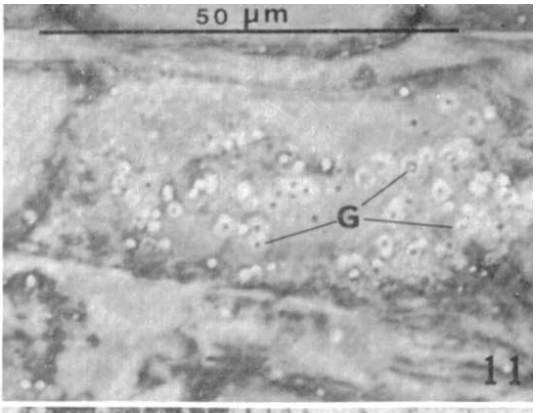
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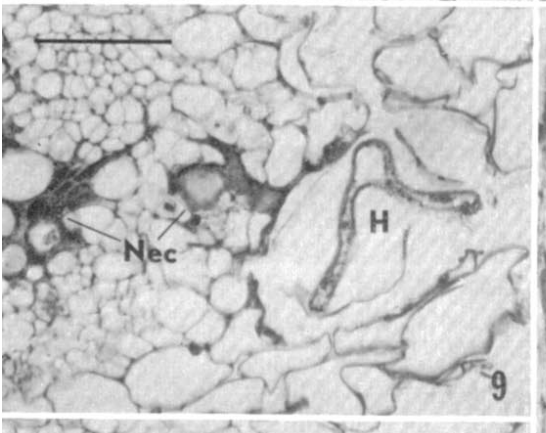
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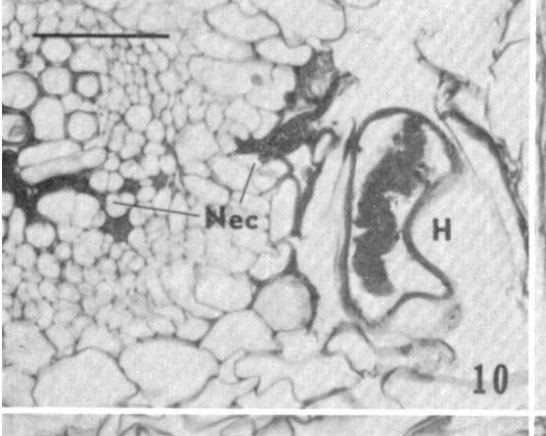
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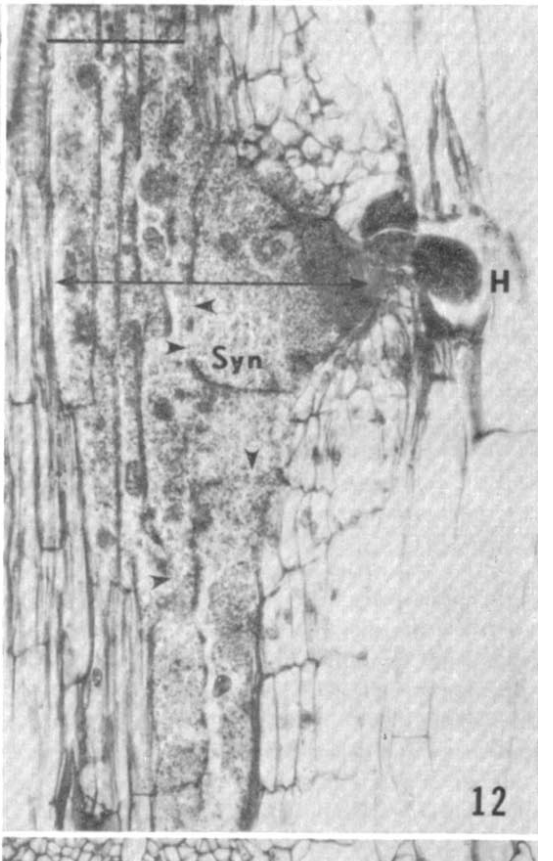
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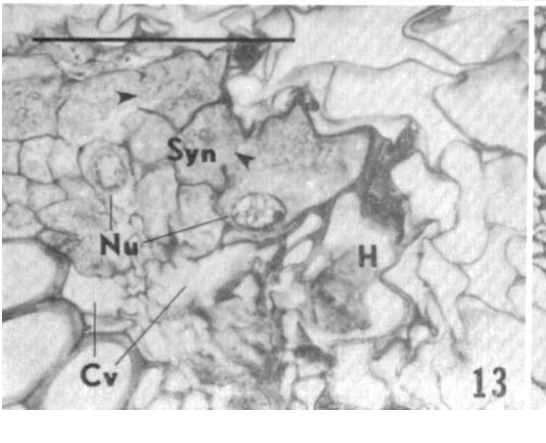
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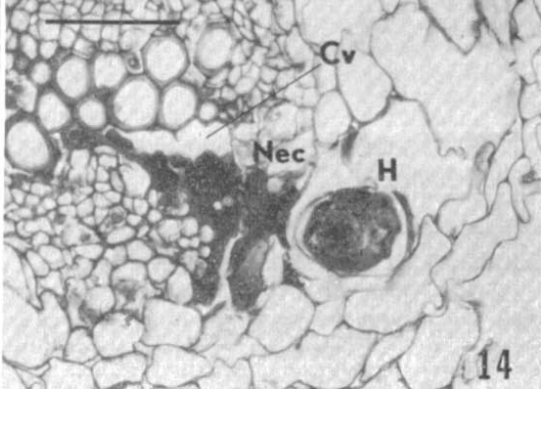
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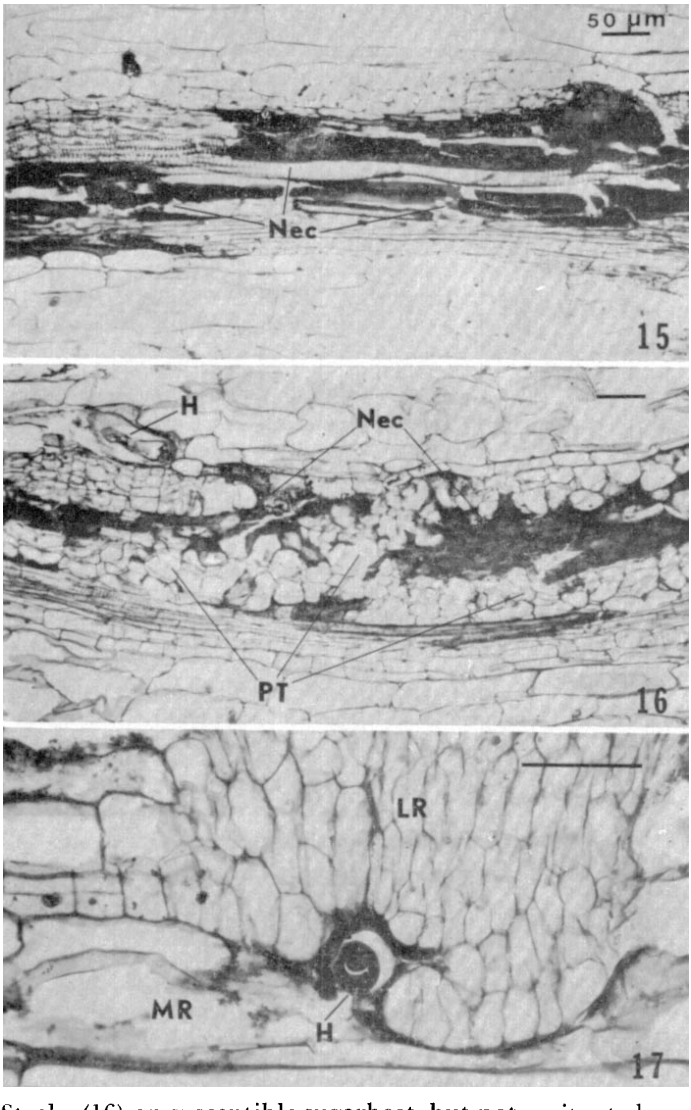

Figs. 15–17. 15) Syncytial necrosis (Nec), 25 d after inoculation. 16) Empty space in root invaded by parenchymatous tissues (PT) after the collapse of the syncytium, 31 d. 17) Nematode (H) at juncture of the lateral (LR) and main (MR) roots, 10 d. Scale bar = 50µm.

Steele (16) on susceptible sugarbeet, but not with research of Raski (11). This orientation phenomenon was also in contrast to that reported for *H. trifolii* Goffart in clover, where the larvae always oriented toward the epicotyl (10).

In this study, many larvae were often situated near the juncture of lateral and main roots (Fig. 17). This may be due to the ease of larval penetration through tissue ruptured by emerging lateral roots, or, conversely, lateral roots may proliferate due to stimulation by larval penetration. The nematode may introduce or stimulate for-

Figs. 8–14. 8–10) Serial sections of a deteriorated female nematode (about 460 µm in length) at 20 µm, 230 µm, and 420 µm, respectively, from the lip region, 31 d after inoculation. The most extensive syncytial necrosis (Nec) is near the feeding point; cephalic framework of nematode is sited right to necrosis (8). Necrotic syncytium separated into two sectors as it extends farther from feeding point; the deteriorated nematode shows cuticular infolding (9) and some deformed organs (10). 11) Crystalline cytoplasmic granules (G) in the syncytium cell under phase microscopy, 4 d. 12) Longitudinal section shows evidence of coalescence of cytoplasm through dissolved cell walls (arrowheads) during syncytial (Syn) development, 14 d. Note the distribution of hyperchromatic cytoplasm and the extensively affected area (arrows) near the feeding point. 13) Syncytium showing partial dissolution of cell walls (arrowheads) and presence of reticulated nuclei (Nu), 20 d. Note the cavities (Cv) between syncytium and vascular elements. 14) Cavity (Cv) between a necrotic syncytium and vascular elements in cross section, 10 d. Scale bar = 50 µm.

mation or redistribution of growth-promoting hormones in its host, thereby inducing formation of new roots (4). Franklin (5) described how lateral roots are formed to take the place of those invaded by *H. schachtii*.

Initially, syncytia in resistant sugarbeet appeared to be similar to that induced in susceptible sugarbeet. Previous research (15) indicated roots of susceptible sugarbeets had more multiple infection syncytial complexes.

Deteriorating syncytia left spaces into which rejuvenated parenchyma cells expanded, thereby separating syncytia into several isolated fragments. Frequently, one fragment was adjacent to the center of the stele and the other near the deteriorated nematode (Figs. 8–10, 16). Because continuous larval infections did not occur in this study, continuous formation of syncytia was not possible and histological damage in roots of resistant plants was eventually repaired by new parenchyma.

Once the nematodes established a permanent feeding site, no further migration occurred. Deteriorated larvae were always associated with advanced necrosis of syncytia. Whether larvae died as a result of the inability of degenerating syncytia to supply nutrients, or syncytia became necrotic as a result of the cessation of larval feeding, could not be ascertained. In *Meloidogyne* sp. infections of tomato, host necrosis varied inversely with larval growth (3). Ross (12) attributed the induction of a hypersensitive reaction in resistant soybean root tissue to the secretions of the soybean cyst nematode. Studies of susceptible sugarbeet (9,15) have shown that syncytia usually did not become necrotic until after the nematode completed its life cycle. This suggests that collapse of syncytia follows, and is perhaps the result of, cessation of feeding.

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