Morphological Changes in Roots of Sugarbeet and Tomato Infected with Heterodera schachtii Schmidt 1871[1]

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The sugarbeet nematode, *Heterodera schachtii* Schmidt 1871 is an obligate sedentary parasite (5,11).[3] Typically, second stage larvae hatch, emerge from cysts, and invade a host-plant's roots. In the course of their development larvae undergo 3 additional moults. Finally, the nematodes rupture through the surface of roots where mating occurs. The adult females remain attached to host roots until death. Although some eggs are deposited in a gelatinous matrix, the majority are retained within the female whose body wall is converted to a protective cyst. Variations from the typical pattern of development have been reported.

Strubell (16) observed that larvae of the sugarbeet nematode frequently do not take up an internal position but may complete their development while attached to very small roots by their heads only. However, Steele (15) found all larval stages of male *H. schachtii* attached to root surfaces of sugarbeet and tomato and suggested that males typically develop semiendoparasitically on the external surfaces of these plants, while endoparasitic females emerge from roots only as fourth-stage larvae or adults. The dissimilar orientation of the sexes of *H. schachtii* may favor the development of males over females under conditions which restrict deep penetration of larvae, such as development of a tough periderm in older roots.

This paper reports information on the interrelationships of larval orientation and histological changes in roots of sugarbeet and tomato.

Materials and Methods

Sugarbeet (*Beta vulgaris* L., Cultivar U.S. 75), tomato (*Lycopersicon esculentum*, Cultivar Pearson A-1) swiss chard (*Beta vulgaris* L.) and wild beet (*B. patellaris* Moq. and *B. webbiana* Moq.) were germinated in sand and transplanted in the cotyledon stage to 50 clay pots containing sterilized soil. Forty cysts with

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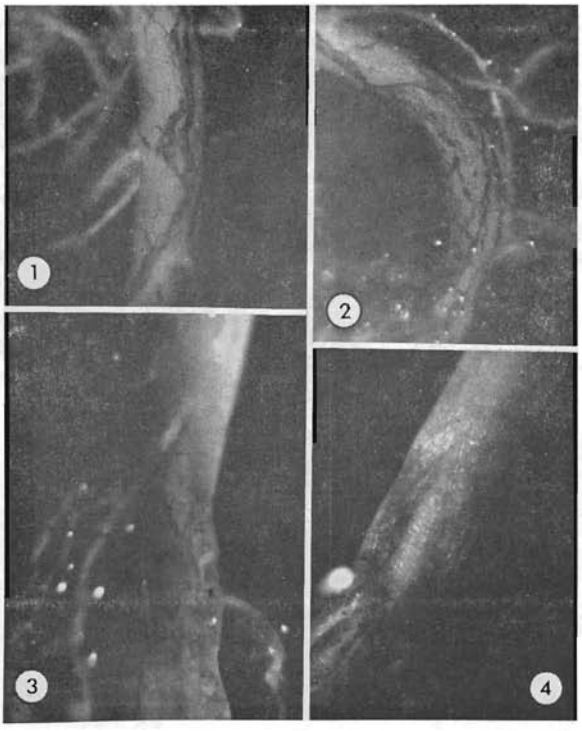
viable eggs of *H. schachtii* were added to the soil of each of 25 pots at the time the seed were planted or when seedlings were transplanted. After 14-82 days the external surfaces of whole unstained roots were examined for nematode larvae and for gross changes in tissue morphology. Similarly treated tomato roots were grown for 21, 30 or 117 days in infested soil. Roots of tomato and sugarbeet infected with *H. schachtii,* and tomato infected with the root-knot nematode, *Meloidogyne incognita acrita,* Chitwood, 1949, were killed and fixed in Nawaschin-type (Craf III) fixative, dehydrated with ethanol, cleared with xylene, and embedded in paraffin. Sections were cut 8 μ thick with a rotary microtome and stained in Safranin and fast green (12). These root sections were examined for their histomorphology and for the orientation of nematodes within the roots.

Results

The surfaces of roots of sugarbeet and swiss chard grown in sterilized and nematode-infested soil showed extensive sloughing of the epidermis (Figures 1-2) and the cortical parenchyma (Figures 5-7). Both infected and non-infected roots exhibited some degree of rift formation that varied from exceedingly shallow fissures (Figure 3) to deeper cracks (Figure 4).

Some rifts on old noninfected plants were always present at the junction of the root and the hypocotyl with more shallow rifts in the smaller roots of 20-day-old plants. However, in infected plants deep cracks occurred in the primary or tap roots, often extending through the cortex to the stele (Figure 4). Cracking frequently occurred in areas where roots were bent or twisted out of line with the root axis. In many instances, swollen female sugarbeet nematodes were found deep within cracks and within the shallow rifts where lateral roots emerged. A total of 12 maturing juvenile males and females were found wihin a single elongated rift in the hypocotyl of a sugarbeet 17 days after transplanting to soil infested with 50 cysts. The plant was extremely stunted, and the roots were heavily parasitized by larvae. In localized areas of the roots, there were often rosettes of proliferated lateral roots; frequently several female nematodes were found near the junctures of lateral and tap roots. Roots of resistant *B. patellaris,* and immune *B. Webbiana* grown in infested or sterilized soil for 20-40 days, showed extensive sloughing of epidermis without sloughing of cortical parenchyma or formation of rifts.

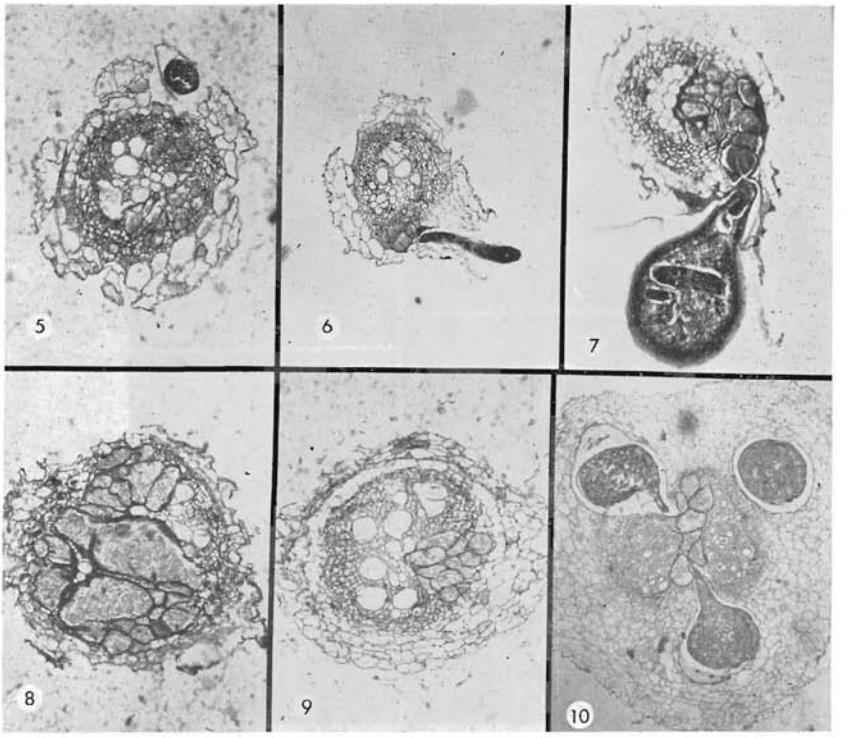
Examination of stained sections of sugarbeet roots revealed that after invasion of the root, larvae usually become oriented parallel to the root axis (Figure 5) but occasionally perpendicular to it (Figure 6). Syncytia (giant cells) initially developed from



Figures 1-2.—Views of 21 day old sugarbeet roots showing sloughing of epidermis.

Figures 3-4.—Views of sugarbeet roots showing degrees of rifting of cortical tissues. Plants infected 22 days with *H. schachtii.*

cells of the pericycle, protophloem, and interfascicular parenchyma (Figures 5-8), which in the hypocotyl and the maturing tap root, gives rise to the primary cambium (1). Sections of roots parasitized for longer periods showed an increase in the number, size and distribution of syncytia which encroached upon and laterally displaced the xylem (Figure 8.) Formation of syncytia within the vascular cylinder was accompanied by enlargement of the stele. This resulted in localized swelling of the roots at sites of invasion both at root tips and in maturing roots quite far removed from the meristematic areas. Stained root sections showed syncytia with dense, granulated cytoplasm and multiple nuclei. Syncytia walls, adjacent to or surrounding the nematode and xylem elements, are thicker and more heavily stained (Figures 7-8), as previously reported by Nemec (9,10).



Figures 5-6.—Cross-sections of roots from sugarbeet plants grown 3 weeks in soil infested with *H. schachtii;* 5 = X75, 6 = X64.

Figures 7-8.—Cross-sections of roots of sugarbeet grown 58 days infested with *H. schachtii;* 7 = X66, 8 = X95.

Figure 9.—Cross-section of root of tomato grown 44 days in soil infested with *H. schachtii;* X71.

Figure 10.—Cross-section of root of tomato plant grown 43 days in soil infested with M. *incognita acrita;* X24.

In roots parasitized by a single nematode, syncytia tend to occupy a limited sector of the root from the periderm inward, sometimes as far as the center of the stele (Figures 5-7). Multiple invasions from opposite sides of the root may result in a spindle-shaped syncytial complex which extends through the stele. Heavily parasitized older roots often exhibit extensive distribution of syncytia, resulting in an almost complete disorganization of root elements (Figure 8).

Infected sugarbeet roots showed extensive sloughing of the epidermis and the cortical parenchyma. Rifts occasionally extended through the cortex to the pericycle at several locations of a given cross section of sugarbeet root (Figures 5-7).

Syncytia formation in tomato infected with the sugarbeet nematode is similar to that in sugarbeets (Figure 9). Giant cells are formed within and adjacent to the pericycle and extend to the center of the stele. However, there is no sloughing of epidermis or corticle parenchyma.

Cross sections of root galls of tomato infected with *Meloidogyne incognita acrita* (Figure 10) showed the formation of syncytia which were restricted to the stele. Proliferation of cortical parenchyma resulted in increased root diameter and in the surrounding of root-knot females throughout development by host tissues. Eggs and larvae were present within the cavities occupied by females. However, corridors connecting the cavities with the root exterior were not found in serial sections of several galls, suggesting that these second generation larvae can reinfect tomato without first entering the soil.

Discussion

Artschwager (1) reported that in the normal course of sugarbeet root development, the central cylinder increases in size, but little growth occurs in the cortex which is at first stretched but later ruptures and collapses, producing fine fissures which gradually widen. Finally, the cortex is sloughed off.

Although small fissures and sloghing of epidermal and cortical tissues were evident even in roots of healthy plants, deep rifts and cracks also were found in roots of infected plants.

According to El-Katten *et al.* (3), cracking in sweet potato is a rupture of the inactive outer tissues due to internal pressure from the expanding vascular cylinder, possibly initiated by rapid influx of moisture following a prolonged drought. However, Krusberg and Neilson (7) obtained a significant correlation between root-knot index and the number of cracks and cracked roots per root system. They postulated that nematodes may inhibit localized cell division in actively growing roots. Continued centripetal cell division and growth of underlying tissues subsequently cause a rupture through the less active infected cortical cells. It is well known that the sugarbeeet nematode stimulates the proliferation of lateral roots (5) which originate in the pericycle region (4). The initial cells of the syncytial complex are also formed in this region. Consequently, rift formation in young sugarbeet may also result from stresses set up in the cortex by growth and reorganization of stelar tissues. The occurrence of all larval stages of males at or near root surfaces suggests that they may not be dependent upon syncytia which are invariably located within the central cylinder of the root (2). On the other hand, females which require a greater intake of

nutrients (6,13) seek the centrally placed vascular cylinder to initiate syncytial formation.

Failure of the sugarbeet nematode to produce galls by proliferation of cortical cells, together with sloughing of the epidermis and the cortical parenchyma as it occurs in sugarbeet, clearly increases the chances for emergence and mating of the nematodes, but also results in the location of syncytia close to the root surface. In young sugarbeets the development of a tough periderm, the lack of sufficient cortical parenchyma, and the presence of the stele (and hence, syncytia) near the root surface possibly make roots less susceptible to penetration and development of late emerging larvae. In tomato, sloughing of the cortical tissues does not occur. Consequently, females would tend to remain trapped within host roots. Nemazi (8) reported that tomato varieties varied in the number of female larvae embedded under the epidermis of roots that were unable to break through to complete their life cycle. In addition, secondary roots with thicker epidermis trapped more females than did the smaller roots.

Factors that restrict either the growth of host roots or the rate at which larvae initially invade the root may therefore influence the number or size of syncytia. This, together with the clearly dissimilar nutritional requirements of males and females, may influence the adult sex ratio by restricting the number of sexually undifferentiated larvae successfully penetrating and developing deep within the root, thereby permitting a proportionately greated number of males to develop at or near the root surfaces. The equilibrium thus established may tend to create an infection threshold or 'ceiling effect' in a given parasite community.

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