# Development of Heterodera schachtii on Large Rooted Crop Plants and the Significance of Root Debris as Substratum for Increasing Field Infestations[1]

A. E. STEELE[2]

*Abstract: Heterodera schachtii* developed to maturity and reproduced on the lateral roots of defoliated sugarbeet which were buried to a depth of 2.5 cm in sterilized soil and inoculated with cysts. Nematodes did not develop on detached lateral roots or on roots of young defoliated beets which did not have a large tap root. The storage roots of large rooted plants were sliced, placed in small jars, inoculated with cysts, covered with moist granulated agar or soil and incubated at 24°C 12-62 days. The sugarbeet nematode developed in root slices of sugarbeet, red table beet, icicle and globe radish, turnip and rutabaga. Only a few males developed on slices of potato tubers. Neither males nor females developed on root slices of carrot, salsify or parsnip. *H. schachtii* also developed on the cut surfaces of growing sugarbeet and radish. *Key Words:* sugarbeet nematode, culture, root slices.

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[1] Cooperative investigations of the Beet Sugar Development Foundation and the Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Salinas, California.

[2] Nematologist, Nematology Investigations, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 5098, Salinas, California 93901. The author is indebted to Madeline Mullins for her assistance. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and it does not imply approval to the exclusion of other products that may also be suitable.

Whole and fragmented storage roots of sugarbeet frequently left in fields after harvest may remain for many weeks without being greatly affected by the decomposing actions of soil organisms. Under optimal conditions of moisture and temperature, these roots or root fragments rapidly can regenerate new rootlets which may continue to grow for a time.

Reports of the successful culture of *Meloidogyne incognita, Pratylenchus brachyurus*, and *Radopholus similis* on carrot

discs (5, 7) and *Ditylenchus destructor* on potato tubers and on the storage roots of carrot, turnip, onion and beet (1, 11) raised the question of whether or not *Heterodera schachtii* can invade and reproduce on post-harvest beet-root debris. The studies reported herein were undertaken to determine whether or not *H. schachtii* can parasitize detached lateral roots, whole storage roots, or the cut surfaces of whole or sliced storage roots of large rooted plants. In addition, several methods of culturing *H. schachtii* on sliced roots were tested to determine conditions which favor parasitism.

## METHODS AND RESULTS

*DEVELOPMENT OF H. SCHACHTII ON DEFOLITATED SUGARBEET:* Thirty-four sugarbeet plants grown 14 days, and 7 plants grown 67 days in sterilized soil, were washed and the foliage removed. The roots were buried to a depth of about 2.5 cm in clay pots containing steam-sterilized soil, and the contents of 50 cysts were added to each pot. The average weight of seedling roots was 29 mg, whereas the older plant roots averaged 7.3 g/root. The roots were incubated in darkness for 31 days at 24 C, and were subsequently washed free of soil and examined for sugarbeet nematode.

Both male and female sugarbeet nematodes developed to maturity on lateral rootlets of defoliated sugarbeet plants which were attached to large fleshy tap roots. No nematodes were observed on detached lateral roots or on roots of young plants which did not have a large tap root.

*DEVELOPMENT OF H. SCHACHTII ON CUT SURFACES OF LARGE-ROOTED PLANTS:* Seed of sugarbeet (*Beta vulgaris* L. 'U.S.-75'), parsnip (*Pastinaca sativa* L. 'Long White'), salsify (*Tragopogon porrifolius* L. 'Sandwich Island'), rutabaga (*Brassica napobrassica* 'American Purple Top') and radish (*Raphanus sativus* L. 'Scarlet Globe' and 'White Icicle') were planted in 20.3-cm clay pots containing steam-sterilized soil. To obtain plants with large storage roots, sugarbeets were grown 63 days, parsnip and salsify for 154 days, and the two radish varieties for 36 days. Small slices of cortical tissue were excised from the large storage roots 2 cm below the crowns of three plants of each variety. Viable eggs and larvae from 50 cysts of *H. schachtii* were added to the soil near the cut or uncut surfaces of individual plants. Small wedges of cortical tissue measuring about 4 cm long, 3 cm wide and 1-2 cm deep were excised below the crown of tap roots of three sugarbeet plants (Fig. 1). *H. schachtii* cysts were added to cut root surfaces, the wedges of tissue replaced and the beets replanted in sterilized soil. The wedges were held in place only by the soil next to the root. The storage roots of three plants of each variety were left intact and inoculated with viable eggs and larvae to serve as controls. The plants were grown for 23 or 30 days in a greenhouse, then examined for the presence of developing sugarbeet nematodes.

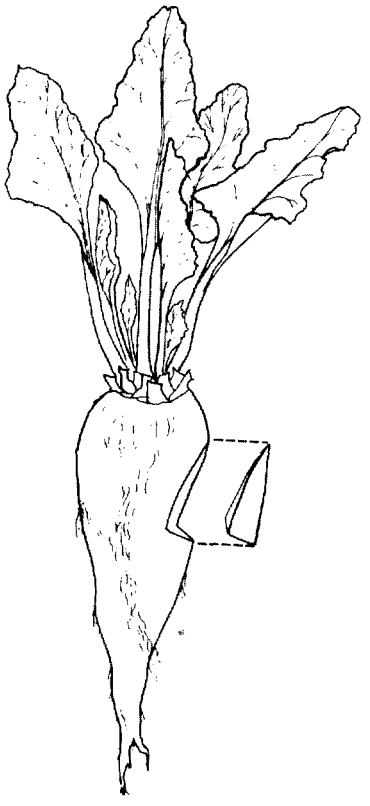
Twenty-three days after inoculation, small white female *H. schachtii* were attached to the cut surfaces of 'White Icicle' and 'Scarlet Globe' radish. At 30 days after inoculation, white females with viable eggs were attached to cut surfaces and small rootlets growing out of the cut surfaces of each radish variety (Fig. 4). The cambium exhibited secondary thickening on the cut surfaces. Nematode invasion of the cut root surfaces of one 'Scarlet Globe' radish plant appeared to have stimulated regrowth of the distal end of the fleshy tap root and stimulated callus tissue growth on the tap root (Fig. 2).

Male and female *H. schachtii* were observed on the cut surfaces of tap roots of sugarbeet plants and on the cut surfaces of small wedges of tissue excised from these plants. Maximum numbers of nematodes found on a single plant were 21 on the tap root and 12 on the excised tissue wedge.

No nematodes were observed on cut surfaces of parsnips or salsify 31 days after inoculation, but extensive callus tissue developed on each.

*DEVELOPMENT OF H. SCHACHTII ON ROOT SLICES:* The plant species listed in Table 1 were grown from seed in crocks containing steam-sterilized soil. When the plants had developed large, well-formed storage roots or tubers, the storage organs were dipped in 95% ethyl alcohol, flamed, and sliced into discs 3-5 cm diam and 0.5-1.5 cm thick. 'Scarlet Globe' radish was sliced longitudinally through the root axis, then cut into lengths of about 5 cm. Three parts by weight of sterile distilled water were added to one part of unheated granulated agar to form a spongy, absorbent medium in which to culture root slices.

Fifty cysts containing eggs and larvae of *H. schachtii* were broken open, and the cysts and



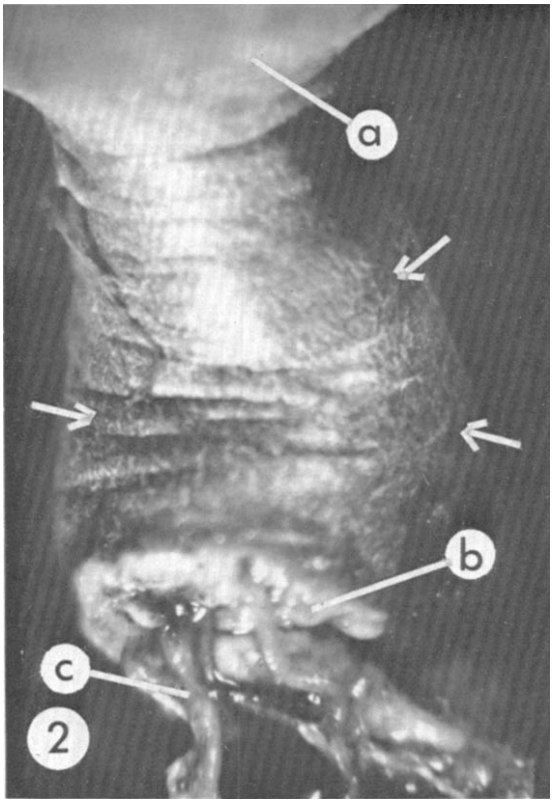




FIG. 1. Drawing of a 94-day-old sugarbeet plant showing excised piece of root which was replaced after inoculation of the cut surface with *Heterodera schachtii*. FIG. 2. Root of 66-day-old 'Scarlet Globe' radish 30 days after inoculation with *Heterodera schachtii*. Apical root axis showing secondary thickening (arrows), callus tissue formation (b) and massive proliferation of secondary rootlets (c). Storage organ of radish (a) was cut and inoculated with *Heterodera schachtii*.

their contents were placed on the upper surface of each of six slices of storage organ. Six slices of each plant species served as uninoculated controls. The slices then were placed in individual jars of 150-ml capacity and covered with moist granulated agar, moist sterilized soil, or left uncovered. Jars not containing agar or soil were loosely capped to reduce drying of the slices. The cultures were incubated without light at 24 C for 12, 15, 20, 25, 33, or 62 days and examined for development of *H. schachtii*.

Many male and female nematodes were present on cut surfaces of slices of sugarbeet, red table beet, turnip and rutabaga 15 days after inoculation. The nematode occurred singly and in groups from the center to the outer edges of longitudinal or transectional slices (Fig. 3-7). Nematodes frequently were near the edge of the secondary cortex just below the peridium or along the radial sectors of secondary xylem, suggesting that these areas are more amenable to invasion than other areas. Nematodes also were on small rootlets which grew out of the cut surfaces of two slices and from the external uncut surfaces of the taproot slices. Slices from near the crown or the root tip (apex) regions were parasitized by nematodes. After 15 days, male and female *H.*

FIG. 3. Small area of red table beet root slice infected with *Heterodera schachtii*. FIG. 4. Root of 'White Icicle' radish sliced parallel to root axis showing *H. schachtii* attached to secondary vascular tissues and to the secondary cortex immediately beneath the periderm. FIG. 5-7. *H. schachtii* attached to cut surfaces of red table beet root slices. FIG. 5. *H. schachtii* distributed in a linear pattern along a ring of secondary cambium. FIG. 6. *H. schachtii* attached to extensive growth of callus tissue. FIG. 7. Isolated group of *H. schachtii* attached to small callus tissue protuberances. FIG. 8. Surface of sugarbeet storage root heavily infected with females (white) and cysts (dark) (Courtesy of L. R. Faulkner).

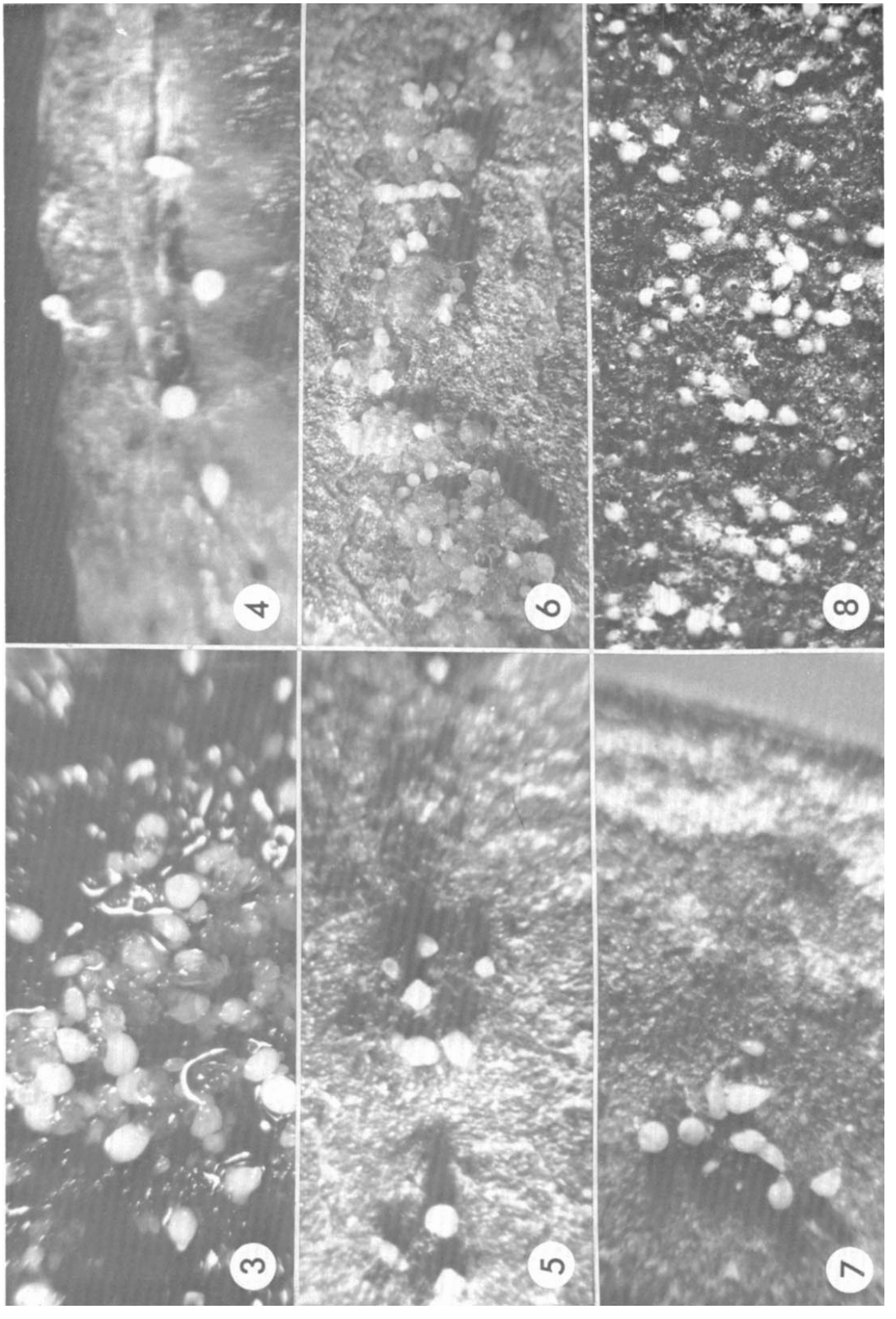

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TABLE 1. Large-rooted and tuberous crop plants rested for in vitro culture of *Heterodera schachtii.*

| Plant species | Commercial cultivar and/or common name[a] | Host[b] status |
|---|---|---|
| *Beta vulgaris* | 'US-75' sugarbeet | + |
| | 'Detroit Dark Red' table beet | + |
| *Brassica napobrassica* | 'American Purple Top' rutabaga | + |
| *B. rapa* | 'Purple Top White Globe' turnip | + |
| *Daucus carota* | Carrot | 0 |
| *Pastinaca sativa* | 'Long White' parsnip | 0 |
| *Raphanus sativus* | 'Scarlet Globe' radish | + |
| | 'White Icicle' radish | + |
| *Tragopogon porrifolius* | 'Sandwich Island' salsify | 0 |
| *Solanum tuberosum* | Potato | 0 |

[a]Except for potato, sugarbeet and carrot, seed of all plant species were obtained from Germain's Inc., Los Angeles, Calif.

[b]+ = host; 0 = nonhost (8).

*schachtii* were larger and were present in larger numbers on slices of red beet than on other plants, suggesting that the nematodes invaded earlier and/or developed more rapidly on this plant. Mature females removed from red beet or turnip 15 days after inoculation contained eggs, presumably fertilized, in early cleavage stages (2 to 16-cell stage). At 20 days, females contained eggs with moving embryos; and by 25 days, all stages of developing nematodes, including a few brown cysts, were observed. Cut surfaces of storage organs exhibited extensive growth of callus tissue regardless of whether or not nematodes were added. Single nematodes and groups of nematodes frequently were attached to callus tissue and to surfaces in areas where no callus tissue was present. Neither males nor females developed on salsify, carrot or parsnip. Two adult male *H. schachtii* were found on a single potato tuber slice 25 days after inoculation.

Many nematodes developed on the cut surfaces of slices of susceptible plant species which were inoculated and covered with moist agar or soil. Moist agar was judged to be a more suitable covering medium in that it provided air space with high moisture content at the cut surfaces. On the other hand, soil particles tended to compress rather tightly to the cut surfaces. Such soil-covered plant materials were less suitable for visual inspection and for microtome sectioning. Slices not covered with soil or agar tended to dry out and supported fewer nematodes. Cut surfaces of sugarbeet not covered by soil or agar became dry and leathery, whereas those of rutabaga were least affected by drying.

Adult male *H. schachtii* developed on 'Scarlet Globe' radish as early as 12 days after inoculation (Table 2). Two hundred forty-one males but only 14 females were found on a single root slice of 'Scarlet Globe' radish at 26 days.

The ratio of male/female *H. schachtii* was greater on tissue slices least suitable for development of females. The ratios found on 'Scarlet Globe' radish, 'White Icicle' radish, and rutabaga were 17.2, 13.0, and 3.0, respectively. 'Scarlet Globe' radish deteriorated rapidly in culture. Rutabaga was more suitable than 'White Icicle' radish for nematode cultures.

## DISCUSSION AND CONCLUSIONS

The successful development of *H. schachtii* on rootlets of defoliated sugarbeet and on the cut surfaces of sugarbeet and other large-rooted host crops strongly suggests that the nematode may increase on post-harvest root debris, particularly in areas favored by combinations of high soil moisture and temperature. Reproduction of the nematode is greatly restricted when the cut surface is dry and leathery or when, as in 'Scarlet Globe' radish, the root fragments undergo rapid decomposition. Although the sugarbeet nematode failed to develop on intact storage organ surfaces, the nematode frequently was attached to cut surfaces immediately below the periderm. These observations indicate that the periderm normally is an effective barrier to invading nematode larvae. Massive parasitization of the surfaces of the storage root of sugarbeet has been observed (L. R. Faulkner,

TABLE 2. Number of male *Heterodera schachtii* emerged from infected root and potato tuber slices.

| Storage organ slice[a] | Number of days after inoculation | | | | | | Total no. males |
|---|---|---|---|---|---|---|---|
| | 12 | 15 | 20 | 25 | 33 | 62 | |
| Carrot discs | – | 0 | 0 | 0 | 0 | 0 | 0 |
| Parsnip discs | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Salsify discs | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Potato discs | – | 0 | 0 | 2 | 0 | 0 | 2 |
| Turnip discs | – | 4 | 21 | 51 | 2 | – | 78 |
| 'Scarlet Globe' radish discs | 1[b] | 173 | – | – | – | – | 174 |
| Longitudinal slice of 'Scarlet Globe' radish | 3 | 164 | 395 | 241 | – | – | 803 |
| 'White Icicle' radish discs | 0 | 119 | 526 | – | – | – | 642 |
| Longitudinal slice of 'White Icicle' radish | 0 | 166 | 360 | 179 | – | – | 705 |
| Sugarbeet discs | 0 | – | 32 | 130 | 26 | – | 188 |
| Longitudinal slice of sugarbeet | 0 | – | 159 | 166 | 35 | – | 360 |
| Red table beet discs | – | 28 | 360 | 251 | 10 | – | 649 |
| Rutabaga discs | – | – | – | – | 123 | – | 123 |

[a]Rutabaga left uncovered — all other root slices covered with moist agar during incubation.
[b]Figures given are for individual cross-sectional or longitudinal slices of root or tubers.

personal communication). However, the surfaces of the storage root appear extensively discolored (Fig. 8), suggesting that necrosis of the peridermal tissue by fungi and/or bacteria may have facilitated invasion of deeper cortical tissues.

The finding that the ratio of male to female *H. schachtii* is greater on root fragments least suitable for development of females is evidence that the sex ratio of sugarbeet nematode probably is related to differential nutritional requirements of the sexes as previously suggested (9, 10).

The appearance of free adult males 11 days after inoculation and of adult females containing developing eggs at 15 days suggests a shorter life cycle than reported by Raski (6). Our results are in essential agreement with reports of sugarbeet nematode development on excised roots (3, 4). However, placement of the nematode inoculum directly on freshly cut host tissue surfaces probably resulted in more rapid invasion of larvae and earlier development of the nematode.

Female *H. schachtii* frequently were attached to clumps of callus tissue which formed on cut surfaces of storage organs (Fig. 6, 7). Similar observations reported for *Meloidogyne incognita* growing on carrot discs were cited as evidence for nematode-induced callus growth (7). Observations in the present test suggest that callus growth occurs at sites most suitable for nematode invasion.

In vivo culture of nematodes on root fragments may offer certain advantages over pot cultures or excised root cultures (1, 2, 5). Some of these advantages are: direct inoculation of nematodes on host plant tissues; close control of temperature, moisture, and size of area available for parasitism; enhanced observation of nematode and disease development; rapid acquisition of high nematode populations; and reduced requirements of space and equipment. In addition, the technique may be of particular value in studies of resistance, the dynamics of intra- and inter-specific competition, and the interrelationships of nematodes and other plant disease pathogens.

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