Histopathology of Soybean Roots Infected with Helicotylenchus dihystera[1]

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Abstract: Soybean roots infected with *Helicotylenchus dihystera* in a greenhouse were stained with acid fuchsin in lactophenol or sectioned and stained with safranin and fast green. Adults and larvae were observed in semi-endoparasitic and endoparasitic feeding positions. Adults, larvae and eggs were observed within the root cortex posterior to the region of maturation. Small brown lesions, affecting the walls of six to ten cells, were observed in the immediate vicinity of the nematode. Endoparasitic nematodes were usually coiled within the walls of one or two cells. Cytoplasm of the infected cells appeared normal, and there was no indication of nuclear proliferation. Walls of the infected cells were thickened and lignified, but there was no indication of swelling or giant cell formation. Uncoiled nematodes usually were aligned parallel to the vascular tissue, but were not consistently oriented with respect to the root apex. Nematodes moved through cell walls rather than between them; however, no persistent burrows were observed. *Key words:* spiral nematode, histopathology, feeding habits.

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The spiral nematode *Helicotylenchus dihystera* (Cobb) Sher has been reported associated with numerous crop plants. Its presence in the southeastern United States in association with soybeans has been confirmed by several workers (5, 7, 8). Furthermore, it has been shown that *H. dihystera* can reproduce rapidly and in great numbers on certain cultivars of soybeans (4). *H. dihystera* has been

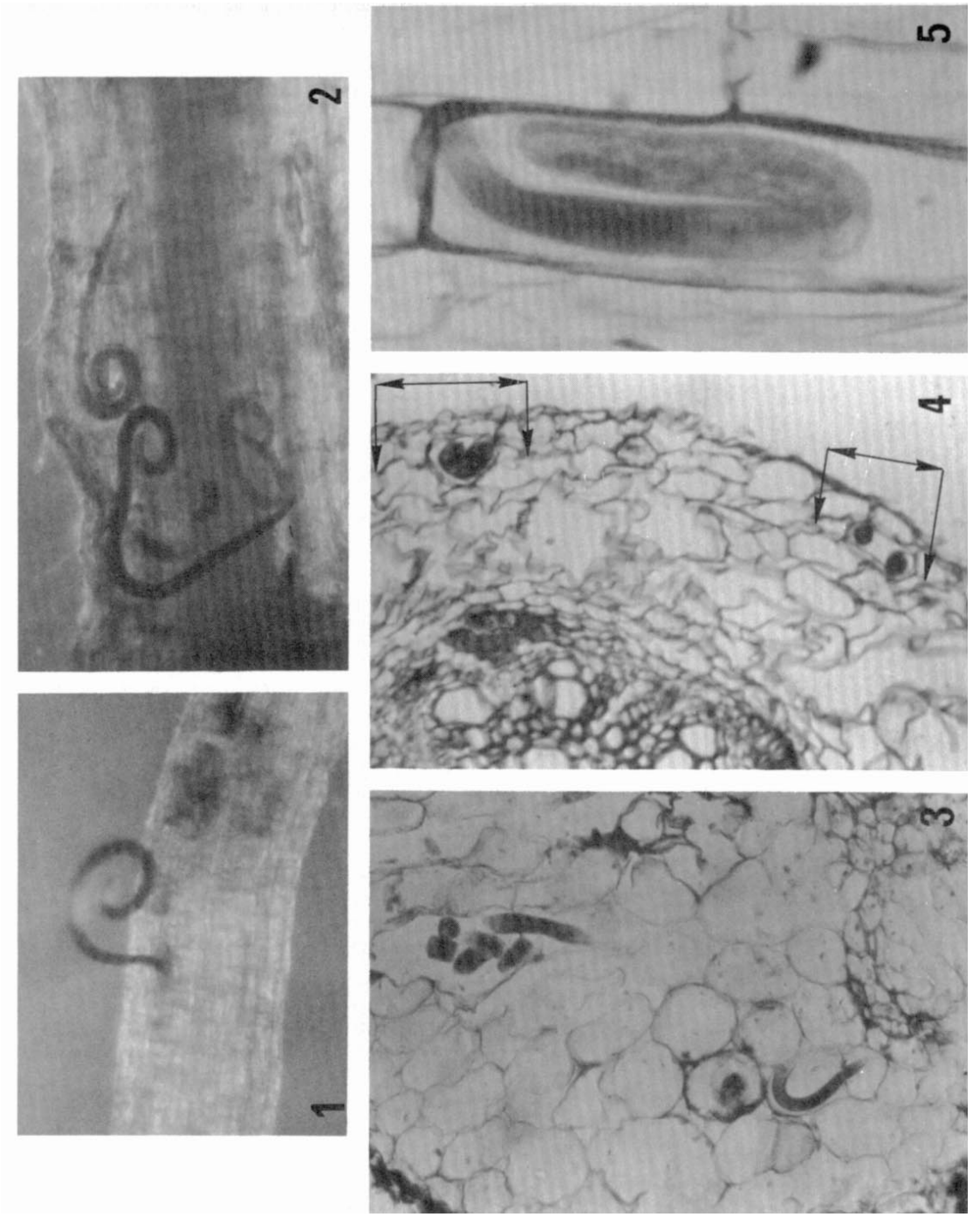

Fig. 1-5. **1.** *Helicotylenchus dihystera* in a semi-endoparasitic feeding position on a soybean root. **2-5.** Soybean roots infected with *Helicotylenchus dihystera*. **2.** Adults, larvae, and eggs within a root. **3.** Cross section showing nematodes within the cortex. **4.** Cross section of a young root showing the nematode in the cortex with arrows indicating the extent of affected tissue. **5.** Longitudinal section of a young root showing a nematode within a cortical cell and thickened cell walls.

described as exclusively ectoparasitic or semi-endoparasitic (2, 9). This study was conducted to determine feeding habits of *H. dihystera* on soybeans and its subsequent effect upon root cells.

MATERIALS AND METHODS

Fifteen southern soybean cultivars were used in this study. They were: *Glycine max* (L.) Merr. 'Bragg', 'Custer', 'D63-7320', 'D64-3646', 'D66-12394', 'Dare', 'Hampton', 'Hardee', 'Harlee', 'Hill', 'Hood', 'Jackson', 'Lee', 'Peking' and 'Pickett'. Two seeds of each cultivar were planted in steamed soil in separate 17-cm plastic pots. Four pots of each cultivar were infested with 2000 *H. dihystera* in 1 ml of water. Two pots of each cultivar were designated as controls and received an equal volume of the supernatant fluid from the nematode suspension. The plants were grown for 6 weeks in a greenhouse, after which the roots were separated from the soil.

One half of the roots of each cultivar were stained with acid fuchsin in boiling acid fuchsin-lactophenol and cleared in lactophenol (1). The cleared roots were observed under a stereo-dissecting microscope to determine the distribution and feeding habits of the nematodes. The other half of the roots were fixed in Navashin's fluid (3). After fixation, selected portions of the roots were dehydrated in tertiary butyl alcohol and embedded in paraffin. Cross and longitudinal sections were cut at 20 μ on a rotary microtome. Sections were mounted and stained with safranin and fast green for observation under the compound microscope.

RESULTS

Adults and larvae of *H. dihystera* were observed in semi-endoparasitic feeding positions on roots of all 15 cultivars (Fig. 1), and adults, larvae and eggs also were observed completely within the roots (Fig. 2). Nematodes were observed attached to and within the roots behind the region of maturation, but none was observed attacking the root tips. Free-hand sections of acid fuchsin-stained roots showed endoparasitic nematodes restricted to the root cortex except for a single specimen found within the stele in one of the 120 root systems examined. Nematodes were observed in the tap roots of all cultivars and within the secondary roots of all but one cultivar. *H. dihystera* was observed in greater numbers in and around tap roots than in and around secondary roots.

Small brown lesions usually were observed around the heads of semi-endoparasitic nematodes in the acid fuchsin-stained roots. These lesions were composed of six to ten cells with discolored walls. No swelling or curvature of the root was noted in connection with these lesions. Nematodes within the roots had similar lesions around them affecting similar numbers of cells.

Paraffin sections of soybean roots stained with safranin and fast green revealed *H. dihystera* in the region of the root cortex (Fig. 3). It was difficult to assess the damage caused by the nematode in mature roots, because expansion of secondary vascular tissue caused a great deal of disruption and splitting of the cortex. Therefore, plant damage and nematode activity were assessed in young roots (Fig. 4, 5).

Cytoplasm of invaded cells appeared normal, and there was no indication of nuclear proliferation. The walls of cells invaded by the nematode and six to ten adjoining cells were thickened and took up safranin dye indicating the presence of lignin (3).

Endoparasitic nematodes were usually coiled within the walls of one or two cells (Fig. 5). Uncoiled specimens were usually aligned parallel to the vascular tissue, but were not consistently oriented with respect to the root apex. Nematodes were aligned with their heads toward the base and apex of the root in about equal numbers.

H. dihystera moved through cell walls rather than between cells, but no persistent burrows were observed. Openings in the root surface indicative of points of entry were not observed. There was no indication of giant cell formation. Infected cells appeared normal except for altered cell walls.

In a few instances, cortical tissues of nitrogen nodules contained endoparasitic *H. dihystera*. The nematode frequently was observed within the crevices of the disrupted cortex of mature primary roots where it did not penetrate the tissue, but fed on exposed cells.

DISCUSSION

H. dihystera can live ectoparasitically, semi-endoparasitically, and endoparasitically on soybeans. However, the factors that determine the mode of parasitism are not clear. Lee soybean was quite heavily infected with endoparasitic *H. dihystera* in this experiment,

but in other tests (6) the roots were not entered. Taylor (10) reported that *H. microlobus* fed endoparasitically on corn and tomato, but not on soybean. He suggested that the mode of parasitism was largely dependent upon the host. Minton and Cairns (4) reported that *H. nannus* penetrated the roots of fescue, but not the roots of Ogden soybean. Apparently some factors other than host determine the mode of parasitism of *H. dihystera* on soybean.

The lack of persistent burrows indicates that the nematode did not migrate far in the root, or that the wound healed rapidly after the nematode passed. The nematode's preference for older root tissue, coupled with minimal damage to the host, may temper the pathogenicity of *H. dihystera*. Effects observed in this investigation occurred in the presence of nematode populations two to four times higher than maximum field populations found by the author (6). Thus it would appear that *H. dihystera* is not a significant pathogen of soybeans when acting alone.

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