RESEARCH NOTES

Histopathology of Rotylenchulus reniformis on Sunflower

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Investigations on the histopathology of *Rotylenchulus reniformis* Linford and Oliveira (1, 2, 3, 4, 6) have involved diverse plant taxons, viz., cotton (Malvaceae), tomato (Solanaceae), castor (Euphorbiaceae), soybean (Leguminosae), mint (Labiatae), papaya (Caricaceae), and cantaloupe (Cucurbitaceae). This study examined the histopathology of reniform nematode infection on sunflower (*Helianthus annuus* L.), a member of the Compositae.

Sunflower seeds were planted in a greenhouse groundbed infested with *R. reniformis* (ca. 20 infective larvae/cm^3 soil). After 18 days, eight plants were harvested; roots were fixed in FAA and examined with a stereomicroscope. Infected regions were identified by maturing or gravid females protruding through the root epidermis. Two infected segments (ca. 0.4 cm long and 0.9 mm in diam) were cut from a region 2–4 cm below the hypocotyl transition zone of each taproot, dehydrated, and embedded in paraffin. Ten segments, two from each of five plants, were sectioned transversely at 10 μm; one segment from each of the remaining plants was sectioned longitudinally. Sections were stained with safranin and fast green (5). After staining, 30 feeding sites were examined at high magnification (×400). Polarized light (Nomarski) was used to identify the birefringent casparian strip (Fig. 1), which is otherwise somewhat indistinct in young sunflower roots. At one feeding site on each of three roots, radial and tangential cell diameters were measured to evaluate pericycle hypertrophy.

Rotylenchulus reniformis females were embedded among disrupted cortical cells, usually with the posterior portion protruding through the root epidermis and the anterior portion perpendicular to the stele. The lips were always pressed against, or proximal to, the outer tangential wall of a large cell containing an enlarged nucleus and darkly stained granular cytoplasm (Fig. 2); this cell will be referred to as the feeding cell. In two instances, the nematode head was located within a secondary root, and the histological origin of the feeding cell could not be determined. The feeding cell was of pericycle origin at two feeding sites, in the cortical layer adjacent to the endodermis at three feeding sites, and of endodermal origin at 23 feeding sites. Where the feeding cell was of endodermal origin, the pericycle cell immediately centripetal to, and 1–9 pericycle cells circumferentially in one or in both directions away from, the feeding cell were hypertrophied

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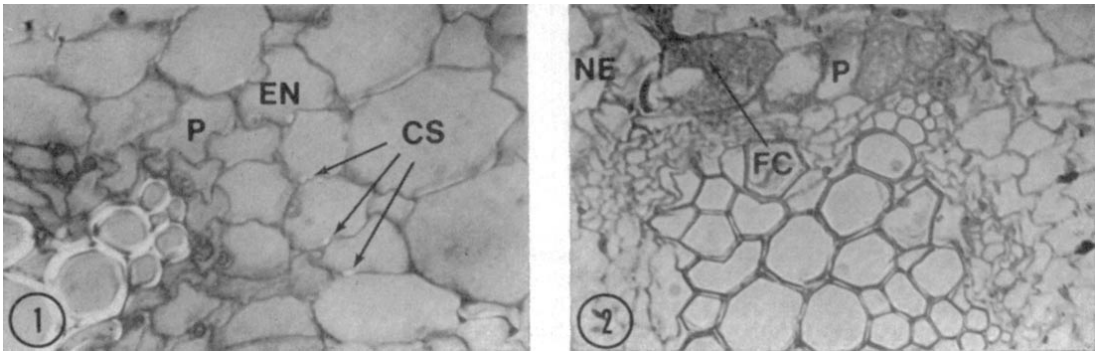

FIGS. 1–2. 1) Casparian strip (CS) demarking endodermis (EN) and pericycle (P). Nomarski at ×560. 2) Hypertrophied and granular pericycle cells (P) extending circumferentially away from feeding cell (FC). Note nematode (NE) with lips against outer tangential wall of feeding cell. ×280.

and contained granular cytoplasm. These cells contained hypertrophied nuclei and densely red-staining hypertrophied nucleoli. Feeding cells and affected pericycle cells were uninucleate. In longitudinal section, granular cytoplasm continued for several cells above and below the feeding site.

Birchfield (1) reported that *R. reniformis* normally penetrated the epidermis, cortex, endodermis, and pericycle of cotton roots before coming to rest and feeding in the phloem. Sivakuma and Seshadrin made similar observations on tomato and papaya. Cohn (2), however, insisted that the nematode invariably fed in the pericycle of tomato, cotton, and mint, with limited phloem involvement. Affected cells were described as hypertrophied, uninucleate, and with intact cell walls. Rebois et al. (4), in a subsequent electron-microscope study on soybean, described *R. reniformis* as generally stopping with the lips pressed against an endodermal cell which he termed the prosyncyte. This event was followed by hypertrophy of the prosyncyte and the adjoining pericycle, a distance, tangentially, of 3–10 cells away. Affected cells were essentially uninucleate but comprised a syncytium formed by the coalescence of cytoplasm, after the partial dissolution of radial pericycle walls. Heald (3), working concurrently with cantaloupe, also described *R. reniformis* as generally feeding on an endodermal cell with pericycle involvement. The feeding cell and pericycle cells were uninucleate with prominent nucleoli.

The results of this study indicate that feeding on, and response by, sunflower are similar to that reported for soybean by Rebois et al. (4) and that reported for cantaloupe by Heald (3), in that affected cells were essentially uninucleate and the feeding cell or prosyncyte was endodermal in 23/28 cases. Apparently normal female development occurred in instances where cortical or pericycle cells served as feeding cells. Thus, direct endodermal feeding may not be necessary for reniform nematode reproduction. The disruption of the endodermis necessary to reach the pericycle may impair the osmotic stability of the vascular cylinder. Densely red-staining intercellular material, apparently necrotic, was observed between the pericycle and endodermis in both instances where the feeding cell was of pericycle origin. The partial dissolution of radial pericycle walls observed with the electron microscope by Rebois et al. (4) on soybean could not be verified with the light-microscope techniques we used on sunflower; however, radial walls of affected cells appeared thinner than the radial walls within normal pericycle.

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