

Reactions of Sweet Potato Root Tissue to the Reniform Nematode

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ABSTRACT

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Roots of the sweet potato cultivar Centennial were severely infected by the reniform nematode, *Rotylenchulus reniformis*, 3 wk after they were inoculated in the greenhouse. Histologic sections showed that the nematode penetrated the cortex of the roots and fed in the endodermal layer. A single endodermal cell hypertrophied into a giant cell. The pericycle next to the giant cell reacted severely to infection and hypertrophied into a curved sheet of syncytia. These cells stained darkly with methylene blue, showing dense, granular cytoplasmic contents. Phloem cells at the infection point enlarged, and the cambium and xylem vessels appeared pressed together. Cortical parenchymal cells around the neck of the nematode were also enlarged and irregularly shaped and looked disorganized.

The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, is semiendoparasitic on a wide variety of crops. This nematode causes considerable damage worldwide and reduces yields of soybeans, cotton, and sweet potatoes. Extensive research has been done on the pathogenesis of the reniform nematode on cotton and soybeans. Severe root pruning, root necrosis, unthrifty growth, dwarfing, and yield loss have been reported (1,5,10). Little information, however, is available on the host-parasite relationship of the reniform nematode on sweet potatoes. Martin (6) and Birchfield and Martin (2) reported that reniform nematodes feed on small sweet potato roots, cause yellowing of foliage, and reduce yields and quality of sweet potatoes. The purpose of the research reported here was to use histologic methods to determine the host-parasite relationships of reniform nematodes in sweet potatoes.

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MATERIALS AND METHODS

Cuttings of sweet potato, *Ipomoea batatas* (L.) Lam. 'Centennial', a cultivar susceptible to reniform nematodes, were planted in infested soil in the greenhouse. After 3 wk, young roots of the sweet potato cuttings were removed from the soil, washed, and observed for nematode infection with a stereoscopic microscope.

Infested sweet potato roots were fixed in 10% paraformaldehyde phosphate buffer for plastic embedding and in chromium-acetic acid formaldehyde for paraffin embedding. The initial fixation was done in a vacuum to facilitate penetration into the root tissues.

After 24 hr, the root sections in paraformaldehyde phosphate buffer were rinsed through three changes of distilled water for 5 min each and dehydrated through an acetone series of 25, 50, 75, 90, and 100% (two changes) at 15 min each. Materials were embedded in Aradite-Epon (Ladd Research Industries, Inc., Burlington, VT) plastic mixtures through a series of 25, 50, 75, and 100% for 24 hr at each concentration (7). After the last plastic change, roots were removed to fresh 100% plastic, cast into molds in an oven overnight at 45 C, and placed in another oven for 3 days at 60 C.

The roots embedded in plastic were cut out, trimmed, and mounted on blocks for sectioning on a Sorvall JB-4A Porter Blum microtome. Sections were cut 3 μ m thick, mounted on glass slides, stained with 1% methylene blue borax, perma-

nently mounted, and sealed with Eukitt.

Roots fixed in chromium-acetic acid formaldehyde for 24 hr were rinsed in running water for 15 min, then dehydrated in an ethyl-tertiary butyl alcohol series of 5, 10, and 20% for 15 min each. Then the roots were processed through 50, 70, 90, and 100% tertiary butyl alcohol series at 2 hr each, infiltrated, and embedded in paraffin. Gradual dehydration and paraffin infiltration were done in an Autotechnicon (The Technicon Co., Chaunfy, NY). Paraffin sections of 7 μ m were cut on a rotary microtome. Serial sections were mounted on slides, dried, stained in toluidine blue, and mounted permanently in Harleco.

Tissues were observed for reactions to the reniform nematodes and were compared with noninfected sections.

RESULTS

Normal 3-wk-old sweet potato roots had well-developed xylem and phloem systems (Fig. 1). The apoplast system consisted of one to three large xylem vessels in a central position, and the smaller vessels formed five radiating xylem poles. The phloem bundles between the xylem poles were separated from the xylem by a layer of actively dividing cambium. A single layer of pericycle encircled the vascular system. A well-developed layer of endodermis is present in sweet potato. The cortical parenchyma is six to eight cells deep, extending from the endodermis in orderly files to the epidermis. Intercellular air spaces occurred between the loose files of cortical parenchyma. These prominent air spaces were situated directly opposite the phloem poles.

Plastic cross sections of infected roots showed severe tissue reactions and distortion of the roots (Fig. 2). The necks of the female nematodes were embedded intercellularly in the cortical cells, and their heads were about one cell away from the endodermal layer. Their bodies, at maturity, formed a kidney shape, were enclosed in a gelatinous matrix, and remained outside the roots.

The cortical parenchyma cells around the neck of the nematode hypertrophied to twice their original size. They appeared to have crushed and pushed the adjoining parenchymal cells out of the orderly files and to have compressed them into irregularly shaped cells. The large air spaces in the infected area were occupied by oversized parenchyma.

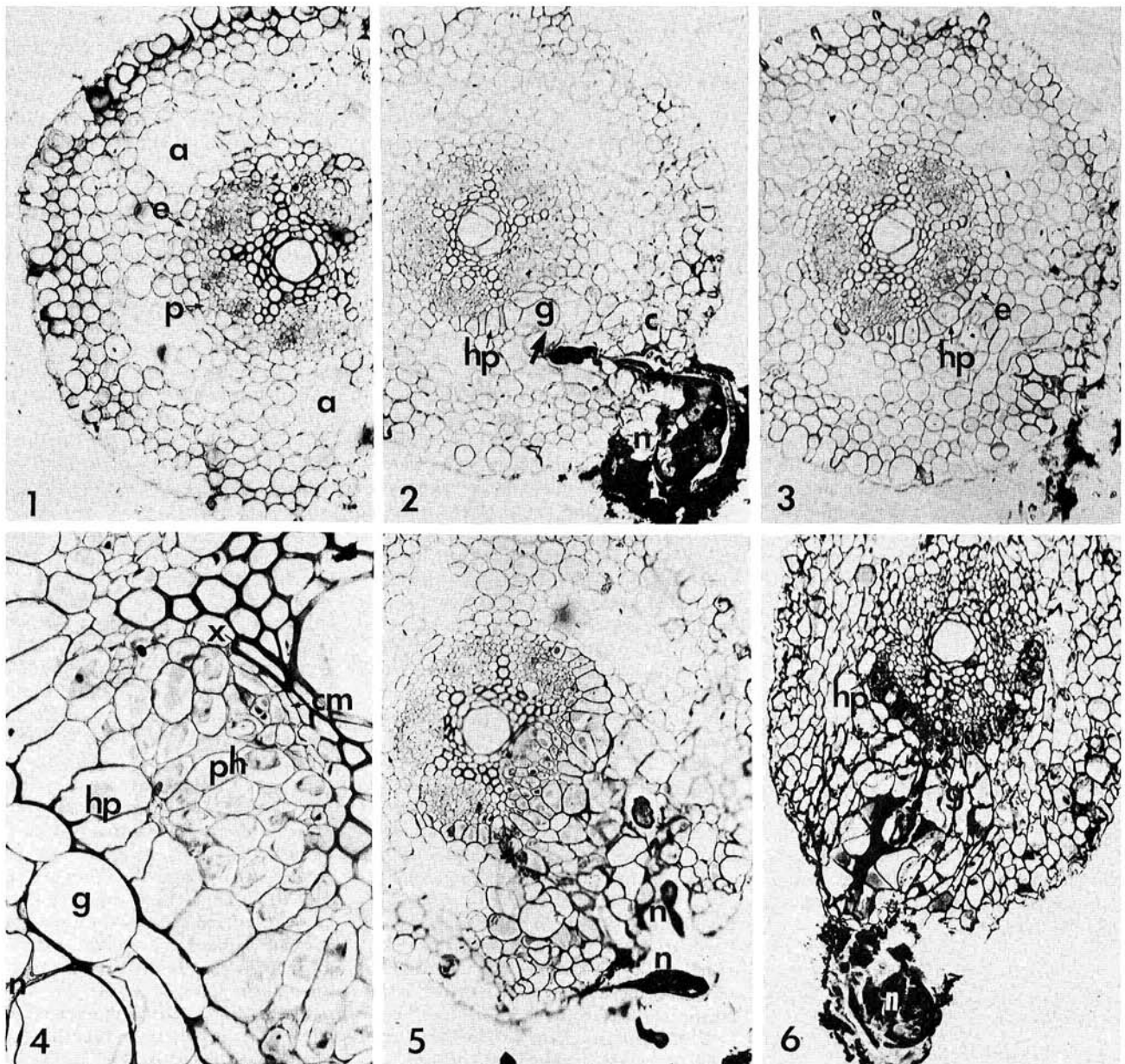
The endodermal cell proximal to the head of the nematode, where the nematode fed, enlarged to three or more times its original size. The adjacent endodermal cells were not affected. When the feeding point was at a cortical cell next to the endodermal cells, the cell wall between the enlarged endodermal and

cortical cells was absent and the area appeared as a large giant cell or "nurse cell." Such giant cells were densely stained. The cell walls were unevenly thickened, but no wall proliferations were observed.

The area most severely affected by the nematodes' feeding was the row of pericycle cells inside the perimeter of the endodermal layer. The pericycle cells immediately next to the transformed endodermal cell hypertrophied. The distortion spread laterally to either side from the point of infection. The rate of enlargement decreased as the distance from the infection increased. This alteration of the pericycle extended to

one-third to two-thirds of the pericycle ring. These cells stained darkly and showed granular thickening of cytoplasmic contents, especially in the paraffin sections (Fig. 6). Cells were not nucleated. In older sections, hyperplasia of the pericycle cells also occurred near the nematodes' feeding sites. Distortion of the pericycle was observed to extend in the longitudinal axis to 10–15 cells from the infection point. Sections at these levels showed that part of the pericycle hypertrophied, while the endodermal cell layer and most of the parenchyma cells of the cortex appeared normal (Fig. 3).

Nematode feeding opposite the xylem or phloem was random, with no



Figs. 1-6. (1) Noninfected root with uniseriate endodermis (e), uniseriate pericycle (p), and large air spaces (a). (2) Root infected with nematode (n). The giant cell (g) was formed from the lysis of cell wall between the hypertrophied cortical cell and a single endodermal cell. Cells of the pericycle hypertrophied (hp). Cortical parenchyma (c) around the nematode hypertrophied and was disorganized. (3) Root section at some distance away from the infection point showed altered pericycle. Endodermis was not affected. (4) A single cell of the endodermis at the head of the nematode enlarged. The layer of pericycle and the phloem bundle (ph) were hypertrophied. The cambium (cm) layer and xylem vessels (x) were crushed and compressed. (5) Root section infected with two nematodes. About half of the cortical parenchyma, endodermis, and pericycle were enlarged and disorganized. The phloem bundle was enlarged, and the xylem vessels were crushed. (6) Paraffin cross section of sweet potato root infected by the reniform nematode. The giant cell at the head of the nematode and the altered curved sheet of pericycle were darkly stained and showed granular cytoplasmic content.

particular preference for either tissue. However, when feeding was opposite the phloem pole, this tissue appeared to be adversely affected by the parasite. Cells of the whole phloem bundle increased to four times their original size (Figs. 4 and 5). Further development of the cambium layer appeared to be limited because of pressure from surrounding hypertrophied tissue or tissue with hyperplasia. Growth of the expanding xylem was apparently impeded, and the thick-walled vessels appeared to be crushed and compressed toward the large central vessels.

The amount of tissue distortion was proportional to the number of nematodes at an infection site. Two to four females were commonly observed at the same infection point; the result, as shown in cross sections (Fig. 5), was that more than half of the root tissues were altered compared with unparasitized tissues.

DISCUSSION

The reniform nematode, like root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp.), induced the formation of syncytia in the host tissues by its feeding (5). However, the syncytia of reniform nematodes do not have wall ingrowths characteristic of the giant cells (nematode-induced transfer cells) of root-knot nematodes.

The row of pericycle cells constituting the source of lateral root development formed a row of syncytia. This was obviously the reason for root formation stopping and consequent dwarfing of the

plants, symptoms that were associated with plants infected with reniform nematodes. This tissue reaction of the pericycle in sweet potato roots to the reniform nematode is similar to findings of others (1,3,4,8-11) working with other crops infected with reniform nematodes.

The growth in size of the sweet potato roots would depend on the normal functioning of the vascular systems. According to our observations, reniform nematode infection caused malformation of the xylem, cambium, and phloem cells, which could result in smaller, lower quality potatoes. These symptoms have been noted in fields with heavy populations of reniform nematodes. However, physiologic tests need to be made to establish this hypothesis as fact.

Apparent crushing of the xylem and cambium has not been described in other plants infected with this nematode. Further tests should be made to verify this phenomenon.

Mechanical damage to the cortex and cortical cellular hypertrophy brought about by the nematodes' penetration probably resulted in root necrosis and cracking observed on infected roots. The openings may have been entry points for secondary infections by pathogenic fungi and bacteria.

A comparison of the two techniques for embedding the diseased materials, sectioning, and staining showed that the paraffin sections gave a better overall picture of the syncytia compared with normal cells. The thinner plastic sections

gave greater detail of alterations at the cellular level.

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