

Histopathology of Okra and Ridgeseed Spurge Infected with *Meloidodera charis*

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Abstract: Histological observations of okra *Abelmoschus esculentus* 'Clemson Spineless' and ridgeseed spurge *Euphorbia glyptosperma* (a common weed) infected with *Meloidodera charis* Hopper, indicated that the juvenile nematode penetrated the roots intercellularly. Within 5 days after plant emergence the nematode positioned its body in the cortical tissue parallel to the vascular system. By 10 days after plant emergence the juvenile had extended its head into the vascular system and initiated giant cell formation, generally in protophloem tissue. Giant cells were one celled and usually multi-nucleate. Eggs were observed in the female body 30 days after plants emerged and juveniles were found within the female body by 40 days. Nematode development progressed equally in the root system of either host plant. Generally, throughout the nematode's life cycle its entire body remained inside the cortical tissue of okra. In ridgeseed spurge, however, the posterior portion of the female erupted through the host epidermis as early as 15 days after plant emergence; only the head and neck remained embedded in the host. The nematode caused extensive tissue disruption in the cortical and vascular system of both plant species. Corn, *Zea mays*, was another host of the nematode.

Key words: *Abelmoschus esculentus*, *Euphorbia glyptosperma*, new host, host range, corn.

Meloidodera charis was described by Hopper (2) from a greenhouse population infecting honey mesquite, *Prosopis juliflora*. Earlier Norton (4) found that the genus *Meloidodera* occurred in 0.3 percent of 2,406 samples collected from 175 Texas counties. Heald and Golden (1) reported the presence of *M. charis* juveniles from soil about the roots of St. Augustinegrass and observed swollen females attached to the roots. Soil samples from a corn field in Nebraska also contained *M. charis* juveniles although roots were not present in the sample (3). *Meloidodera* larvae are frequently found in cultivated fields and native habitats throughout south Texas. Females are easily dislodged from roots, therefore, they are seldom found unless roots are carefully removed from soil. Two common hosts heavily infected under field conditions are okra, *Abelmoschus esculentus*, and ridgeseed spurge *Euphorbia glyptosperma*, a common weed.

The objective of this study was to describe the histopathology of *M. charis* in

the root systems of okra and ridgeseed spurge, to examine the nematode's host range and identify other hosts.

MATERIALS AND METHODS

Histopathology: One hundred sixty pots 15 cm in diameter were filled with a steam sterilized sandy clay loam soil and placed on a greenhouse bench. Two okra and two spurge seeds were planted in each of 80 pots. *Meloidodera charis* juveniles were collected by the Baermann funnel technique from soil about the roots of mesquite trees growing in soil boxes in the greenhouses. Ten thousand juveniles in 20 ml water were added to the soil in each pot through five holes 5 cm deep spaced evenly about the planted seeds. At 5-day intervals from emergence, and continuing for 60 days, the roots from five pots of each host species were harvested (care was taken to avoid dislodging the nematode) and washed. The roots from three pots of each plant species were fixed in FAA (formalin-acetic acid-alcohol) for at least 48 hours, dehydrated in tert-butyl alcohol and embedded in paraffin (melting point 56-58 C). Roots were sectioned 10-12 μ m thick, stained in saf-

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ranin-fast-green and examined microscopically. The remaining roots were stained in lactophenol cotton blue or lactophenol acid fuchsin for 3 to 6 hours and destained in lactophenol.

Host range: Twenty plant species (Table 1) were planted each in 12-m rows, in an area previously cropped to okra and heavily infested with *M. charis*. After 66 days, soil and root samples were taken from all species and examined for the nematode. The same species were tested in the greenhouse by planting seeds in 15-cm pots (four pots/plant species) and infesting the potting soil with 10,000 *M. charis* juveniles/pot. Sixty days after inoculation roots were removed from the soil, stained in lactophenol as described above and examined microscopically. Plants were considered to be hosts when all developmental stages of the nematode were attached to roots and females contained eggs.

RESULTS AND DISCUSSION

Histopathology and nematode development: Within 5 days of inoculation of okra, *M. charis* juveniles penetrated roots, primarily immediately proximal to the root cap in the area of differentiation. The abundance of disrupted cortical cells indicates juvenile migration inside the root is intercellular. By 10 days after inoculation juveniles became sedentary with their heads extended into the vascular system at nearly right angles. Initial stages of giant cell formation occurred at this time. Occasionally, juveniles became sedentary in the cortex with their bodies coiled around the vascular cylinder and their heads extended into it (Fig. 1). Giant cells generally formed in the protophloem, however, in some cases it originated from metaphloem. By 10 days after inoculation the giant cell had reached maturity; there were no noticeable differences in giant cells among the 10, 15, or 20 day sections. Giant cells were single celled and contained from one to five nuclei with

prominent nucleoli. Generally the giant cell was oval in the middle and tapered to a conoidal shape at either end (Fig. 2). As the posterior portion of the female began to swell (10 days), cortical cells were crushed causing considerable cell destruction. In okra, with few exceptions, the body of the female was contained within the root system (Fig. 3); occasionally the enlarged female body ruptured the epidermis of the root. Infected roots did not appear swollen or galled. Thirty days after plant emergence eggs were observed within the female body and after 40 days hatched juveniles were found within the female body. In spurge, *M. charis*' development was similar to that in okra with the following exceptions. In many cases the giant cells involved much of the vascular system (Fig. 4). Fifteen days after plant emergence, after the female body had begun to swell, the posterior portion of the nematode erupted through the host epidermis. Only the head and neck portion of the nematode remained embedded in the cortex and vascular system (Fig. 5). This process appeared to be a function of the size of the spurge roots which are very small; the mature female is often equal to or larger in diameter than the root. As the female matured and erupted from the root cortical and vascular tissue became exposed.

These observations are consistent with Ruehle's (5) on slash and loblolly pine roots infected by *M. floridensis*. Some giant cells in pine developed in the cortex, protophloem and protoxylem. I did not observe giant cells in the cortex of okra or spurge; most were in the protophloem.

Meloidodera charis is often found in non-cultivated areas associated with mesquite. Less frequently, juveniles occur in cultivated fields but not usually associated with a crop. Spurge is a very common weed in south Texas which could account for the presence of *M. charis* in cultivated fields.

Host range: Of the species tested, only

FIGS. 1-5. Histopathological studies of okra and ridgeseed roots infected with *Meloidodera charis*. 1) Cross section of okra root showing a developing female (N) embedded in the cortex, feeding from a giant cell (G) in the vascular cylinder. 2) Longitudinal section of okra root containing a giant cell (G) with two nuclei (NU). 3) Cross section of okra root showing female body (N) contained within the cortex and giant cell in the vascular tissue. 4) Cross section of ridgeseed spurge root showing nematode (N) feeding in giant cell (G) which involves the major portion of the vascular cylinder. 5) Cross section of ridgeseed root after female body (N) was erupted from the root; giant cell (G).

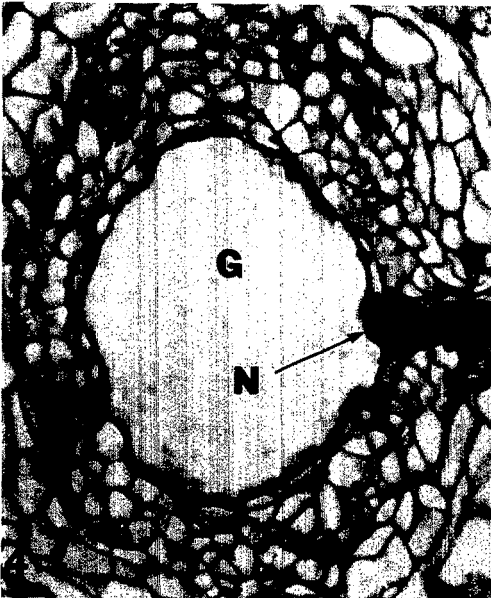
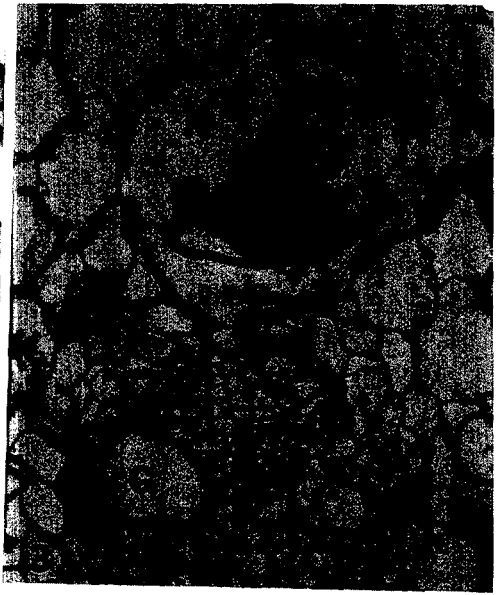
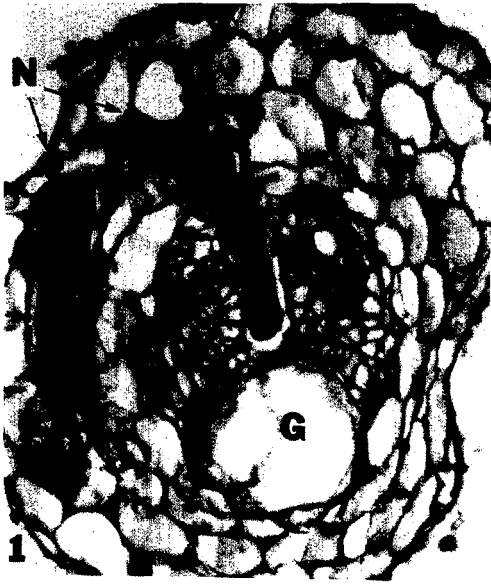


TABLE 1. Limited host range test with *Meloidodera charis*.

Plant identification	Plant response*
Bean, bush lima (<i>Phaseolus limensis</i> 'Henderson')	-
Beet (<i>Beta vulgaris</i> 'Detroit')	-
Cantaloupe (<i>Cucumis melo</i> 'Perlita')	-
Carrot (<i>Daucus carota</i> 'Scarlet Nantes')	-
Corn (<i>Zea mays</i> 'Green Velvet')	+.
Cotton (<i>Gossypium hirsutum</i> 'Stoneville 7A')	-
Cucumber (<i>Cucumis sativa</i> 'Ashley')	-
Jungle rice (<i>Oryza sativa</i>)	-
Lettuce (<i>Lactuca sativa</i> 'Great Lakes 659')	-
Okra (<i>Abelmoschus esculentus</i> 'Clemson Spineless')	+
Onion (<i>Allium cepa</i> 'Yellow Granex')	+
Cowpea (<i>Vigna unguiculata</i> subsp. <i>unguiculata</i> 'Blackeye')	-
Pigweed (<i>Amaranthus hybridus</i>)	-
Purple nightshade (<i>Solanum margaranthus</i>)	-
Purslane (<i>Portulaca oleracea</i>)	-
Ragweed (<i>Parthenium husterophorus</i>)	-
Ridgeseed spurge (<i>Euphorbia glyptosperma</i>)	+
Sorghum (<i>Sorghum vulgare</i> 'H-89')	-
Sunflower (<i>Helianthus giganteus</i>)	-
Tomato (<i>Lycopersicon esculentum</i> 'Monte Grande')	-

* + = host; - = nonhost.

corn, okra and spurge were hosts of *M. charis* (Table 1).

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