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# Observations on the Mode of Parasitism and Histopathology of *Meloidodera floridensis* and *Verutus volvingentis* (Heteroderidae)[1]

E. Cohn,[2] D. T. Kaplan,[3] and R. P. Esser[4]

*Abstract:* Some aspects of the host–parasite relationships of two heteroderid nematodes are described. *Meloidodera floridensis* induced formation of single uninucleate giant cells in the stelar parenchyma tissue of sand pine (*Pinus clausa*) roots. Wrinkling and yellowing of the cuticle were associated with maturation of the adult female (cystoid stage). The mode of parasitism of different life stages of *Verutus volvingentis* on buttonweed (*Diodia virginiana*) is described. The nematode caused extensive necrosis during penetration and the formation of a large feeding site consisting of non-hypertrophied parenchyma cells with enlarged nuclei and thickened cell walls in the cortex. Walls between cells within the feeding site degenerated, resulting in the formation of a syncytium. Two citrus rootstocks, rough lemon (*Citrus limon*) and trifoliate orange (*Poncirus trifoliata*), were not hosts of *V. volvingentis*.

*Key words:* histopathology, pine cystoid nematode, buttonweed, syncytium, uninucleate giant cell.

*Meloidodera floridensis* Chitwood, Hannon and Esser, 1956 (1), and *Verutus volvingentis* Esser, 1981 are sedentary plant parasitic nematodes apparently indigenous to Florida. *Meloidodera floridensis* parasitizes several species of pine (3,10) along the eastern coast of North America (9,11,16). However, few details concerning its parasitic habits have been described, and two histopathological studies provide only limited information on the nematode's feeding site (14,15).

A description of *V. volvingentis* from roots of buttonweed, *Diodia virginiana* L. (4), and its distribution, host range, and pathogenic potential (5) were recently reported; however, cellular responses to parasitism have not been described. The feeding behavior and development of this nematode on its type host are reported in a separate paper (6).

Some aspects of the mode of parasitism and host responses to these two heteroderid nematodes are dealt with here.

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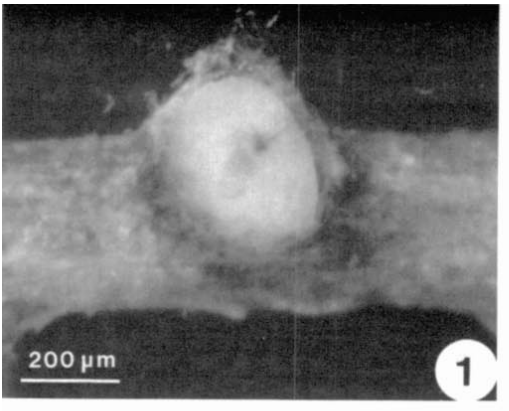
Figs. 1–5. *Meloidodera floridensis* infected roots of *Pinus clausa*. 1) Light micrograph (LM) of adult female (white). 2) LM of yellow cystoid body showing numerous small white elliptical internal ova. 3) Scanning electron micrograph (SEM) of adult female. Note subcrystalline layer separating from body. 4) SEM of cystoid body. Note smooth surface lacking annulation. 5) Subcrystalline layer detaching from the cuticle of adult female in Figure 3.

Figs. 6–12. LM of *Verutus volvingentis* parasitizing *Diodia virginiana*. 6) Female on thin root stained with acid fuchsin. 7) Females on a thin root. 8) Females and groups of eggs (arrows) on a thick root. 9) Females on a rhizome. 10) Nematodes (arrows) on a stem which was in contact with soil. 11) Single female on a stem with necrosis (arrow) around infection site. 12) Acid fuchsin-stained female on young root with superimposed cuticle (arrows), attached debris, and egg.

Figs. 13–17. SEM of *Verutus volvingentis* infected roots of *Diodia virginiana*. 13) Infective juvenile (left) and adult female. 14) Female with remnants of the subcrystalline layer and excreta on body surface. 15) Female covered with detritus adhering to the subcrystalline layer (arrow indicates uncovered vulval region). 16) Vulva with exposed protruding prolapsed vaginal tissue of an adult female. 17) Adult female dissected from tissue with egg (arrow) attached to subcrystalline layer in vulval region.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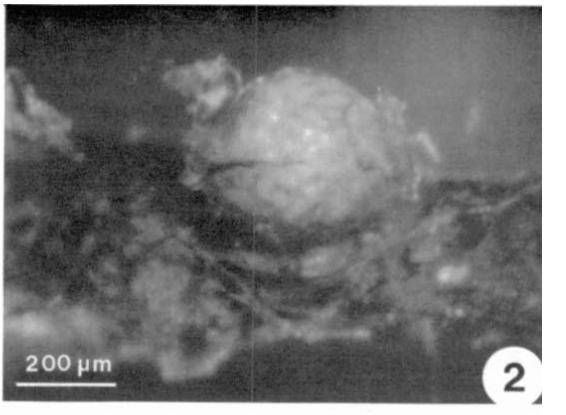
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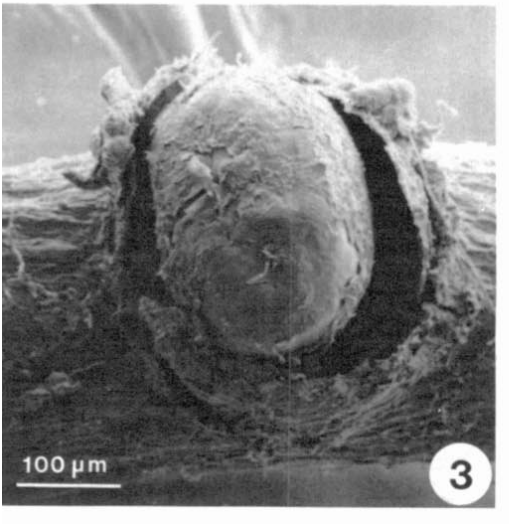
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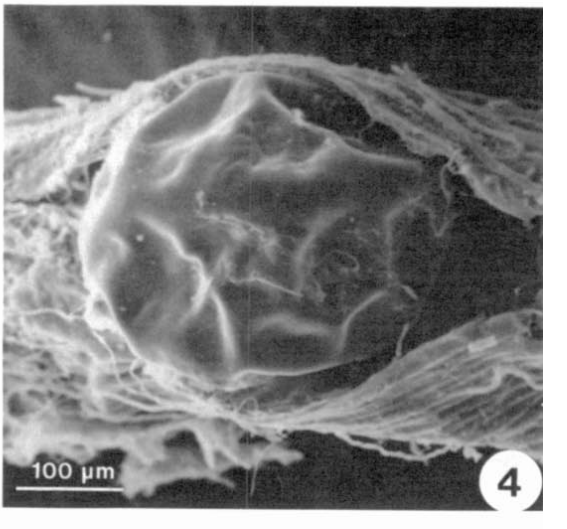
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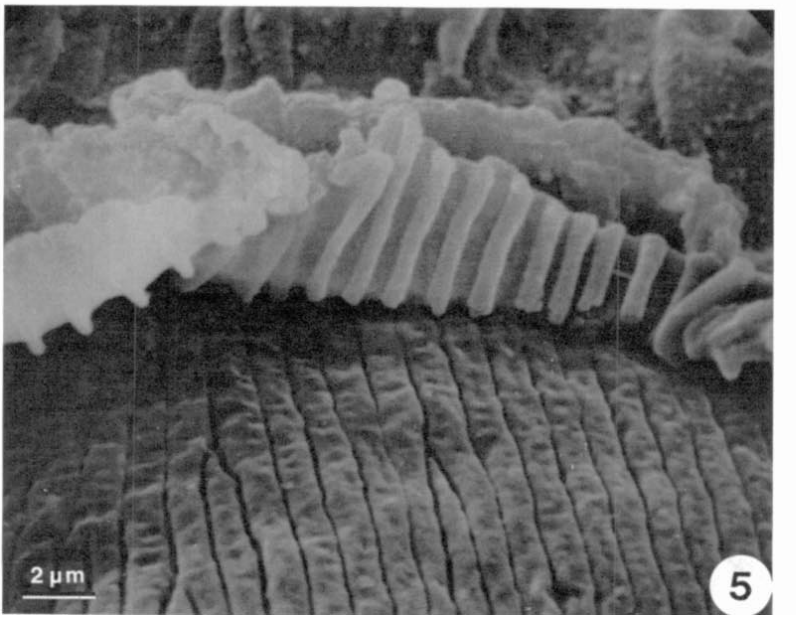
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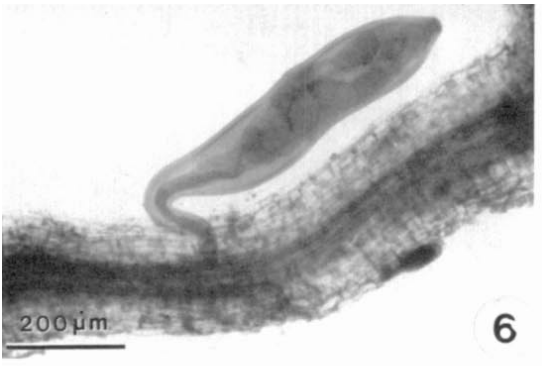
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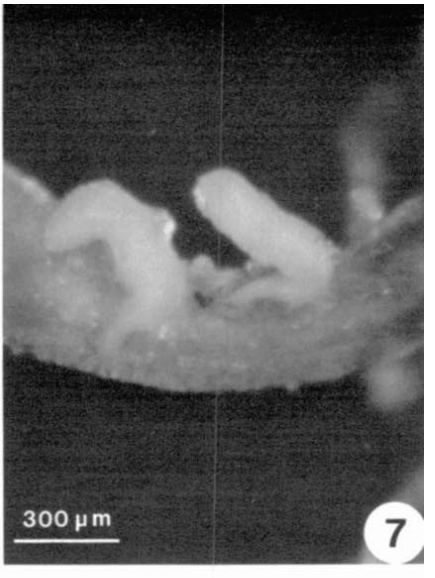
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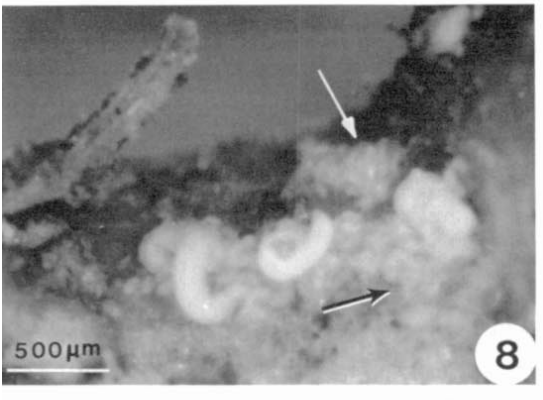
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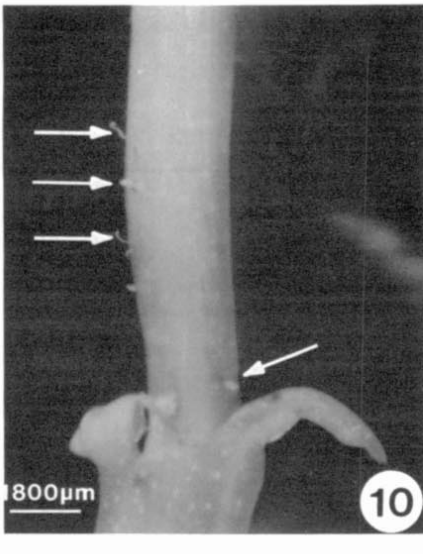
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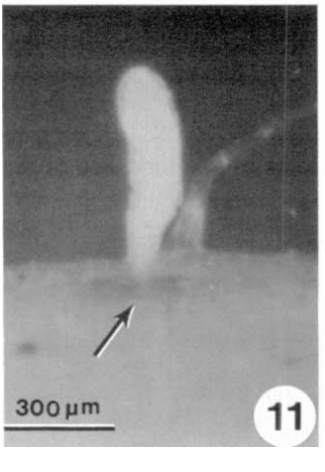
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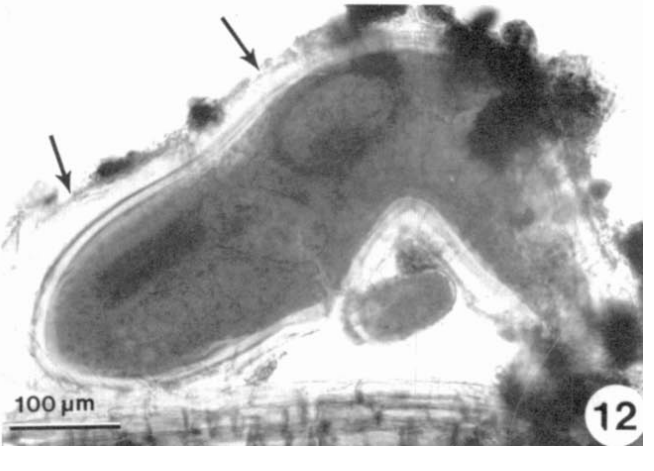
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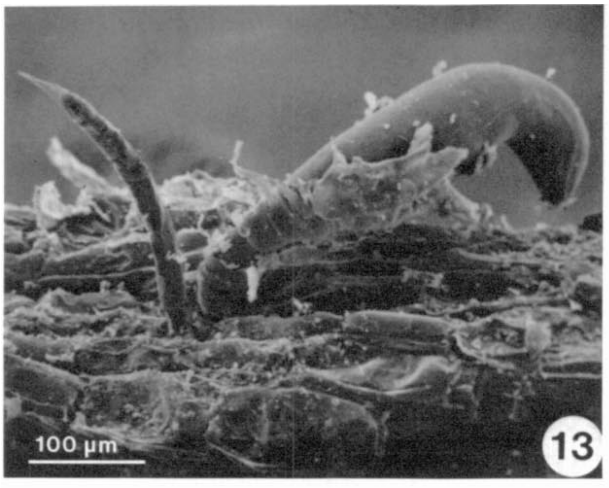
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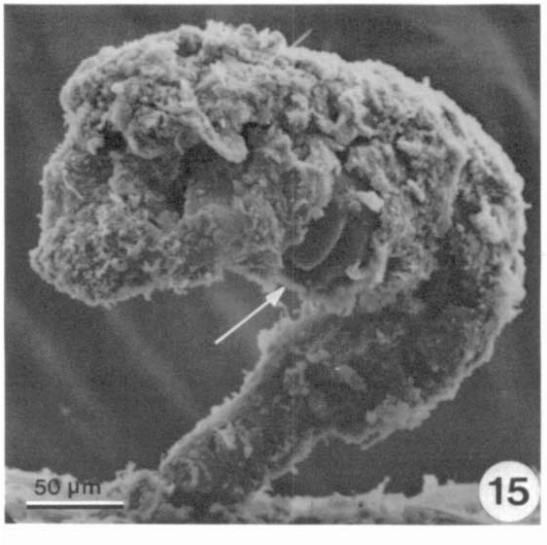
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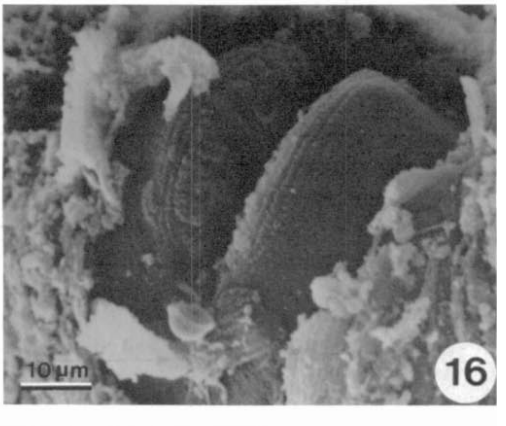
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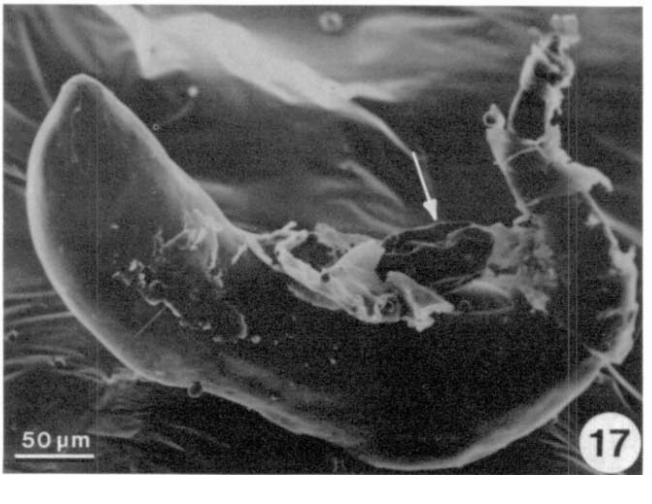
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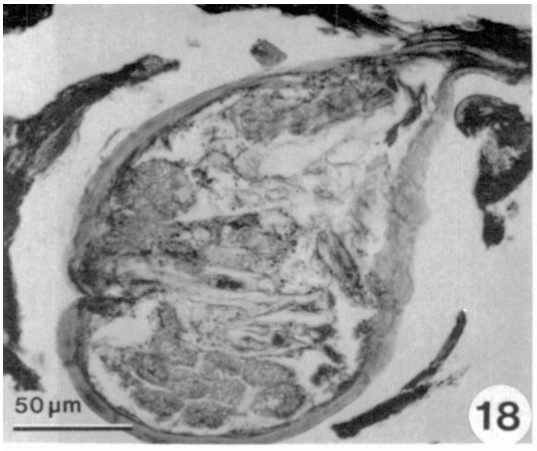
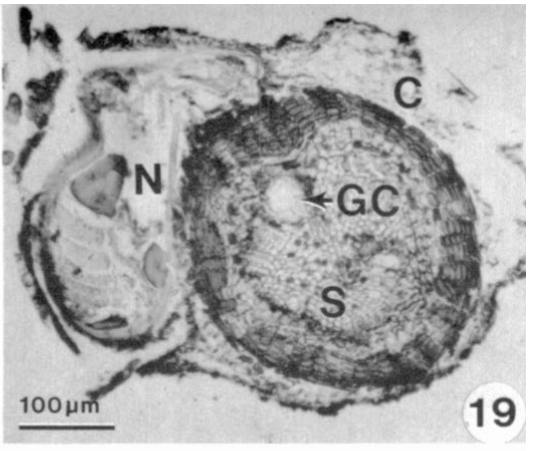
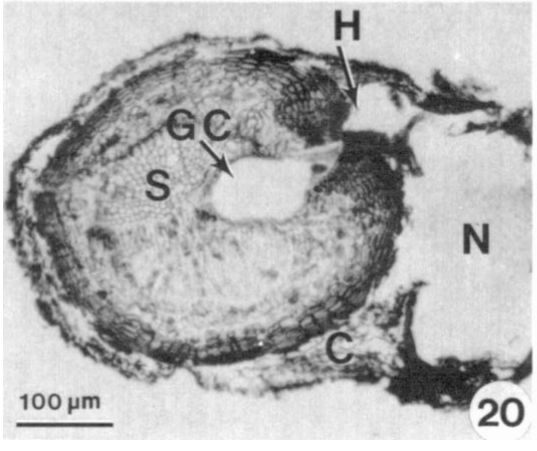
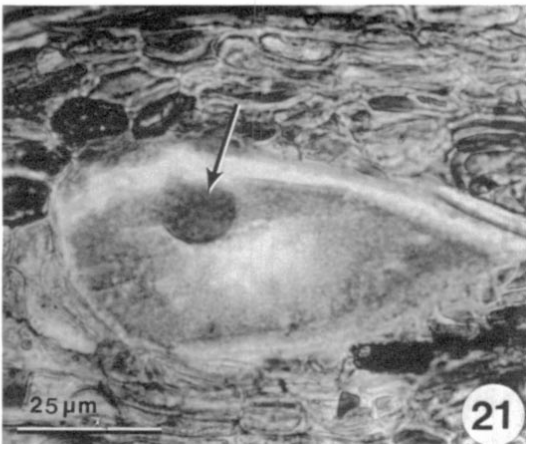



FIGS. 18–21. Cross sections of roots of *Pinus clausa* infected with *Meloidodera floridensis* (LM). 18) Adult female within root cortex. 19, 20) Location of nematode site (N) in cortex (C), giant cell (GC) within the stele (S), and point of contact between nematode head (H) and giant cell. 21) Single uninucleate giant cell with dense cytoplasm and enlarged nucleus (arrow).

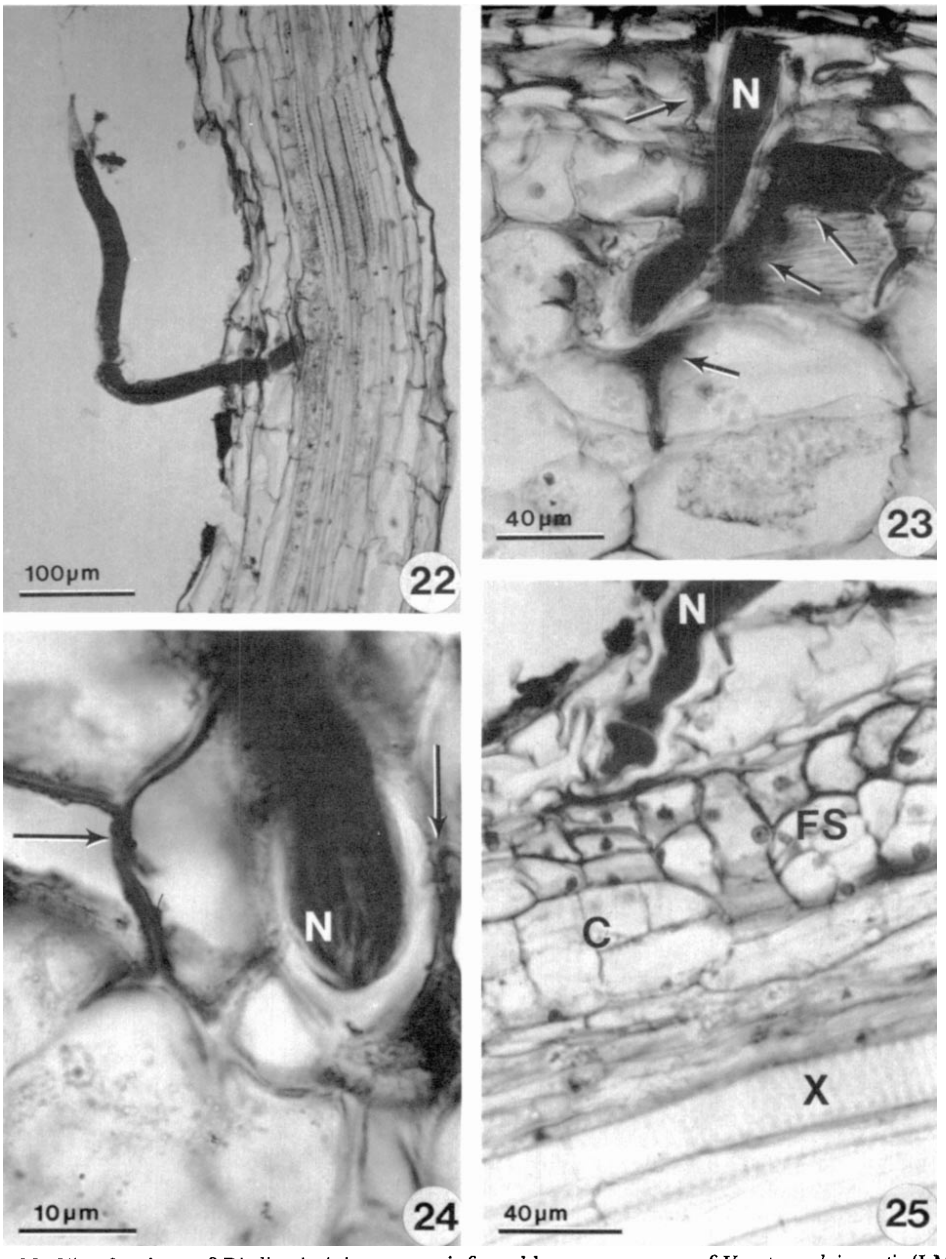
## MATERIALS AND METHODS

*Meloidodera floridensis* was collected from roots of sand pine (*Pinus clausa* [Chapm.] Vasey) and loblolly pine (*P. taeda* L.) located in Sanlando Park, Altamonte Springs, Florida, and from roots of loblolly and slash pine (*P. elliottii* Engelm.) collected in Gainesville, Florida. Buttonweed infected by *Verutus volvingentis* was collected near Clermont, Florida.

Living nematode-infected plant material was fixed in 10% alcoholic formalin (1:9, formalin:95% EtOH, v/v) or stained for 1 minute with hot acid fuchsin in lactophenol and destained with clear lactophenol. Selected stained roots were hand sectioned to observe the location of nematode feeding sites within plant tissues. For histological examination, segments of *M. floridensis*-infected roots of sand pine and *V. volvingentis*-infected roots of buttonweed were fixed as previously described, dehydrated through a tert-butyl alcohol series, and embedded in paraffin. Serial sections (12 µm) were stained with safranin–fast green and photographed using a light microscope.

Scanning electron microscope (SEM) observations were made on nematode-infected root segments fixed in 3.5% glutaraldehyde in 0.2 M 2,4,6-collidine buffer (pH 7.5), postfixed in 1% osmium tetroxide, dehydrated with acetone, critical point dried, mounted on stubs, and coated with carbon. The specimens were viewed and photographed with a JEOL JSM-35 scanning microscope.

To determine if *V. volvingentis* could parasitize citrus, five rough lemon (*Citrus limon* [L.] Burm. f.), five 'Flying Dragon' trifo-

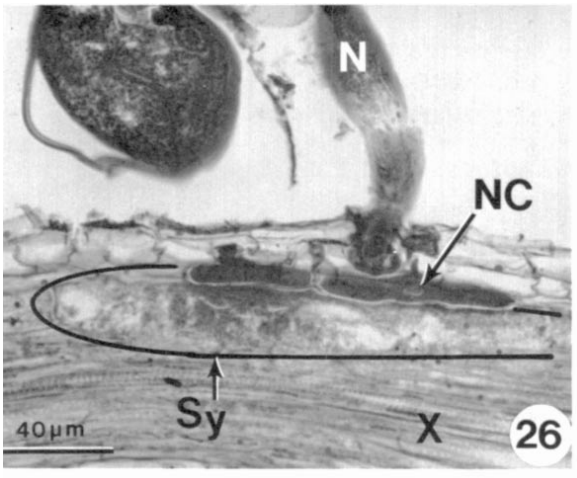


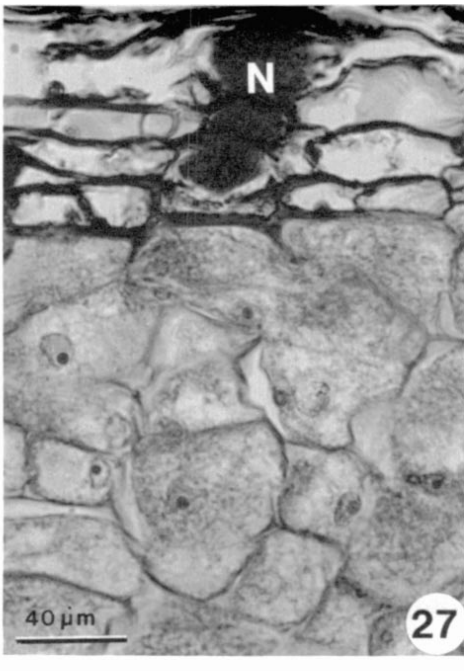

FIGS. 22–25. Sections of *Diodia virginiana* roots infected by young stages of *Verutus volvingentis* (LM). 22) Infective juvenile in longitudinal section of thin root. 23) Juvenile penetrating cortical parenchyma of thick root (cross section) showing necrosis of adjacent cells (arrows). 24) Nematode head (N) positioned in empty cortical cells. Note deeply stained cell walls (arrows). 25) Feeding site (FS) established by a young female (N) within cortex (C). X = xylem.

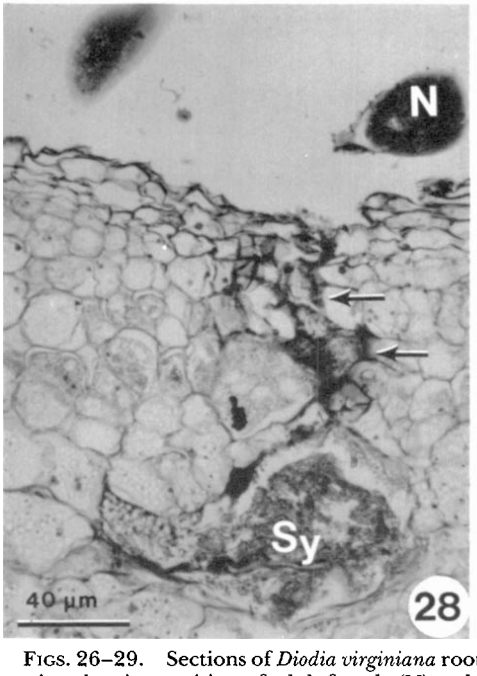
liate orange (*Poncirus trifoliata* [L.] Raf.) seedlings, and five nematode-free rooted cuttings of buttonweed were grown for 16 weeks in a glasshouse in 1-liter clay pots containing nematode-infested soil. Plants were harvested and roots examined at 31.5–50.0 × for adult females and for egg masses.

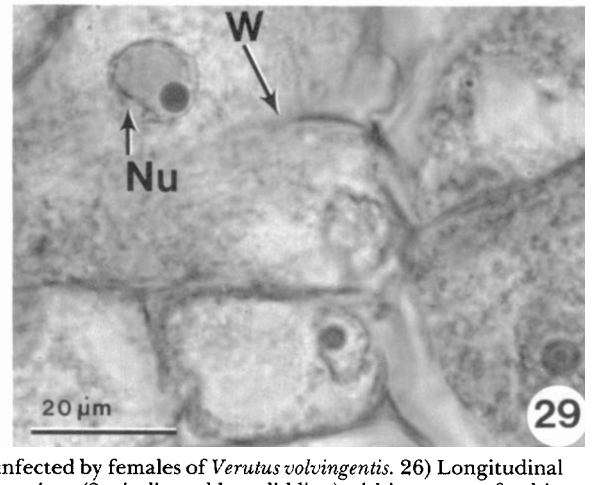
## RESULTS AND DISCUSSION

*Observations on root surfaces:* Isolated *M. floridensis* females occurred on roots of all three *Pinus* spp. Young females, devoid of uterine eggs, were white and smooth coated (Figs. 1, 3), whereas older, egg-bearing females in the cystoid stage were yellowish and appeared soft and wrinkled at 31.5 ×




FIGS. 26–29. Sections of *Diodia virginiana* roots infected by females of *Verutus volvingentis*. 26) Longitudinal section showing position of adult female (N) and syncytium (Sy, indicated by solid line) within cortex of a thin root. Note necrotic cells (NC) near root surface. X = xylem. 27) Large feeding site of young female (N) in cross section of a thick root. Feeding site is comprised of cortical parenchyma cells with dense cytoplasm and enlarged nuclei and nucleoli. The cells are primarily discrete. 28) Adult female (N) in cross section of thick root showing syncytium (Sy) formed within cortex. Note necrosis along path of nematode penetration (arrows). 29) Enlargement of part of Figure 27 showing limited cell wall breakdown (W) and nuclear and nucleolar hypertrophy (Nu).

(Figs. 2, 4). In addition to this color change, SEM observations indicated that weakening of the cuticular striation was related to aging. The subcrystalline layer was also shed (Figs. 3, 5). Hence, while genuine cysts were not observed, it appears that transformation of the female into the cystoid body involves some structural changes in the cuticle, as in other cyst and cystoid-forming nematodes (7).

The infective stage of *V. volvingentis* was an early vermiform juvenile with a pointed tail tip (Figs. 13, 22). The nematodes infected fine roots (Figs. 6, 7), thick fleshy roots (Fig. 8), rhizomes (Fig. 9), and even lower stem parts (Fig. 10). Root penetra-

tion was relatively superficial and, generally, more than two-thirds of the nematode body protruded from the root surface (Fig. 6). Vermiform juveniles appeared to penetrate the tissue intracellularly, and infection sites were often necrotic (Fig. 11). Young nematodes were firmly positioned in roots, but as they enlarged with age they became more easily dislodged from the root tissue.

Superimposed cuticles were observed in many juvenile and adult specimens (Fig. 12). A subcrystalline layer, which was usually sloughing off, covered most of the adult female body (Figs. 13, 15). Soil particles, debris, and eggs often adhered to this layer (Figs. 12, 15, 17). In most specimens the vulva could be seen protruding through this layer, and in some the prolapsed vaginal tissue was observed (Fig. 16). Occasionally, specimens nearly lacking a subcrystalline layer were encountered (Figs. 6, 14). As previously reported (4), the nematodes usually occurred in colonies where sticky excretus covered parts of the root surface to which clusters of singly laid eggs were attached (Fig. 8). This would appear to be a unique mechanism for maintaining eggs on the rhizoplane. Many females were observed with up to three eggs adhering to their bodies in the ventrally curved vulval region (Figs. 12, 17). Males were not observed feeding on roots in this or in previous studies (6).

*Histopathology:* Each *M. floridensis* female, generally located in the root cortex of sand pine, produced a single giant cell in the stelar parenchyma region (Figs. 19, 20). The giant cell often contained dense cytoplasm, a markedly hypertrophied nucleus and nucleolus, and a thick cell wall (Fig. 21). Walls were thicker in areas proximal to the nematode head. The wall of the giant cell where the nematode stylet made contact was slightly indented.

Our observations of the host–parasite relationship between *M. floridensis* and *P. clausa* are similar to those described for this nematode in loblolly pine (14) and in loblolly and slash pine (15). Thus, our findings support the suggestion of Mundo-Ocampo and Baldwin (14) that the nematode feeding site in Ruehle's illustrations (15) is not properly identified.

In general size and structure, the uninucleate giant cell induced in pine by *M. floridensis* resembles the uninucleate giant cells associated with *Hylonema ivorense* (17) and *Sarisodera hydrophila* (13). It is considerably different from the giant cell created by *Rotylenchulus macrodoratus,* which is larger and contains a more amoeboid, lobed nucleus and has prominent wall ingrowths (2).

Plant response to parasitism by *V. volvingentis* in *D. virginiana* was markedly different from that of *M. floridensis* in *P. clausa.* Infective juveniles of *V. volvingentis* ruptured many cells during penetration of buttonweed roots, causing necrosis in both penetrated cells and cells adjacent to the path of penetration (Figs. 23, 28). The nematodes penetrated the cortex in fine and fleshy roots, inducing a permanent feeding site composed of many cortical parenchyma cells (Figs. 25, 27). These cells were similar in size to normal parenchyma cells, but they contained dense cytoplasm and enlarged nuclei and nucleoli as described elsewhere (6) (Figs. 27, 29). The walls of some affected cells were slightly thickened and stained positively with safranin, particularly near the nematode head which was situated within an empty, thick-walled cortical cell (Fig. 24). In older feeding sites some cell wall disintegration was observed (Fig. 29), resulting in well-defined, multinucleate syncytia (Figs. 26, 28) being associated with adult females.

One interesting aspect of this nematode–plant relationship is that the syncytium is formed entirely within the root cortex. This is in contrast to the location of syncytia formed by other heteroderid nematodes whose feeding sites occur primarily in the stele (12). No feeding sites of *V. volvingentis* were observed within the stele of thin or thick roots. The lack of enlargement of cells within the feeding site is reminiscent of the feeding site "nurse cells" induced by *Tylenchulus semipenetrans* (8), another sedentary cortical parasite. However, the *Verutus*-induced cells fuse at a later stage to form a syncytium.

*Host range:* Neither of the two rutaceous plant species (*C. limon* or *P. trifoliata*) supported any life stages of *V. volvingentis* in their roots, while roots of *D. virginiana* cuttings in similar tests were heavily infected. Citrus apparently is not a host, which supports the proposal by Esser (5) that *V. volvingentis* is highly host specific.

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