Histopathology of Pinus ponderosa Ectomycorrhizae Infected with a Meloidogyne Species

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ABSTRACT

An undescribed *Meloidogyne* sp. was found infecting ectomycorrhizae of mature *Pinus ponderosa* in southwestern New Mexico in 1963. Nematode larvae penetrated the ectomycorrhizae, migrated to the stelar region, and developed into adults with their heads embedded in vascular tissues. As the female developed, cortical cells and the associated Hartig net at and near its body were compressed and collapsed. Fungal mantles were ruptured, and a gelatinous matrix containing many nematode eggs protruded from the mantle surface. Giant cells developed as a cluster of multinucleate cells in the vascular tissues immediately adjacent to the head of the nematode. Cytoplasm of actively functioning giant cells was dense, very granular in texture, and contained greatly enlarged nuclei with irregularly lobed membranes. As the giant cells became senescent, their cytoplasm became highly vacuolated, deteriorated, and cavities usually appeared in vascular tissues originally occupied by the giant cells. Xylem tracheids in the immediate vicinity of the giant cells were distorted, crushed, and even scattered in irregular isolated patches.

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Additional key words: root-knot nematode, forest nematology, host-parasite relationships, ponderosa pine.

Root-knot nematodes, genus *Meloidogyne*, are distributed worldwide and are probably the best known of the plant-parasitic nematodes. Many tree species are susceptible to these endoparasites. A review of the literature revealed four or more *Meloidogyne* spp. associated with the roots of 14 *Pinus* spp. (Table 1). Most of these reports do not indicate whether the nematodes completed their life cycle and reproduced on the trees. However, two papers reported *Meloidogyne* parasitizing and damaging ectomycorrhizae of *Pinus*. Riffle & Lucht (23) found an undescribed *Meloidogyne* species parasitizing ectomycorrhizae of mature *P. ponderosa* Laws. growing in southwestern New Mexico, and

recovered all stages of the nematode from infected ectomycorrhizae. Donaldson (9) found gravid *M. javanica* (Treub) Chitwood in swollen ectomycorrhizae of *P. elliottii* Engelm. var. *elliottii* seedlings in Florida. Since the effects of *Meloidogyne* on *Pinus* mycorrhizae are not well known, a histopathological study was undertaken to determine the effects of a *Meloidogyne* sp. (undescribed according to Gerald Thorne, *personal communication*, 27 November 1963) on the anatomy of *P. ponderosa* growing in natural forest stands.

MATERIALS AND METHODS.–*Pinus ponderosa* ectomycorrhizae and rhizosphere soil were collected from the Gila National Forest in southwestern New Mexico in November and December 1963, December 1964, October 1965, and May 1966. The mycorrhizae were washed in a gentle spray of tap water to remove adhering organic matter and soil, and carefully examined using a dissecting microscope. Nematode-infected and noninfected root pieces about 1 to 4 cm long were killed in FAA fixative, dehydrated in tertiary butyl alcohol, embedded in paraffin, sectioned at 12 μ on a rotary microtome, and stained with safranin and fast green (13). Additional root segments infected with nematodes were stained in lactophenol-acid fuchsin and mounted in glycerin (18). The centrifugal flotation technique (6) was used to recover nematodes from the rhizosphere soil.

RESULTS.–Ectomycorrhizae found on the root systems of *P. ponderosa* were unbranched, dichotomously branched once or twice (Fig. 1), or coralloid types formed with unknown fungal symbionts or *Cenococcum graniforme* (Sow.) Ferd. & Winge. The fungal mantle of the ectomycorrhizae consisted of a compact layer of tightly interwoven mycelia ranging in thickness from 16 to 70 μ (Fig. 2, 3). One layer of compressed and heavily stained cortical cells (tannin layer) was located immediately beneath the mantle (Fig. 2, 3). Cells of the outer two or three tiers of turgid cortex were surrounded by the Hartig net (Fig. 2, 3).

Second-stage *Meloidogyne* larvae were found in rhizosphere soil associated with various types of mycorrhizae, and in undifferentiated tissues of meristematic root apices of many nonmycorrhizal and mycorrhizal roots (Fig. 4). The larvae apparently penetrated the fungal mantle (Fig. 5) at or near the meristematic root apex, or at tissues ruptured by emergence of secondary roots. No cell necrosis was observed in areas where nematodes penetrated.

Larvae migrated through undifferentiated tissues in the meristematic root apices (Fig. 4), or through cortical parenchyma and became established with their heads buried in the vascular tissues (Fig. 6). The body of the female increased in width and length, became obese and developed a protruding neck region (Fig. 6). The posterior end of mature females was located near (Fig. 6) or protruded from the mantle (Fig. 7, 8). Mature females deposited eggs in a gelatinous egg sac that surrounded their posterior ends (Fig. 8, 9). Sand granules and organic matter readily adhered to the gelatinous material causing it to become crusty in appearance.

Adult male nematodes were commonly found in the gelatinous material in association with the egg masses. They also occurred in rhizosphere soil, and in a few instances were found in undifferentiated meristematic tissues of the mycorrhizae.

Feeding and subsequent development of the females caused many abnormalities in the external morphology of the ectomycorrhizae. Many mycorrhizae were markedly distorted and thickened (Fig. 10) as a result of hypertrophy and hyperplasia of cortical and vascular tissues and swelling of females. Some nematode-infected mycorrhizal apices were swollen into spherical galls (Fig. 11). Swollen mycorrhizae usually branched prolifically (Fig. 12).

Internal anatomy of the ectomycorrhizae was also severely altered by developing females. The most notable abnormality was the occurrence of giant cells that were found in the vascular cylinder (Fig. 6, 13, 14, 15) and in undifferentiated tissues of root apices, but not in the cortex. The giant cells developed in clusters near or immediately adjacent to the lip region of the sedentary females. Three to nine, but more commonly four to five, giant cells occurred around a single developing female (Fig. 13, 14). These cells were thick-walled (Fig. 15), somewhat circular or ellipsoidal in cross section (Fig. 15), elongate or fusiform in longitudinal section (Fig. 16), and were

TABLE 1. Reported *Meloidogyne - Pinus* associations

Nematode species	Associated with	Literature citation
Meloidogyne arenaria	*Pinus elliottii* var. *elliottii*	27
	Pinus sp.	11
	Pinus taeda	27
Meloidogyne incognita acrita	*Pinus densiflora*	10
	Pinus sp.	11
	Pinus thunbergii	10
Meloidogyne javanica	*Pinus elliottii* var. *elliottii*	9
Meloidogyne sp.	*Pinus densiflora*	14
	Pinus echinata	25, 26
	Pinus elliottii var. *elliottii*	5, 7, 26
	Pinus lambertiana[a]	31
	Pinus palustris	5, 7
	Pinus ponderosa	23
	Pinus radiata	7, 30
	Pinus resinosa	29
	Pinus rigida	15
	Pinus spp.	1, 4, 7, 11, 19
	Pinus strobus	15
	Pinus sylvestris	29
	Pinus taeda	28
	Pinus thunbergii	14

[a] Tyler (31, page 54) reports unpublished data in files of Division of Nematology that H. N. Hanson in 1939 collected *P. lambertiana* seedlings heavily infected with *Meloidogyne* growing on a garbage dump at 6,000 elevation in Tuolumne County, California.

approximately two to six times larger than normal cells.

Cytoplasm of newly formed and actively functioning giant cells was dense and very granular (Fig. 17), whereas cytoplasm of older cells was vacuolated (Fig. 18). Cytoplasm deteriorated in senescent giant cells leaving only cavities (Fig. 19).

The giant cells were multinucleate. The nuclei of these cells, about twice as large as those of unaffected cells, were clustered in groups (Fig. 20), had irregularly lobed membranes (Fig. 17, 21), and contained many enlarged nucleoli and large amounts of chromatin. The nucleoli were stained bright red by the safranin. Nuclei in parenchyma around the giant cells were nearly 1.5 times larger than nuclei in normal cells. In highly vacuolated giant cells, the nuclear membranes were concave and deteriorated (Fig. 18, 22).

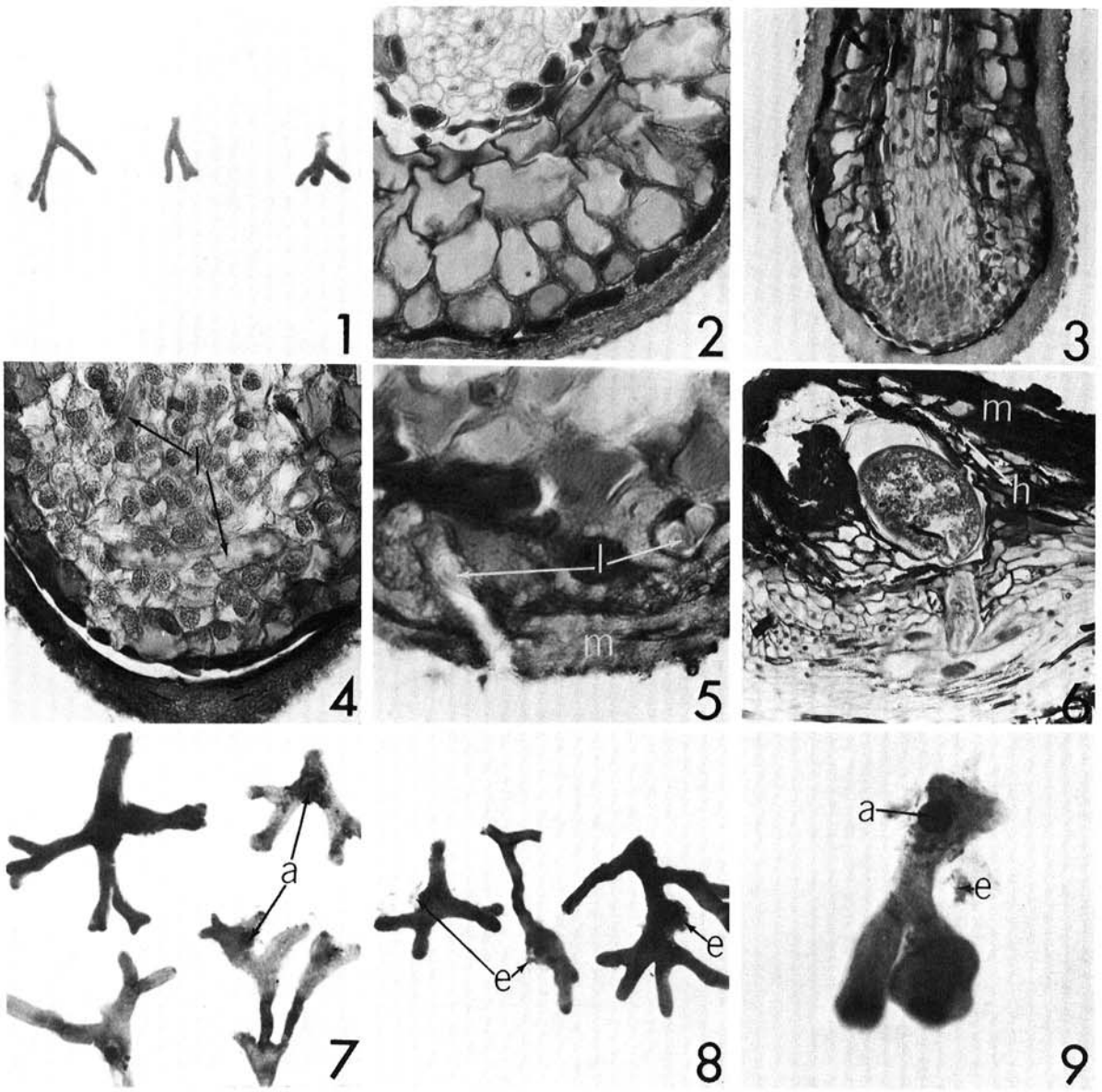

Fig. 1-9. Noninfected and *Meloidogyne*-infected *Pinus ponderosa* ectomycorrhizae. 1 = *Meloidogyne* larvae; m = fungal mantle; h = Hartig net; a = *Meloidogyne* adult; and e = nematode egg mass. **1)** Noninfected ectomycorrhizae (×4). **2)** Cross section showing fungal mantle, Hartig net, cortex, and stele (×242). **3)** Longitudinal section showing fungal mantle, Hartig net, cortex, and stele (×95). **4)** Longitudinal section showing location of *Meloidogyne* larvae in undifferentiated meristematic tissues (×240). **5)** Penetration of fungal mantle by *Meloidogyne* larvae (×372). **6)** *Meloidogyne* female with neck region protruding into vascular tissues (×97). **7, 8)** Ectomycorrhizae dichotomously branched once or twice with ruptured mantles and *Meloidogyne* adults visible from mycorrhizal surfaces (×7). **9)** *Meloidogyne* adult and egg mass protruding from ectomycorrhizae (×14).

Vascular tissues were disorganized in the immediate vicinity of the giant cells. Xylem tracheids around the periphery of these cells were distorted, collapsed, or ruptured (Fig. 23, 24). In some root sections, the vascular tissues were dislodged and scattered in irregular patches, causing a discontinuity in the vascular column.

Hypertrophy and hyperplasia occurred in cortical and vascular parenchyma, and was most notable in areas immediately adjacent to or near giant cells.

Development of the female body compressed and collapsed the surrounding cortical parenchyma with the associated Hartig net (Fig. 6, 25, 26), and, in some roots, stretched and ruptured the fungal mantle (Fig. 6, 26, 27).

DISCUSSION.—This is the first report on the histopathology of a *Meloidogyne* species on a *Pinus* species (22). One reason for a dearth of investigations

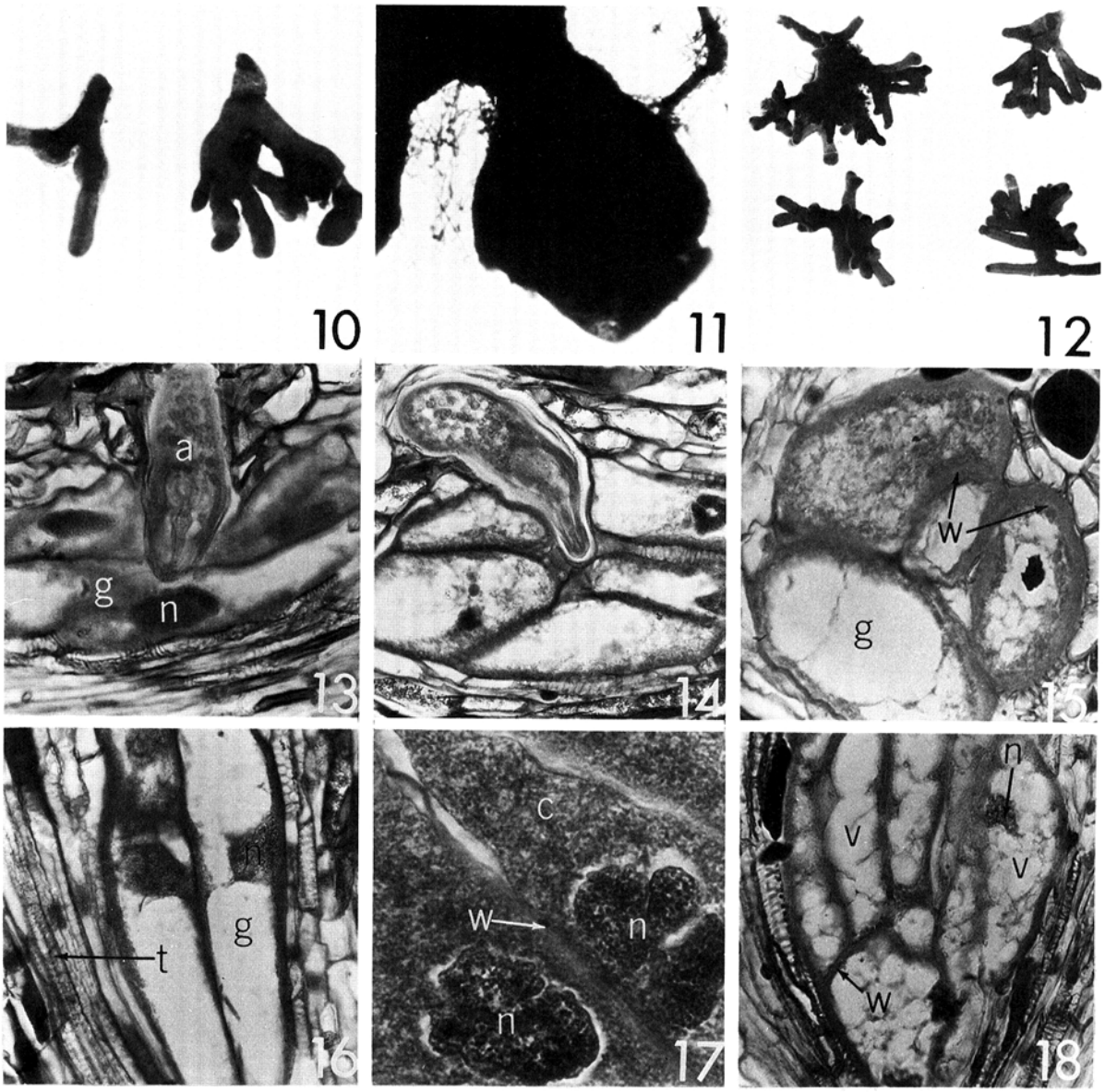

Fig. 10-18. *Meloidogyne*-infected *Pinus ponderosa* ectomycorrhizae. g = giant cell; a = *Meloidogyne* adult; n = cell nuclei; w = cell walls of giant cell; t = xylem tracheids; c = giant cell cytoplasm; and v = vacuoles in giant cell cytoplasm. **10)** Distortion and hypertrophy of ectomycorrhizae (×8). **11)** Spherical gall of *Cenococcum graniforme* ectomycorrhiza (×39). **12)** Proliferation of lateral branching of ectomycorrhizae caused by nematode infection (×6). **13, 14)** Giant cells immediately adjacent to lip region of sedentary nematodes (×234). **15)** Circular to oblong giant cells in cross section, showing thick cell walls (×240). **16)** Thick-walled elongate giant cells as seen in longitudinal section (×234). **17)** Granular cytoplasm and lobed nuclei in actively functioning giant cells (×528). **18)** Vacuolated cytoplasm and deteriorating nuclei of senescent giant cells (×232).

in this area is that many *Pinus* species appear to be resistant or immune to *Meloidogyne*. Hume (12) reported never having seen harmful *Meloidogyne* infestation on conifers, and even suggested that heavily infested nursery land may be freed of nematodes in 6 to 7 years by planting pine trees to shade the soil, to keep green weeds out, and to cover the ground densely with needles. Donaldson (9) examined past records of the Division of Plant Industry, Florida State Department of Agriculture, and found eight samples from which *Meloidogyne* larvae were recovered from *P. elliottii* var. *elliottii*, but in no case were mature *Meloidogyne* females recorded as having been seen or dissected from the

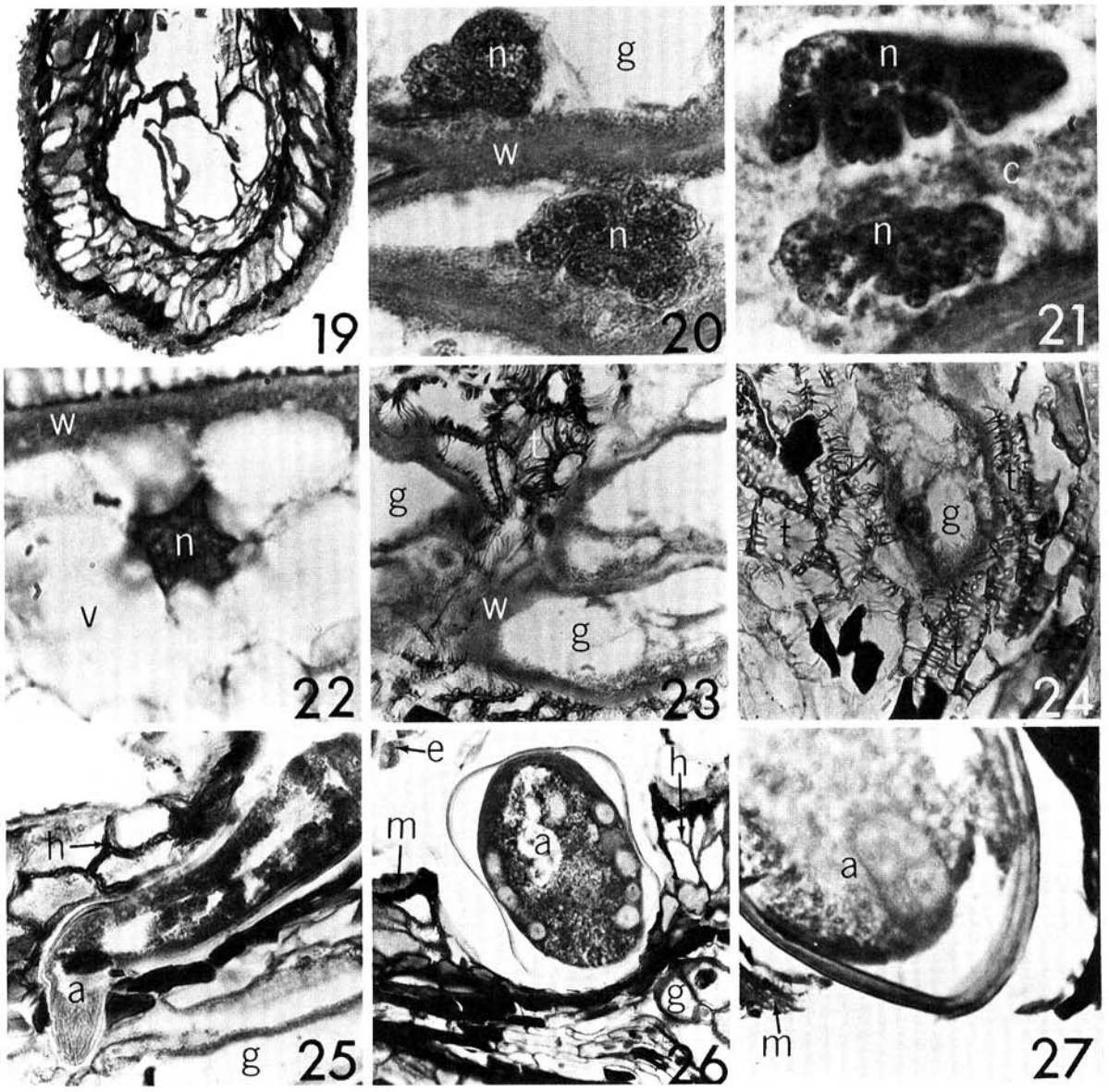

Fig. 19-27. *Meloidogyne*-infected *Pinus ponderosa* ectomycorrhizae. g = giant cell; n = cell nuclei; w = cell walls of giant cells; c = giant cell cytoplasm; v = vacuoles in giant cell cytoplasm; t = xylem tracheids; h = Hartig net; m = fungal mantle; e = nematode egg mass; and a = *Meloidogyne* adult. **19)** Cavities in vascular tissue originally occupied by giant cells (×99). **20)** Giant cell nuclei clustered in groups (×433). **21)** Nuclei of actively functioning giant cells with irregularly lobed membranes (×935). **22)** Nuclei of vacuolated giant cells with concave membranes (×742). **23, 24)** Compression, distortion, and collapse of xylem tracheids in or immediately adjacent to giant cells (×244). **25)** Compression and collapse of cortical parenchyma and associated Hartig net by invading *Meloidogyne* female (×247). **26)** Rupture of fungal mantle and collapse of cortical parenchyma by developing *Meloidogyne* female (×96). **27)** Rupture of fungal mantle by fully developed *Meloidogyne* female (×238).

roots. Sutherland (*personal communication*, 16 September 1970) has often found *Meloidogyne* larvae in forest nurseries in British Columbia, but they do not appear to feed or reproduce on the conifers.

There are no reports in the literature establishing that a *Meloidogyne* sp. completed its life cycle on a pine species. In unreported greenhouse studies with 86 *Pinus ponderosa* seedlings in 21 pots, the *Meloidogyne* species investigated herein completed its life cycle and reproduced on five seedlings in one pot. In another greenhouse study, this nematode failed to reproduce on *P. edulis* Engelm., *P. flexilis* James, *Juniperus monosperma* (Engelm.) Sarg., *J. deppeana* Steud., *Picea engelmanni* Parry, *P. pungens* Engelm., *Abies concolor* (Gord. & Glend.) Lindl., and *Pseudotsuga menziesii* (Mirb.) Franco.

In unsuitable hosts, *Meloidogyne* spp. produce few or no mature females and no prominent galls on the roots (20). Differences in gall size in gardenia (*Gardenia jasminoides* Veitchi) infected with three different species of *Meloidogyne* was reported to be dependent upon the extent of cellular hypertrophy (8). Since hypertrophy and hyperplasia of *P. ponderosa* tissues occurred only in the vicinity of the giant cells, perhaps this localized swelling accounts for the absence of conspicuous galls characteristic of *Meloidogyne* infections of other plant species.

The irregularly lobed nuclear membranes and enlarged nuclei reported in this investigation appear to be common phenomena in giant cells in roots of several host plants (2, 21).

Roots morphologically altered by mycorrhizal symbionts apparently have little effect on giant cell development because the granular to vacuolated texture of *P. ponderosa* giant cell cytoplasm is similar to the cytoplasm in tomato giant cells. Changes in texture of this cytoplasm may be related to the stimulus received from invading nematodes. Bird (3), working with *M. javanica* in tomato and bean, found that giant cells under constant stimulation from the nematode had a dense and granular cytoplasm with distinct nuclei and no vacuoles. However, cytoplasm of giant cells in which the nematode had been removed became vacuolated, had indistinct and weakly stained nuclei, deteriorated, and was eventually encroached upon by the surrounding cells of the host.

The histopathology of *Meloidogyne* on *P. ponderosa* ectomycorrhizae is similar in many respects to the histopathology of *Meloidodera floridensis* Chitwood, Hannon, & Esser on *P. elliottii* var. *elliottii* or *P. taeda* L. mycorrhizae (24). Both of these endoparasites cause the formation of giant cells in undifferentiated tissue in meristematic root apices of mycorrhizae, and hypertrophy and hyperplasia of vascular and cortical parenchyma, but do not extensively gall the rootlets. Mycorrhizae infected with *Meloidogyne* can be distinguished by the fact that giant cells also form in vascular tissues but not in the cortical tissues, as is characteristic of *Meloidodera* infections (24).

The severe disorganization of conducting tissues adjacent to giant cells in roots of *P. ponderosa* probably impairs the translocation of water and nutrients. Such disorganization may be highly significant during prolonged periods of low available soil moisture and result in reduced tree vigor as well as growth increment.

It has been established that some ectomycorrhizae function as biological deterrents to pathogenic root infections (16). Marx & Davey (17) reported that fully formed, naturally occurring *Pinus echinata* Mill. ectomycorrhizae were resistant to infection by *Phytophthora cinnamomi* Rands, but those mycorrhizae with incomplete fungal mantles at the root meristem were infected. In addition, mycorrhizae with artificially exposed cortical tissues were resistant to infection by this pathogen, but zoospores were attracted to excised root tips with exposed vascular tissues. The *Meloidogyne* sp. reported herein severely altered the normal development of vascular tissues, ruptured the fungal mantle, and in some cases exposed vascular tissues. Such findings indicate that damage by *Meloidogyne* may alter the role of ectomycorrhizae as biological deterrents to pathogenic root infections.

LITERATURE CITED

1. ALEXANDER, P. M. 1963. Stylet-bearing nematodes associated with various plants in South Carolina, 1959-1962. Plant Dis. Reptr. 47:978-982.
2. BIRD, A. F. 1961. The ultrastructure and histochemistry of a nematode-induced giant cell. J. Biophys. Biochem. Cytol. 11:701-715.
3. BIRD, A. F. 1962. The inducement of giant cells by Meloidogyne javanica. Nematologica 8:1-10.
4. BROWN, J. G. 1948. Root-knot in Arizona. Ariz. Agric. Exp. Stn. Bull. 212. 40 p.
5. BUHRER, EDNA M. 1938. Additions to the list of plants attacked by the root-knot nematode (Heterodera marioni). Plant Dis. Reptr. 22:216-234.
6. CAVENESS, F. E., & H. J. JENSEN. 1955. Modification of centrifugal-flotation technique for isolation and concentration of nematodes and their eggs from soil and plant tissues. Proc. Helminthol. Soc. Wash. 22:87-89.
7. CESNIK, R. 1962. Nematodeos em essencias florestais. Revista Agric. 37:181-187.
8. DAVIS, R. A., & W. R. JENKINS. 1960. Histopathology of gardenia (Gardenia jasminoides Veitchi) infected with three species of Meloidogyne. Nematologica 5:228-230.
9. DONALDSON, F. S., JR. 1967. Meloidogyne javanica infesting Pinus elliottii seedlings in Florida. Plant Dis. Reptr. 51:455-456.
10. HASHIMOTO, H. 1962. Some observations on the damage caused by nematodes in the forest nurseries in Fukuoka Prefecture. J. Jap. For. Soc. 44:248-252.
11. HOPPER, B. E. 1958. Plant-parasitic nematodes in the soil of southern forest nurseries. Plant Dis. Reptr. 42:308-314.
12. HUME, H. H. 1937. Special discussion of nematode hosts. p. 118. *In* H. P. Barss, S. A. Wingard, E. M. Buhrer, G. Steiner, & J. Tyler [ed.]. Proceedings of the root-knot conference. Plant Dis. Reptr. Suppl. 102:97-122.
13. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co., Inc., New York. 522 p.
14. KIYOHARA, T. 1970. Distribution of plant parasitic nematodes associated with coniferous seedlings in

Kyúshú, Japan. Bull. Gov. For. Exp. Stn. No. 232:1-12.

15. MAI, W. F., H. W. CRITTENDEN, & W. R. JENKINS. 1960. Distribution of stylet-bearing nematodes in the Northeastern United States. N. J. Agric. Exp. Stn. Bull. 795. 62 p.

16. MARX, D. H. 1971. Ectomycorrhizae as biological deterrent to pathogenic root infections. p. 81-96. *In* E. Hacskaylo [ed.]. Mycorrhizae. First North American Conference on Mycorrhizae Proceedings, April 1969, U.S. Dep. Agric., Forest Serv., Misc. Publ. 1189. 255 p.

17. MARX, D. H., & C. B. DAVEY. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. IV. Resistance of naturally occurring mycorrhizae to infections by Phytophthora cinnamomi. Phytopathology 59:559-565.

18. MC BETH, C. W., A. L. TAYLOR, & A. L. SMITH. 1941. Note on staining nematodes in root tissues. Proc. Helminthol. Soc. Wash. 8:26.

19. MINTON, N. A., E. J. CAIRNS, E. B. MINTON, & B. E. HOPPER. 1963. Occurrence of plant-parasitic nematodes in Alabama. Plant Dis. Reptr. 47:743-745.

20. NEMEĆ, S., & L. S. MORRISON. 1972. Histopathology of Thuja orientalis and Juniperus horizontalis plumosa infected with Meloidogyne incognita. J. Nematol. 4:72-74.

21. PAULSON, R. E., & J. M. WEBSTER. 1970. Giant cell formation in tomato roots caused by Meloidogyne incognita and Meloidogyne hapla (Nematoda) infection. A light and electron microscope study. Can. J. Bot. 48:271-276.

22. RIFFLE, J. W. 1972. Histopathology of Pinus ponderosa ectomycorrhizae infected with a Meloidogyne species. Phytopathology 62:785 (Abstr.).

23. RIFFLE, J. W., & D. D. LUCHT. 1966. Root-knot nematode on ponderosa pine in New Mexico. Plant Dis. Reptr. 50:126.

24. RUEHLE, J. L. 1962. Histopathological studies of pine roots infected with lance and pine cystoid nematodes. Phytopathology 52:68-71.

25. RUEHLE, J. L. 1962. Plant-parasitic nematodes associated with shortleaf pine showing symptoms of littleleaf. Plant Dis. Reptr. 46:710-711.

26. RUEHLE, J. L. 1964. Plant-parasitic nematodes associated with pine species in southern forests. Plant Dis. Reptr. 48:60-61.

27. RUEHLE, J. L., & J. N. SASSER. 1962. The role of plant-parasitic nematodes in stunting of pines in southern plantations. Phytopathology 52:56-68.

28. SOUTHERN COOPERATIVE SERVICE BULLETIN. 1960. Distribution of plant-parasitic nematodes in the South. Reg. Proj. S-19, No. 74. 72 p.

29. SUTHERLAND, J. R. 1965. Nematodes associated with coniferous nursery seedlings in Quebec. Phytoprotection 46:131-134.

30. TURICA, A. 1951. Comprabación de un ataque de Heterodera marioni Goody a sauceálamos del delta. Idia (Buenos Aires) 4:5-6.

31. TYLER, JOCELYN. 1941. Plants reported resistant or tolerant to root-knot nematode infestation. U.S. Dep. Agric. Misc. Publ. 406. 91 p.