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Histopathology and Host Range Studies of the Redwood Nematode *Rhizonema sequoiae*[1]

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Abstract: Second-stage larvae of *Rhizonema sequoiae* Cid del Prado Vera et al. tunnel through the cortex of the redwood *Sequoia sempervirens* (D. Don) Endl. root to the vascular tissue where each developing female induces a single ovoid or occasionally spherical giant cell with a single ovoid to spherical nucleus containing one to four enlarged nucleoli. Nematode tunnels are filled with a gel material and often contain second-stage larvae and males. There is tissue necrosis around females, and cortical tissue is destroyed after infection by many second-stage larvae. *R. sequoiae* females developed to maturity on *S. sempervirens, Acer macrophyllum* Pursh, *Alnus rhombifolia* Nutt., *Libocedrus decurrens* Torr, *Pseudotsuga menziesii* (Mirb.) Franco, and *Sequoiadendron giganteum* (Lindl.) Decne. In the Marin County, California, forest mature females were also found naturally infecting *Lithocarpus densiflorus* (Hook & Arn.) Rehd., *Umbellularia californica* (Hook & Arn.) Nutt., and *Arbutus menziesii* Pursh.

Key words: Rhizonema, pathology, host range.

Vascular alterations and cellular responses caused by *Heterodera* spp. have been reviewed by Endo (4,5). Damage involves the direct penetration by the second-stage larvae, the formation of galleries in the cortex, and the initiation of giant cells in the vascular tissue. There is evidence that this histopathology is a prerequisite to nematode reproduction (1,3,7). Host histopathology has also been described for species in some other genera of the Heteroderidae. *Meloidodera floridensis* induces giant cell formation associated with mycorrhizae in pine root tips, but the nuclear

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TABLE 1. Extent of development of *Rhizonema sequoiae* in roots of woody plants native to California. Plants were examined 8 months after inoculation with 1,000 second-stage larvae.

Plant	Total number of nematodes recovered from three root systems			
	3rd stage	4th stage	Adult male	Adult female
Abies concolor white fir	2	0	1	0
Abies magnifica red fir	0	0	0	0
Acer macrophyllum big-leaf maple	3	0	1	1
Aesculus californica California buckeye	0	0	0	0
Alnus rhombifolia white alder	13	0	30	9
Arbutus menziesii madrone	0	0	0	0
Baccharis pilularis coyote bush	0	0	0	0
Cercis occidentalis California redbud	0	0	0	0
Libocedris decurrens incense cedar	0	0	0	7
Lithocarpus densiflorus tanbark oak	0	0	0	0
Pinus ponderosa ponderosa pine	0	0	0	0
Photinia arbutifolia toyon	0	0	0	0
Pseudotsuga menziesii douglas fir	5	0	0	2
Quercus dumosa scrub oak	0	0	0	0
Salix lasiolepis arroyo willow	1	0	0	0
Sequoia sempervirens coast redwood	1	0	1	16
Sequoiadendron giganteum giant sequoia	0	0	2	3
Umbellularia californica California bay	0	0	0	0

condition of these giant cells has not been reported (10). *Hylonema ivorense* induces a uninucleate giant cell in roots of *Turraeanthus africana* Pellegr (9). *Sarisodera hydrophila* also induces a uninucleate hypertrophied giant cell with a hypertrophied nucleus.

This paper describes the histopathology of *R. sequoiae* on Coast Redwood (*Sequoia sempervirens* (D. Don) Endl.) and reports the host suitability of other plants in the redwood ecosystem.

MATERIALS AND METHODS

Histological effects: Roots of Coast Redwood infected with *R. sequoiae* were collected near Lake Lagunitas, Marin County Water District, California. Secondary roots were cut and fixed in FAA for 7 days, dehydrated in an ethanol : butanol : distilled water series (25:15:60; 30:25:45; 30:40:30; 25:55:20; 20:70:10; 15:85:0; 100 cc), 2 hours each, embedded in Paraplast tissue-embedding medium, sectioned at 12 μm, stained with safranin and fast green, and photographed with a photomicroscope.

Host suitability: Seventeen native California trees and one native California shrub (Table 1) were inoculated with 1,000 second-stage larvae of *R. sequoiae* and placed in a lathhouse at Davis, California. There were three replicates for each plant species. Eight months later the roots were collected, macerated in 200 cc of water in a blender for 2 minutes, and the resulting suspension was poured through a series of sieves with openings of 0.83, 0.25, 0.15, 0.07, and 0.04 mm (20, 60, 100, 200, and 325 meshes/inch). *R. sequoiae* stages were picked from the contents of the four finer sieves using a stereoscopic microscope. Nematodes present in the soil were extracted using the centrifugal-flotation technique of Jenkins (6).

RESULTS

Histological study: Single giant cells were found in the parenchyma of the xylem and phloem of the vascular tissue of Coast Redwood (Fig. 1A, B) near the heads of the females of *R. sequoiae.* These giant cells were swollen to an ovoid shape (Fig. 1C–F), 231 (89–460) μm in length and 61 (37–105) μm in width, and each had one large nucleus, a variable number of nucleoli, and dense cytoplasm. The large nuclei were usually elongate in shape, less commonly amoeboid or nearly spherical, 43 (21–95) μm long and 24 (11–45) μm wide (Fig. 1C–E). The enlarged nucleoli were one to four in number, 12 (5–21) μm in length, and 8 (5–18) μm in width. Normal cells adjacent to the giant cell were 41 (39–229) μm long and 9 (11–26) μm wide (Fig. 1F). Their

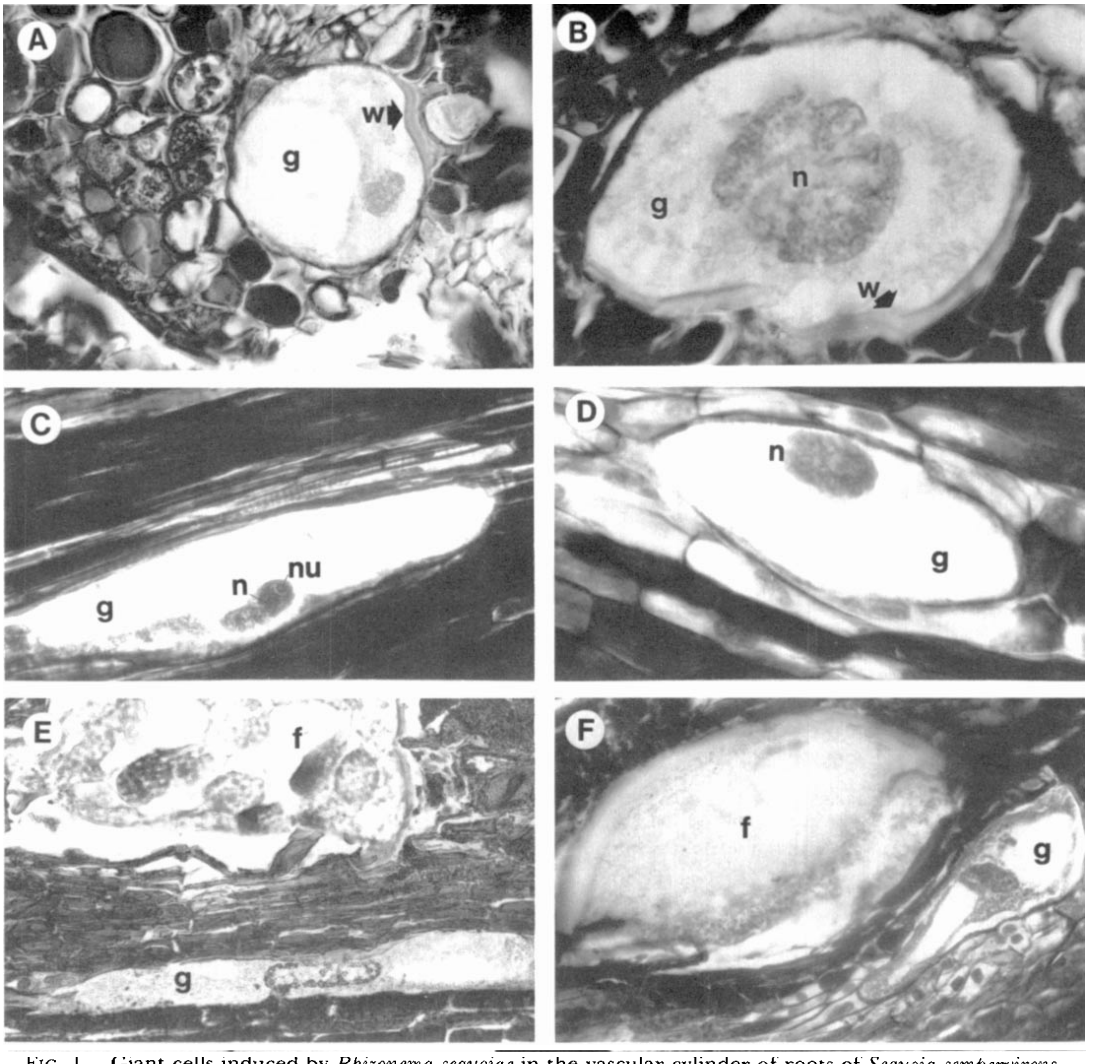

FIG. 1. Giant cells induced by *Rhizonema sequoiae* in the vascular cylinder of roots of *Sequoia sempervirens*. A–B) Transverse sections. C–F) Longitudinal sections (g = giant cell; n = enlarged nucleus; nu = enlarged nucleolus; w = cell wall; f = female).

nuclei were 17 (11–37) μm long and 11 (5–18) μm wide, and their nucleoli were 7 (3–18) μm long and 6 (3–13) μm wide. A secondary wall thickening lined each giant cell. Most commonly the thickening occurred on the side of the cell where the nematode fed (Fig. 1A, B). In the approximately 100 infected sections of roots studied, no case of multiple giant cells around a single female was found. There were cases of multiple infection in the same piece of root, but each female was associated with a single giant cell. Females were found in the cortical tissue of the roots, but giant cells were not observed in this area. These females in the cortex contained eggs and larvae. Third-stage larvae were also observed in the cortical tissue, with no giant cells in association with them; in the vascular tissue they were always associated with giant cells.

Wide tunnels were generally observed posterior to the females and extending from the point of nematode penetration of the root to the female body (Fig. 2A). The tunnels were completely full of gel material in which second-stage larvae were commonly found (Fig. 2B, C). Cell wall debris, but no soil fungi, was observed in the tunnels.

Severe damage to the cortical tissue was

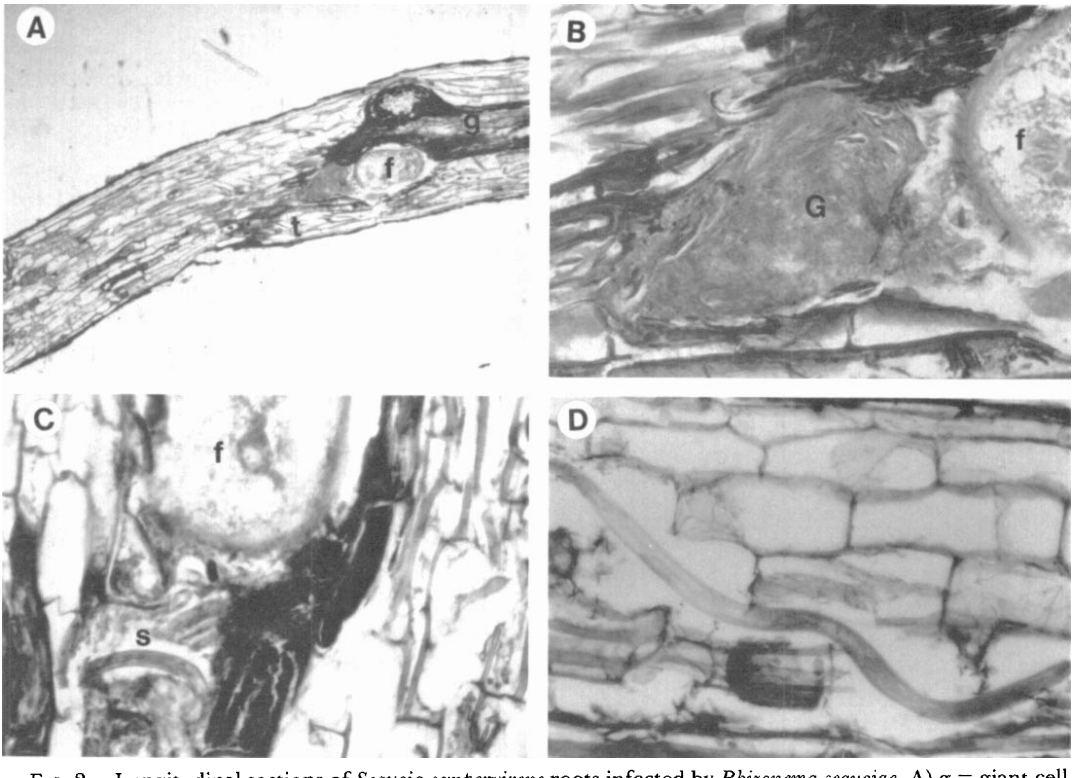

FIG. 2. Longitudinal sections of *Sequoia sempervirens* roots infected by *Rhizonema sequoiae.* A) g = giant cell f = female; G = gel material; t = tunnel extending to root surface. B–C) Longitudinal sections showing f = female; G = gel material; s = second-stage larvae in gel material. D) Second-stage larva in the cortical tissue.

associated with the second-stage larvae. Cell walls were destroyed, and cells close to the larvae were necrotic (Fig. 2D). In a few cases, small necrotic areas on the surface of the root were associated with females inside the root.

Host suitability test: Adult *R. sequoiae* females developed in 6 of the 18 plant species tested (Table 1). Some development occurred in *Abies concolor* (Gord & Glend.) Lindl. and *Salix lasiolepis* Benth, but there was no sure evidence the nematodes would reach maturity on these hosts.

Mature females of *R. sequoiae* were found in roots of naturally infected *Lithocarpus densiflorus, Umbellularia californica,* and *Arbutus menziesii* collected in the Marin County forest but not on roots infected in the lathhouse test.

DISCUSSION

A giant cell with a single enlarged nucleus has been associated previously with three species of plant-parasitic nematodes, *Rotylenchulus macrodoratus* (2), *Hylonema ivorense* (9), and *Sarisodera hydrophila* (8). In the case of *R. macrodoratus,* the giant cell has amoeboid nuclei and the cell walls are well differentiated with irregular thickenings. In *H. ivorense* the giant cell is reported to have ovoid to spherical enlarged nuclei and no cell walls (9). Host response induced by *R. sequoiae* is similar to that seen with *H. ivorense,* except for the occurrence of multiple nucleoli in *R. sequoiae* and the reported lack of a wall around the cell induced by *H. ivorense.* The small swellings, small necrotic depressions, and disoriented xylem tissue seen in roots infected with *H. ivorense* were not seen in the roots infected by *R. sequoiae.* The host responses to *R. sequoiae* most closely resemble the histological symptoms induced by *Sarisodera hydrophila.* The giant cell is induced in the vascular cylinder, the hypertrophied nucleus is amoeboid or lobate in shape, the giant cell wall is thicker in the region where the nematode has fed and thin on the op-

posite side, and the number of nucleoli varies from one to several.

Infection of plants in the host suitability test was low, even for the type host (Coast Redwood), which is often heavily infected in nature. Since mature females of *R. sequoiae* were found on several trees which are not related botanically or geographically to Coast Redwood, it is possible that *R. sequoiae* may eventually be found to have a broad host range.

We do not know the real function of the gel material. However, during the dissection of roots, females were associated with abundant transparent gel-like substance, in which we found second-stage larvae and males. It is possible that this gel material is used to provide an escape tunnel for the larvae after their hatch inside the females and that females release "sexual pheromones" into the gel which attract the males.

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