The Influence of Ectotrophic Mycorrhizal Fungi on the Resistance of Pine Roots to Pathogenic Infections. V. Resistance of Mycorrhizae to Infection by Vegetative Mycelium of Phytophthora cinnamomi

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ABSTRACT

Mature ectomycorrhizae formed aseptically on roots of *Pinus echinata* seedlings by *Thelephora terrestris* and *Pisolithus tinctorius* were resistant to infection by vegetative mycelium of *Phytophthora cinnamomi*. Nonmycorrhizal short and lateral roots were infected heavily by mycelium of the pathogen. Both the fungal mantle and Hartig net of ectomycorrhizae appear to function as barriers to infection by the pathogen. Phytopathology 60:1472-1473.

Additional key words: Biological control, symbiosis, root inoculation techniques.

Ectomycorrhizae perform several important physiological functions in the survival, growth, and development of trees (1). Recently, ectomycorrhizae were reported to function also as biological deterrents to infection of feeder roots by pathogenic fungi (2, 3, 5, 6, 7). The pathogen used in the initial root protection investigations (2, 3, 6, 7) was *Phytophthora cinnamomi* Rands, the primary cause of littleleaf disease of shortleaf (*Pinus echinata* Mill.) and loblolly (*P. taeda* L.) pines and many other root diseases. In these studies, zoospores were used as inocula, as they were considered the primary agents of initial feeder root infection by the pathogen. Recently, vegetative mycelium of *Phytophthora cinnamomi* proved highly infectious to nonmycorrhizal feeder roots of shortleaf and loblolly pine seedlings (4). Histological studies of infected roots revealed vegetative mycelium penetrating the root epidermis directly and ramifying through the cortical and stelar tissues of roots even more extensively than infectious hyphae from zoospores. The realization that the mycelium of *P. cinnamomi* could infect nonmycorrhizal feeder roots of pine suggested further studies to determine the infective potential of vegetative mycelium of the pathogen on ectomycorrhizal roots of pine.

MATERIALS AND METHODS.—Ectomycorrhizae were formed aseptically on roots of shortleaf pine seedlings with *Thelephora terrestris* (Ehrh.) Fr. and *Pisolithus tinctorius* (Pers.) Coker & Couch (6, 7). Ectomycorrhizae, short roots, and lateral root tips intact on seedlings were inserted in glass cells (25 mm outside diam. × 1 cm deep with slits 1 mm wide × 5 mm deep to accommodate the root) and inoculated (4) with (i) a mycelial strip of *Phytophthora cinnamomi* with sporangia (formed in nonsterile soil leachate [NSSL] after 5 days at 25 C) in 2.5 ml of autoclaved soil leachate (ASL); (ii) a mycelial strip without sporangia in 2.5 ml of ASL; (iii) a mycelial strip without sporangia in 2.5 ml of NSSL; (iv) 2.5 ml of ASL without mycelial strip (ASL control); and (v) 2.5 ml of NSSL without mycelial strip (NSSL control). Microorganisms in soil leachate induce production of sporangia and zoospores in *P. cinnamomi*, but in their absence the fungus produces abundant vesicles on mycelium and no sporangia (4, 8). All roots were examined histologically (6) after 12 days' incubation.

RESULTS.—All nonmycorrhizal roots inoculated with *P. cinnamomi* were infected (Table 1). Infection from zoospores and from vegetative mycelium was similar to that previously reported (4). The nonmycorrhizal roots which were infected by vegetative mycelium had abundant vesicles on the root surfaces and intracellularly in the cortical and stelar tissues (Fig. 1-A).

Mature ectomycorrhizae formed by either fungal symbiont were resistant to infection from zoospores and from vegetative mycelium of *P. cinnamomi* (Table 1). Fungus mantles of ectomycorrhizae from the NSSL treatments with *P. cinnamomi* were covered with sporangia, while mantles from the vegetative mycelium treatments in ASL were covered with vesicles (Fig. 1-B). Thickness of fungus mantles of ectomycorrhizae formed by *T. terrestris* averaged 29 μ; those formed by *Pisolithus tinctorius* averaged 76 μ. The few ectomycorrhizae infected by zoospores or vegetative mycelium lacked the complete covering of the fungus mantle at the meristems. Infection of these ectomycorrhizae was limited to meristematic tissues, and did not penetrate into cortical cells surrounded by the Hartig net. The Hartig net around cortical cells in all ectomycorrhizae penetrated to the endodermis.

DISCUSSION.—The results demonstrate that ectomycorrhizae of shortleaf pine are resistant to infection by both zoospore and vegetative mycelium inocula of *Phytophthora cinnamomi* when mantle covering is complete. Apparently, the fungus mantle of ectomycorrhizae provides a mechanical barrier preventing penetration of root tissues by the highly infective vegetative mycelium and germ tube penetration by zoospores of the pathogen. The Hartig net appears to be another barrier, since the pathogen did not spread from meristem to cortex tissues which were surrounded by a Hartig net. The Hartig net barrier could be either physical or due to chemical inhibitors produced by the host cortical cells, as discussed earlier (6, 7). Neither *T. terrestris*

TABLE 1. Infection of aseptic feeder roots of shortleaf pine (*Pinus echinata*) seedlings by *Phytophthora cinnamomi* in the presence and absence of sporangial-inducing soil leachate[a]

	Mycelial strip with preformed sporangia		Mycelial strip and nonsterile soil leachate (NSSL)		Mycelial strip and autoclaved soil leachate (ASL)	
	Seedlings with ectomycorrhizae formed by:					
	Thelephora terrestris	*Pisolithus tinctorius*	*Thelephora terrestris*	*Pisolithus tinctorius*	*Thelephora terrestris*	*Pisolithus tinctorius*
No. lateral roots inoculated	9	10	11	8	9	11
% Lateral roots infected	100	100	100	100	100	100
No. short roots inoculated	18	21	26	17	23	24
% Short roots infected	100	100	100	100	100	100
No. ectomycorrhizae inoculated	48	58	86	119	74	109
% Ectomycorrhizae infected[b]	9	0	5	3	8	4

[a] Several feeder roots of each type incubated with nonsterile and autoclaved soil leachates without *P. cinnamomi* were free of infection and are not presented in this table.

[b] Infection of ectomycorrhizae limited to root meristems without Hartig nets and not covered by fungus mantle of mycorrhizal fungus.

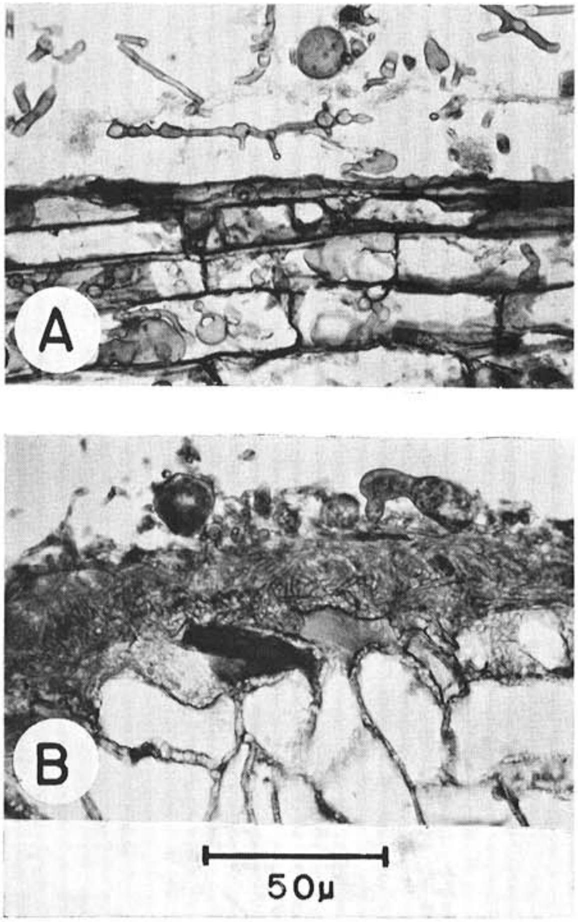

Fig. 1. Tangential section of nonmycorrhizal root (A) and ectomycorrhiza (B) of *Pinus echinata* inoculated with vegetative mycelium of *Phytophthora cinnamomi*. Note vesicles of pathogen on root surface and abundant intracellular cortex infection in (A). Note vesicles on fungus mantle and absence of intracellular cortex infection in B.

nor *Pisolithus tinctorius* produces antibiotics inhibitory to zoospores or vegetative mycelia of *Phytophthora cinnamomi* (2, 7); therefore, this mechanism cannot be implicated.

The resistance of ectomycorrhizae to infections by all known infectious stages of *P. cinnamomi* strengthens the concept that ectomycorrhizae function as biological deterrents to infection of feeder roots by pathogenic fungi.

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