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Phialophora melinii: Inoculations in Wounded Red Maple

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ABSTRACT

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Isolations from discolored wood associated with wounds previously inoculated with *Phialophora melinii* in *Acer rubrum* showed that the fungus was present in all 1.5- to 26-mo-old wounds. Hymenomycetes from natural sources were not isolated from wounds less than 4 mo old. Wounds on trees inoculated with *P. melinii* yielded fewer Hymenomycetes than did noninoculated wounds. The fungus thus acted as an aggressive invader and a long-term inhabitant of discolored wood.

Additional key words: discoloration and decay, successions.

Phialophora melinii (Nannfeldt) Conant occurs frequently in discolored wood surrounding columns of decay or associated with young or old wounds in red maple, *Acer rubrum* L. (11). Results of other studies indicate that the fungus is well suited to the role of pioneer invader of wounds in red maple. It can accumulate manganese in its mycelium (16), produce black-green pigmentation in response to changes in concentration of manganese (14), utilize and alter wound-initiated phenolic compounds (17), grow in media containing high concentrations of microelements (14) and any of a variety of nitrogen sources (12), and produce phialospores in discolored wood in living trees (11). We attempted to determine (i) whether *P. melinii* can invade fresh wounds, and if so, how long it remains in the wood, and (ii) what effects such an invasion has on invasion by other fungi, especially Hymenomycetes.

MATERIALS AND METHODS

Study area.—Red maples in a 5-hectare (ha) forested area at Alfred, Maine, were selected for freedom from mechanical wounds or large or open branch stubs. The trees were about 50 yr old, 15 to 25 cm in diam at 1.4 m above ground, and 15 m tall. Before the study, dissections of many trees in the area showed them to be relatively free of internal defects, and growing at a uniform rate.

Inoculation procedures.—All inoculations were made in March 1970. Wounds were made with a flame-sterilized drill after the bark was washed with 70% alcohol.

In one group of 16 trees (Group A), 278 wounds were inflicted: eight trees each received seven to 24 drill-bit wounds, 1.4 cm in diameter and 5 cm deep; eight other trees each received eight to 24 chisel wounds, 1.5 cm wide and 5 cm deep. All wounds were 0.5 to 2 m above ground. No two wounds were aligned in the same vertical plane. About half the wounds on each tree were inoculated, giving group totals of 138 inoculated and 140 noninoculated. The trees were felled for dissection and isolations after 3, 4, 5, 6, 8, 10, 14, 17, and 22 mo.

In another group of 15 trees (Group B), 180 wounds were inflicted. Each tree received two whorls of 6 equally spaced drill wounds, at 0.5 m and 1.5 m above ground. The bark was washed and flamed before the holes were made. The lower six wounds were not inoculated and were left unsealed; the upper six were treated variously: five were fitted with an air filter assembly (10) designed to allow change of air and to delay natural infection for a few weeks; the remaining hole was plugged with a rubber serum-bottle stopper (Fig. 1). Rubber stoppers in the upper whorl were sealed at their margins with epoxy glue.

The inoculum.—The isolate of *P. melinii* used in this study was obtained from discolored wood associated with a natural wound in red maple from the study area. The chip that yielded *P. melinii* was placed in an agar medium consisting of 10 g malt extract and 2 g yeast extract. The bacteria that grew from the same wood chip that yielded *P. melinii* also were used for inoculations. They were included in the inoculations only because they were associated with the isolate of *P. melinii* used for the study. They were not identified. *Phialophora melinii* alone, the bacteria alone, and *P. melinii* and the bacteria were grown in 250-ml Erlenmeyer flasks each containing 25 ml of a medium consisting of 5 g glucose and 1 g yeast extract per liter of water. The cultures were incubated at 25 C for 2 wk. The contents of two flasks containing *P. melinii* were washed twice with sterile distilled water, drained, reconstituted to 50 ml with sterile water, and triturated for 5 sec in a Waring Blendor. Sterile water was added to the contents of two flasks of bacteria alone and to two flasks of *P. melinii* with bacteria, to make 50 ml, and each was triturated for 5 sec.

Inoculations were made with a hypodermic needle forced through the rubber stopper. Each tree received 1 ml of *P. melinii* inoculum in one hole, 1 ml of *P. melinii* and bacteria in a second hole, and 1 ml of bacterial

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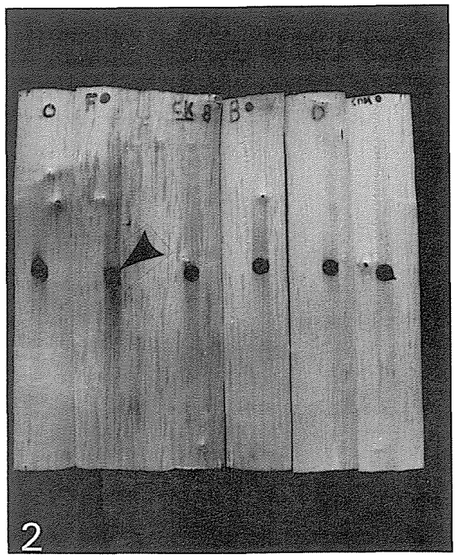

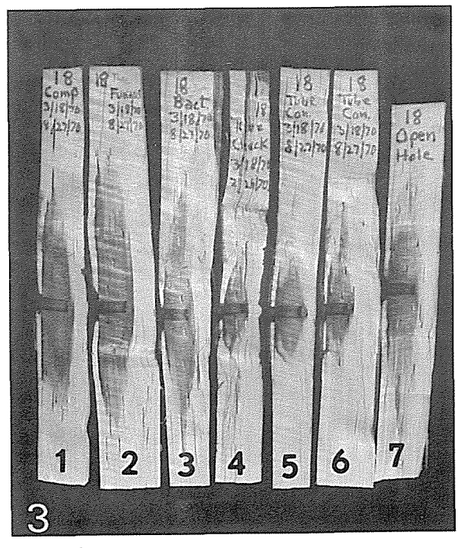

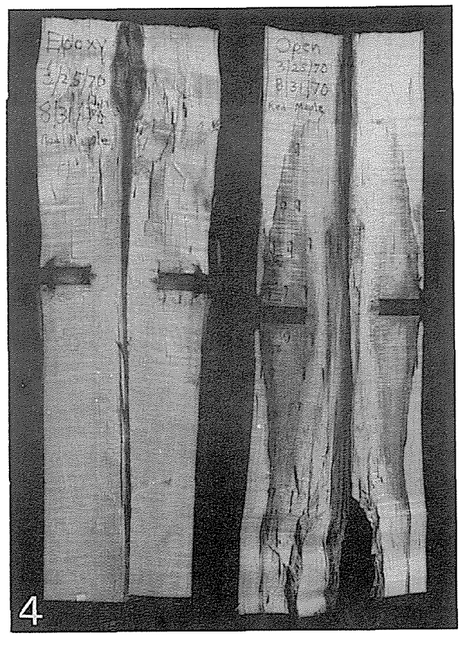

Fig. 1-4. Experimental inoculation of red maple with *Phialophora melinii*. **1)** Drilled holes sealed with filter-tube apparatus and lower whorl of six open holes (arrow) on a red maple. **2)** Surface view (with bark removed) of six billets showing dark streaks associated with 4-mo-old wounds. The wound inoculated with *P. melinii* (arrow) had the darkest and longest discolored streak. **3)** Radial view of discolored columns associated with 5-mo-old wounds: billet 1, inoculated through rubber stopper with bacteria and *P. melinii*; billet 2, inoculated through rubber stopper with *P. melinii*; billet 3, inoculated through rubber stopper with bacteria; billet 4, noninoculated hole sealed with rubber stopper; billets 5 and 6, hole sealed with filter-tube apparatus; and billet 7, hole left open. **4)** Two 5-mo-old wounds on the same tree; left, sealed and glued; right, open.

inoculum in a third hole. Two holes were each injected with 1 ml of sterile water, and the plugged hole was not treated. The trees were harvested 1.5, 2, 4, 5, 9, 15, 16, 21, 24, and 26 mo later.

Additional controls.—Five trees (Group C) each received 5, 8, 16, or 20 chisel wounds, or 12 drill wounds. A scattered pattern of wounding, as described above, was used. The wounds were not inoculated and were left unsealed. These trees were harvested after 4, 7, 7, 17, and 18 mo, respectively.

Another group of four trees (Group D) each received 12 drill wounds. On each tree, six wounds were plugged immediately with the filter tube apparatus, three wounds were sealed with rubber serum-bottle stoppers and coated with epoxy glue, and three were left open. These trees were harvested after 1, 2, 3, and 4.5 mo, respectively.

Two vigorous trees, free from external signs of internal defects, made up Group E. They were harvested and 180 chips were taken from nondiscolored wood for isolations.

Harvest procedures.—The trees were cut 10 cm above ground and a 2-m bolt from each was taken to the laboratory within a few hours. On the same day, the bark was stripped from the bolt and, under clean conditions, billets 8 × 8 × 35 cm were cut, each including a wound in its center (Fig. 2). Each billet was split through the wound with a flamed ax (Fig. 3).

Isolation procedures.—Immediately after each billet was split, a total of 12 chips of wood was removed with a gouge so as to sample discolored wood, decayed wood (when present), and the contiguous nondiscolored wood (Fig. 3). The chips were placed in two culture plates (13, 15).

The medium consisted of 10 g malt extract, 2 g yeast extract, and 20 g agar per liter of distilled water. The chips, each 3 × 3 × 10 mm, were inserted vertically into the agar, until each touched the bottom of the plate (13, 15). Plates were incubated at 25 C and examined after 7 and 14 days for identification of the organisms that grew on the medium.

RESULTS

Trees with chisel and drill wounds noninoculated and inoculated with Phialophora melinii, Group A.—Of 140 control wounds on 16 trees, 27 wounds on nine trees yielded Hymenomycetes after 4 mo. The wounds that yielded the Hymenomycetes were 4 to 22 mo old. Of 138 inocuiated wounds on 16 trees, seven wounds on four trees yielded Hymenomycetes. These wounds were also 4 to 22 mo old. The chisel and drill wounds yielded Hymenomycetes equally. All but two of the 27 control wounds that yielded Hymenomycetes also yielded *P. melinii*, and 18 of these 25 wounds had *P. melinii* associated with less than half of the column of discolored and decayed wood.

Trees with filter assembly, Group B.—After 1.5 to 26 mo, *P. melinii* was isolated from all inoculated wounds. All except three inoculated wounds yielded the fungus from throughout the discolored column.

The surface of the wood exposed after removal of bark was streaked darkly above and below the inoculated wounds (Fig. 2). The internal column of discolored wood associated with wounds inoculated with *P. melinii* had discolored streaks that were longest in the most recently formed growth rings (Fig. 3). Until 5 mo after inoculation, the discolored column associated with wounds inoculated with *P. melinii* alone and *P. melinii* plus bacteria were longest. After 5 mo all columns were about the same length. Only one of 15 wounds inoculated with *P. melinii* alone yielded a Hymenomycete. Up to 26 mo, no wound inoculated with *P. melinii* plus bacteria yielded Hymenomycetes. After 6 mo, 24 of 60 open, noninoculated control wounds yielded Hymenomycetes. All wounds on one tree after 9 mo and on another after 23 mo failed to yield Hymenomycetes. *Phialophora melinii* was isolated from many noninoculated wounds, but was found throughout the discolored column in only 20 of 150 such wounds. In only six of 33 wounds that yielded a Hymenomycete was *P. melinii* isolated from chips taken throughout the column. Bacteria were the organisms most frequently isolated from open control wounds, especially those less than 4 mo old. Hymenomycetes were not isolated from wounds less than 4 mo old. The Hymenomycete isolated most frequently was *Polyporus versicolor* (L.) Fr. [*Coriolus versicolor* (L. ex Fr.) Quel.]. Nonhymenomycetous fungi isolated most frequently were of the genera *Fusarium*, *Cytospora*, *Cephalosporium*, *Gliocladium*, and *Graphium*.

Noninoculated wounds, Group C.—Of the five trees felled after 4 to 18 mo, 32 of 61 wounds yielded Hymenomycetes. Ten of the 32 wounds did not yield *P. melinii*, whereas 18 others yielded *P. melinii* only from the margins of the columns.

Sealed noninoculated wounds, Group D.—After 2 mo only a few chips from wounds sealed with rubber stoppers on four trees yielded bacteria and fungi, whereas all of the chips from the open wounds yielded bacteria, fungi, or both (Table 1). After 4 mo, more chips from the sealed wounds yielded microorganisms, but the frequency was still not so high as that from the open holes (Table 1). After 5 mo, wood associated with some sealed wounds was nondiscolored (Fig. 4).

Nonwounded trees, Group E.—No bacteria or fungi grew from chips taken from nonwounded trees. Detailed data on isolations and lengths of each column can be obtained from the author.

DISCUSSION

All trees with wounds inoculated with *P. melinii* in March yielded the organism from wood chips taken 1.5 to 26 mo later. Thus, this fungus acted as a pioneer invader and a long-term inhabitant of discolored wood associated

TABLE 1. Percentage of chips that yielded microorganisms from sealed and open noninoculated wounds in red maples of Group D

Tree number	Age of wounds (mo)	Wounds sealed with:		Open wounds
		Filter[a]	Rubber stopper[b]	
1	1	4	3	66
2	2	10	3	100
3	3	25	47	100
4	4.5	72	50	100

[a]Six wounds, 72 isolations per tree.
[b]Three wounds, 36 isolations per tree.

maple. *Ascocoryne sarcoides* (Jacq. d Wilson, a common inhabitant on was isolated from all wounds ed with it in jack pine, *Pinus*). Also, *Trichoderma viride* Pers. ex n all wounds previously inoculated le (8). These results contrast with *llinus igniarius* (L. ex Fr.) Quel. .) Gill], and *Oxyporus populinus* k [*Fomes connatus* (Weinm.) Gill] in nly one chip of 874 taken from trees ed *F. connatus* after 3 mo (13). Only ioculated with several species of lded the organisms after 5 yr (15). report results of Hymenomycete from no reisolation (7), to less than sive inoculations (1, 3, 6, 18, 19, 20,

ycetes were isolated from wood nds inoculated with *P. melinii* than ids. This pattern occurred with losed with the filter assembly, as well ounds that were left open. Though *P. melinii* delayed colonization by e latter eventually invaded. This hat for wounds inoculated with *T.* e, Hymenomycetes were not isolated (8), but some were recovered after 2

ds (4, 5) suggested that *T. viride* ion by other wood-decay fungi by the supply of nonstructural wood. Perhaps *T. viride* dominates associated with a wound only after it hed through massive inoculations, m isolated from discolored wood nds in red maple (11). *Phialophora* n differently, since it is isolated nds on red maple. Its ability to alter olic compounds in discolored wood as a pioneer invader. These phenolic ss toxic to *P. melinii* than to specially to *F. connatus* (17). lic compounds by a pioneer invader ition by Hymenomycetes and other ora. Perhaps this is why *P. melinii* y from recently inoculated wounds, tremities of the columns even after l invaded.

ymenomycetous fungi, and ll are presumed to have equal onize a fresh wound surface. They mselves and with the tree at the other microorganisms are Hymenomycetes, decay results. *Phialophora melinii*, however, maintains its position in the extremities of the columns.

LITERATURE CITED

1. BASHAM, J. T. 1975. Heart rot of jack pine in Ontario IV: Heartwood-inhabiting fungi, their entry and interactions within living trees. Can. J. For. Res. 5:706-721.
2. ETHERIDGE, D. E. 1961. Ascocoryne sarcoides (Jacq. ex Gray) Groves and Wilson and its association with decay of conifers. Pages 19-26 *in* Fonds Rech. For. Univ. Laval Bull. 13. 43 p.
3. HIRT, R. R. 1949. Decay of certain northern hardwoods by Fomes igniarius, Poria obliqua, and Polyporus glomeratus. Phytopathology 39:475-480.
4. HULME, M. A., and J. K. SHIELDS. 1970. Biological control of decay fungi in wood by competition for non-structural carbohydrates. Nature 227:300-301.
5. HULME, M. A., and J. K. SHIELDS. 1972. Interaction between fungi in wood blocks. Can. J. Bot. 50:1421-1427.
6. LINZON, S. N. 1962. Artificial inoculation of wet and dry heartwood of living eastern white pine trees. For. Sci. 8:163-167.
7. MALOY, O. C. 1973. Reducing decay in grand fir. J. For. 71:706-707.
8. POTTLE, H. W., and A. L. SHIGO. 1975. Treatment of wounds on Acer rubrum with Trichoderma viride. Eur. J. For. Pathol. 5:274-279.
9. SAFFORD, L. O., A. L. SHIGO, and M. ASHLEY. 1974. Gradients of cation concentration in discolored and decayed wood of red maple. Can. J. For. Res. 4:435-440.
10. SHARON, E. M., and A. L. SHIGO. 1974. A method for studying the relationship of wounding and microorganisms to the discoloration process in living trees. Can. J. For. Res. 4:146-148.
11. SHIGO, A. L. 1965. Decay and discoloration in sprout red maple. Phytopathology 55:957-962.
12. SHIGO, A. L. 1965. Organism interactions in decay and discoloration in beech, birch, and maple. U.S. Dep. Agric., For. Serv. Res. Pap. NE-43. 23 p.
13. SHIGO, A. L. 1974. Relative abilities of Phialophora melinii, Fomes connatus, and F. igniarius, to invade freshly wounded tissues of Acer rubrum. Phytopathology 64:708-710.
14. SHIGO, A. L. 1974. Effects of manganese, calcium, zinc, and iron on growth and pigmentation of Trichocladium canadense, Phialophora melinii, Hypoxylon rubiginosum, Daldinia concentrica, and Cytospora decipiens. Mycologia 66:339-341.
15. SHIGO, A. L., and E. M. SHARON. 1968. Discoloration and decay in hardwoods following inoculations with Hymenomycetes. Phytopathology 58:1493-1498.
16. SHORTLE, W. C., and A. L. SHIGO. 1973. Concentrations of manganese and microorganisms in discolored and decayed wood in sugar maple. Can. J. For. Res. 3:354-358.
17. SHORTLE, W. C., T. A. TATTAR, and A. E. RICH. 1971. Effects of some phenolic compounds on the growth of

22. TOOLE, E. R. 1965. Inoculation of bottom land red oaks with Poria ambigua, Polyporus fissilis, and Polyporus hispidus. Plant Dis. Rep. 49:81-83.
23. WHITNEY, R. R., and W. B. DENYER. 1970. Inoculations with Stereum sanguinolentum and Fomes pini in black spruce and balsam fir. For. Sci. 16:160-164.