

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-month-old wounds. Hymenomyces from natural sources were not isolated from wounds less than 4 mo old. Wounds on

trees inoculated with *P. melinii* yielded fewer Hymenomyces 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 Hymenomyces.

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

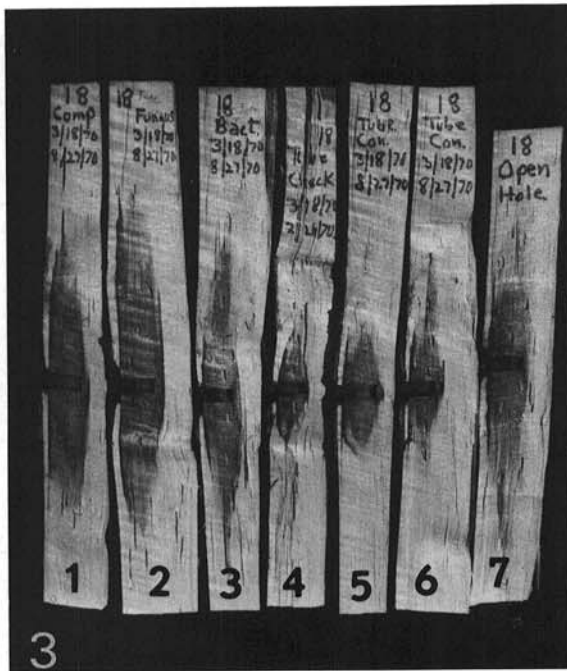
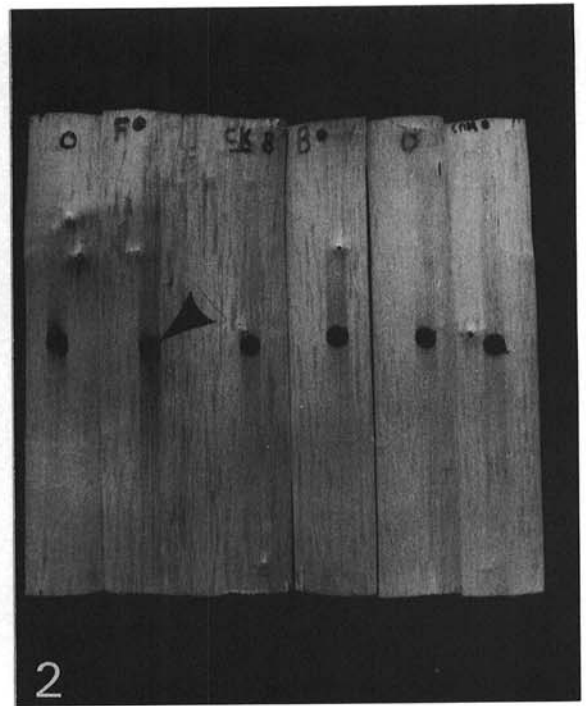


Fig. 1-4. Experimental inoculation of red maple with *Phialophora melinii*. 1) Drilled holes sealed with filter-tube apparatus and lower whorl of six open holes (arrow) on a red maple. 2) Surface view (with bark removed) of six billets showing dark streaks associated with 4-mo-old wounds. The wound inoculated 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 Hymenomyces after 4 mo. The wounds that yielded the Hymenomyces were 4 to 22 mo old. Of 138 inoculated wounds on 16 trees, seven wounds on four trees yielded Hymenomyces. These wounds were also 4 to 22 mo old. The chisel and drill wounds yielded Hymenomyces equally. All but two of the 27 control wounds that yielded Hymenomyces 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 Hymenomyces. Up to 26 mo, no wound inoculated with *P. melinii* plus bacteria yielded Hymenomyces. After 6 mo, 24 of 60 open, noninoculated control wounds yielded Hymenomyces. All wounds on one tree after 9 mo and on another after 23 mo failed to yield Hymenomyces. *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 Hymenomyces 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. Hymenomyces were not isolated from wounds less than 4 mo old. The Hymenomyces 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 Hymenomyces. 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:		
		Filter ^a	Rubber stopper ^b	Open wounds
1	1	4	3	66
2	2	10	3	100
3	3	25	47	100
4	4.5	72	50	100

^aSix wounds, 72 isolations per tree.

^bThree wounds, 36 isolations per tree.

with wounds in red maple. *Ascocoryne sarcoides* (Jacq. ex Gray) Groves and Wilson, a common inhabitant on many conifers (2), was isolated from all wounds previously inoculated with it in jack pine, *Pinus banksiana* Lamb. (1). Also, *Trichoderma viride* Pers. ex Fr. was isolated from all wounds previously inoculated with it in red maple (8). These results contrast with inoculations of *Phellinus igniarius* (L. ex Fr.) Quel. [*Fomes igniarius* (L.) Gill], and *Oxyporus populinus* (Schum. ex Fr.) Donk [*Fomes connatus* (Weinm.) Gill] in red maple in which only one chip of 874 taken from trees with 72 wounds yielded *F. connatus* after 3 mo (13). Only 20% of wounds inoculated with several species of Hymenomycetes yielded the organisms after 5 yr (15). Other investigators report results of Hymenomycete inoculations ranging from no reisolation (7), to less than 100% even after massive inoculations (1, 3, 6, 18, 19, 20, 21, 22, 23).

Fewer Hymenomycetes were isolated from wood associated with wounds inoculated with *P. melinii* than from control wounds. This pattern occurred with inoculated wounds closed with the filter assembly, as well as with inoculated wounds that were left open. Though inoculations with *P. melinii* delayed colonization by Hymenomycetes, the latter eventually invaded. This result is similar to that for wounds inoculated with *T. viride*; in that instance, Hymenomycetes were not isolated during the first year (8), but some were recovered after 2 yr.

Hulme and Shields (4, 5) suggested that *T. viride* interferes with invasion by other wood-decay fungi by rapidly depleting the supply of nonstructural carbohydrates in the wood. Perhaps *T. viride* dominates the discolored wood associated with a wound only after it has become established through massive inoculations, otherwise it is seldom isolated from discolored wood associated with wounds in red maple (11). *Phialophora melinii* may function differently, since it is isolated frequently from wounds on red maple. Its ability to alter and utilize the phenolic compounds in discolored wood may explain its role as a pioneer invader. These phenolic compounds are less toxic to *P. melinii* than to Hymenomycetes, especially to *F. connatus* (17). Alteration of phenolic compounds by a pioneer invader could favor colonization by Hymenomycetes and other successional microflora. Perhaps this is why *P. melinii* was isolated not only from recently inoculated wounds, but also from the extremities of the columns even after Hymenomycetes had invaded.

Bacteria, nonhymenomycetous fungi, and Hymenomycetes, all are presumed to have equal opportunities to colonize a fresh wound surface. They interact among themselves and with the tree at the infection court, but growth into the tree is another matter. In red maple, *P. melinii* apparently can grow rapidly from the infection court into the tree. In doing so, it becomes dominant in wood containing wound-initiated substances (17) that may aid it by serving as nutrients and by inhibiting invasion by competitive microorganisms. The discolored wood contains a high concentration of microelements (9); *P. melinii* grows well under these conditions (14). When *P. melinii* has utilized and altered substrates sufficiently, it can no longer compete with other microorganisms and inhibit invasion by them. If the

other microorganisms are Hymenomycetes, decay results. *Phialophora melinii*, however, maintains its position in the extremities of the columns.

LITERATURE CITED

- 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.
- 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.
- HIRT, R. R. 1949. Decay of certain northern hardwoods by *Fomes igniarius*, *Poria obliqua*, and *Polyporus glomeratus*. *Phytopathology* 39:475-480.
- 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.
- HULME, M. A., and J. K. SHIELDS. 1972. Interaction between fungi in wood blocks. *Can. J. Bot.* 50:1421-1427.
- LINZON, S. N. 1962. Artificial inoculation of wet and dry heartwood of living eastern white pine trees. *For. Sci.* 8:163-167.
- MALOY, O. C. 1973. Reducing decay in grand fir. *J. For.* 71:706-707.
- POTTLE, H. W., and A. L. SHIGO. 1975. Treatment of wounds on *Acer rubrum* with *Trichoderma viride*. *Eur. J. For. Pathol.* 5:274-279.
- 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.
- 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.
- SHIGO, A. L. 1965. Decay and discoloration in sprout red maple. *Phytopathology* 55:957-962.
- 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.
- 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.
- SHIGO, A. L. 1974. Effects of manganese, calcium, zinc, and iron on growth and pigmentation of *Trichocladium canadense*, *Phialophora melinii*, *Hypoxyylon rubiginosum*, *Daldinia concentrica*, and *Cytospora decipiens*. *Mycologia* 66:339-341.
- SHIGO, A. L., and E. M. SHARON. 1968. Discoloration and decay in hardwoods following inoculations with Hymenomycetes. *Phytopathology* 58:1493-1498.
- 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.
- SHORTLE, W. C., T. A. TATTAR, and A. E. RICH. 1971. Effects of some phenolic compounds on the growth of *Phialophora melinii* and *Fomes connatus*. *Phytopathology* 61:552-555.
- SILVERBORG, S. B. 1959. Rate of decay in northern hardwoods following artificial inoculation with some common heart-rot fungi. *For. Sci.* 5:223-228.
- SILVERBORG, S. B., and M. J. LARSEN. 1967. Rate of decay in *Pinus strobus* following artificial inoculation with *Fomes pini*. *Plant. Dis. Rep.* 51:829-831.
- TOOLE, E. R. 1961. Rates of wood decay behind open and closed wounds. *Plant Dis. Rep.* 51:600.
- TOOLE, E. R. 1964. Progress of oak heart-rot varies with height in trees. *Plant Dis. Rep.* 48:585.

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.