Relative Abilities of Phialophora melinii, Fomes connatus, and F. igniarius to Invade Freshly Wounded Tissues of Acer rubrum

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The author acknowledges the assistance of James Murphy and Peter Olesky.
Accepted for publication 7 December 1973.

ABSTRACT

Phialophora melinii, Fomes connatus, and *F. igniarius* were inoculated into wounds inflicted in *Acer rubrum* in June. After 12 wk, *P. melinii* was isolated from 50 of 54 wounds inoculated with it, *F. connatus* was isolated from one wood chip of 874 from 72 wounds inoculated with it, and *F. igniarius* was not isolated. Bacteria, *Graphium* sp. and *Cytospora* sp. were isolated frequently. *Phialophora melinii, Graphium* sp., *Cytospora* sp., and bacteria were pioneer microorganisms infecting the type of wounds inflicted in *A. rubrum* in June, while *F. connatus* and *F. igniarius* were not.

Phytopathology 64:708-710.

Additional key words: wood discoloration and decay, succession of microorganisms.

The classical concept of decay of wood in living trees implies that Hymenomycetes infect wounds, and then invade until the wood decays (1). This concept has been expanded to include bacteria and nonhymenomycetous fungi, which are frequently the first to infect fresh wounds (3). To determine which fungi were the first to infect, fresh wounds in red maple, *Acer rubrum* L. were inoculated with two Hymenomycetes [*Fomes connatus* (Weinm.) Gill. and *F. igniarius* (L.) Gill.] commonly associated with advanced decay in red maple; and with the nonhymenomycete *Phialophora melinii* (Nannfelt) Conant isolated commonly from discolored wood surrounding areas decayed by *F. connatus* and *F. igniarius* (2, 5, 6).

MATERIALS AND METHODS.—Wounds were inflicted on four trees on 15 June 1970 on the Massabesic Experimental Forest, Alfred, Maine; on six trees on 16 June 1970 on the Bartlett Experimental Forest, Bartlett, New Hampshire; and on 11 trees on 17 June 1970 on the Hubbard Brook Experimental Forest, West Thornton, New Hampshire. The trees ranged 20-30 cm in diam at 1.5 m above ground level.

Six wounds, each 5 cm deep, 1 cm wide, and, at a 45-degree angle downward into the trunk, were made by pounding a screwdriver into the wood. A set of these wounds were spaced evenly around the trunk at each of three heights, 1, 1.5, and 2 m aboveground. The six wounds at each height were numbered clockwise from 1 to 6 and spaced so that they were not directly above or below wounds at other heights. Each tree had 18 wounds. The wounds in each of the three heights were positioned so that like numbers were not in the same vertical position.

Wounds 1 and 3 received 1 ml of sterile water immediately after they were inflicted. Wounds 2 and 5 were inoculated with *P. melinii*; wounds 3 and 6 on the Massabesic Experimental Forest and Hubbard Brook Experimental Forest were inoculated with *F. connatus*; and wounds 3 and 6 on the Bartlett Experimental Forest were inoculated with *F. igniarius*. On 20, 21, and 22 July, 1970, wounds 5 and 6 on all trees were inoculated again with the Hymenomycete that was first inoculated into that tree, and wounds numbered 4 were inoculated for the first time with the same Hymenomycete inoculated in the other wounds on each tree.

Fresh cultures of *P. melinii, F. connatus,* and *F. igniarius* from discolored and decayed wood in red maple were used for inocula. The inocula were prepared from cultures growing in 25 ml of a medium (10 g malt extract and 2 g yeast extract/liter of distilled water) in 250-ml Erlenmeyer flasks for 3 wk at 25 C in darkness. Cultures from two flasks were washed thoroughly in sterile distilled water and chopped for 5 s in a blender with sterile water added to make 25 ml. The procedure was repeated to make several vials of inoculum of each fungus. One ml of the inoculum was used per wound.

Three wounded trees chosen at random from each area were cut from 12 to 17 August 1970 and 2.5 m basal trunk sections were taken to a laboratory in Durham, New Hampshire. Billets 30 × 5 × 5 cm, with the wound in the center, were split from the trunk sections. In a clean room, and with a sterile ax, each billet was split longitudinally through the wound (Fig. 1). Immediately after the billets were split, six chips of wood, ca. 3 × 3 × 10 mm, were cut out with a sterile gouge from above and six chips from below the wound. The chips were taken systematically from 1-10 cm above and below the wound. Six chips per dish were placed in petri dishes containing a medium for isolation (10 g malt extract, 2 g yeast extract, and 20 g agar/liter of water). The microorganisms growing from the 1944 chips at 25 C in darkness were recorded after 2 wk, and longer for slow-growing organisms.

The same procedures were carried out on two vigorous nonwounded red maple trees from the study area on the Massabesic Experimental Forest, and 180 chips of unaffected, clear wood were incubated in the agar medium. Also, after 12 wk, samples of the original batches of inocula were spread on agar medium and incubated to determine whether they were still viable.

RESULTS AND DISCUSSION.—*Phialophora melinii* was isolated from 50 of 54 wounds inoculated with it, from 28 of 54 wounds not inoculated with it, and from 21 of 54 wounds inoculated with *F. connatus* and *F. igniarius* (Table 1). Although *P. melinii* was isolated from wounds other than those inoculated with it, the number of chips yielding it was much greater from inoculated wounds than from noninoculated wounds (Table 1). Bacteria were isolated frequently from all wounds. *Fomes connatus* was isolated from one chip of 874 from 72 wounds inoculated with it. *Fomes igniarius* was not isolated from any of the wounds to which it had been introduced. Many species of fungi were isolated. Those isolated most frequently were species of *Cytospora, Graphium,* and *Ceratocystis* (*C. pluriannulata* and *C. coerulescens*).

The four wounds inoculated with *P. melinii* that did not yield *P. melinii* after 12 wk were from two trees on the Bartlett Experimental Forest. Three of the wounds were on one tree, and the chips from discolored wood

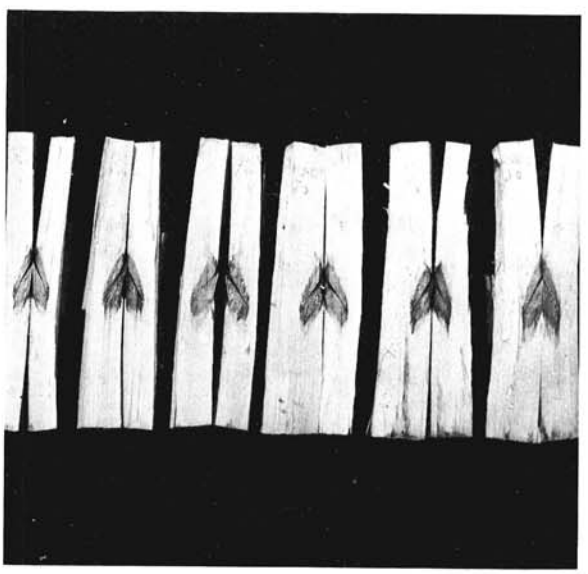

Fig. 1. Discolored wood associated with 12-wk-old inoculated and noninoculated wounds inflicted in June on red maple. The split billets show six wounds at one level.

TABLE 1. Fungi isolated most frequently from discolored wood associated with 12-wk-old June wounds in red maple

Fungus and location[a]	Treatment[b]					
	1	2	3	4	5	6
Phialophora melinii						
HB	3-8-27[c]	3-9-49	3-7-10	2-5-22	3-9-66	2-4- 8
B	1-2- 7	3-6-32	1-2- 2	1-1- 3	3-8-39	1-2- 4
M	3-7-17	3-9-71	3-9-39	2-5-19	3-9-57	2-3-15
Cytospora sp.						
HB	3-9-64	3-8-36	3-9-60	3-9-62	3-6-28	3-9-55
B	2-2- 3	0-0- 0	1-1- 3	0-0- 0	0-0- 0	0-0- 0
M	2-3- 5	1-1- 1	3-3- 7	1-2- 5	0-0- 0	1-1- 1
Graphium sp.						
HB	2-3-11	2-3- 8	2-2- 4	2-2- 7	2-4- 6	2-6-24
B	3-9-55	3-6-36	3-7-33	3-9-50	3-7-21	3-9-58
M	3-9-66	3-4-15	3-8-36	3-9-46	3-4-16	3-7-37

[a]HB = Hubbard Brook Experimental Forest, B = Bartlett Experimental Forest, and M = Massabesic Experimental Forest.
[b]Treatments: 1, water-inoculated control; 2, *Phialophora melinii*-inoculated; 3, Hymenomycete-inoculated, *Fomes connatus*-inoculated at HB and M, and *F. igniarius*-inoculated at B; 4, same as 1; 5, same as 2; and 6, same as 3 for 6 wk, and then all inoculated with *F. connatus* at HB and M, and *F. igniarius* at B.
[c]Number of trees from total of three, number of wounds from total of nine, and number of wood chips from total of 108, respectively, that yielded the fungus.

associated with these wounds primarily yielded rapid-growing isolates of *Ceratocystis coerulescens*.

Discolored wood was associated with every inoculated and noninoculated wound. Although initially all wounds were similar in size, and after 12 wk their sizes varied greatly due to bark cracks and cambial death, the vertical extensions of discolored wood were fairly uniform at 2 cm above and below the wounds (Fig. 1). The 12-wk-old inocula grew on the malt-yeast agar medium. No organisms grew from the 180 chips from the two control trees.

These results indicate that with the type of wounds made in June on red maple, *P. melinii, Cytospora* sp., *Graphium* sp., and bacteria were aggressive pioneer invaders of recently exposed wood, whereas *F. connatus* and *F. igniarius* were not, even though the latter were introduced into the wounds at two different times. Even so, the relatively common occurrence of decay associated with these Hymenomycetes in red maple indicates that they must also be aggressively invasive fungi, but probably at a later period after the wood exposed by wounds has been altered in some way. Shortle et al. (5) showed that *P. melinii* tolerated and utilized phenolic compounds that were toxic to *F. connatus*.

Fomes igniarius did not grow on blocks of heat-killed, sterile, clear wood in bottles, while similarly treated blocks of discolored and slightly decayed wood were decayed further, indicating that *F. igniarius,* like *F. connatus,* requires wood altered in some way before it can invade (4). The results reported here give additional support to the view that successions of microorganisms are involved in discoloration and decay of red maple.

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