Sequence of Microorganisms and Changes in Constituents Associated with Discoloration and Decay of Sugar Maples Infected with Fomes connatus

Terry A. Tattar, Walter C. Shortle, and Avery E. Rich

Graduate Student, Former Graduate Research Assistant, and Plant Pathologist, respectively, New Hampshire Agricultural Experiment Station, Department of Botany, University of New Hampshire, Durham 03824. Present address of second author: Department of Plant Pathology, North Carolina State University, Raleigh 27607.

Published with approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 532. Supported in part by National Defense Education Act Fellowship Grant 68-02747.

Based on a portion of a Ph.D. thesis by the senior author.

The counsel and advice of Alex Shigo of the Northeastern Forest Experiment Station are acknowledged with gratitude.

Accepted for publication 15 December 1970.

ABSTRACT

Discolored and decayed tissue associated with *Fomes connatus* in sugar maple showed a pattern of physical changes and microbial successions. The lignin-to-cellulose ratio remained approximately the same in clear, discolored, and decayed tissue. No quantitative change occurred in the amount of total extractives in all tissues. The pH and ash concentration increased and total phenolic compounds decreased as tissues became discolored and decayed. Microorganisms were in discolored tissue that showed qualitatively altered extractives and increases in pH. *Fomes connatus* was in a narrow zone of discolored tissue at the border of discolored and decayed tissue. Microorganisms were in decayed tissue that showed qualitative changes in extractives, increases in pH, and substantial decreases in amount of lignin and cellulose. Phytopathology 61:556-558.

Additional key words: Acer saccharum, succession of microorganisms, white-rot.

Decay in living sugar maple (*Acer saccharum* Marsh.) results from processes in which woody tissues first become discolored (8, 13). The discoloration process involves host response to wounding and the activity of microorganisms. Discolored tissue may then be decayed by hymenomycetous fungi. Decay of sugar maple associated with *Fomes connatus* (Weinm.) Gill. causes severe structural damage resulting in economic losses (10). Previous descriptions and classifications as a white-rot were made on the basis of color and texture of the decayed tissue (3, 17). Analyses of tissues altered by white-rot fungi were confined mainly to decay tests on wood blocks (5, 12). Moreover, white-rot fungi used in these studies were mainly those that decay dead wood.

In order to better understand the nature of decay in living trees, we studied decay columns in sugar maple trees by mapping natural wounds infected with *F. connatus* (15). This was followed by studying the relationships between microorganisms and chemical changes in discolored and decayed wood.

MATERIALS AND METHODS.—*Material preparation.*—Thirteen sugar maple trees, 8- to 15-cm diam, 1.2 m above ground, bearing fruit bodies of *F. connatus,* were cut. Logs were cut transversely through the sporophores and at 10-cm intervals above and below until the columns of decay and discoloration ended. The column sections were dissected aseptically and mapped for microorganisms. Chips of wood 3 × 10 mm were excised from the column and incubated on a medium consisting of 10 g malt extract, 2 g yeast extract, and 20 g agar/liter (13). Approximately 800 chips were cultured.

Samples of clear, discolored, and decayed tissue were obtained from seven trees. Samples were ground to pass a 40-mesh but not a 60-mesh screen. Approximately 10 g of air-dried, clear, discolored, and decayed tissue were obtained from each column.

Determination of ash concentration.—One-g air-dried samples were ashed in a muffle furnace at 600 C for 24 hr. Amount of ash in the crucible was recorded as total ash/g. Extracted wood samples equivalent to 1 g oven-dried unextracted tissue were ashed, and the amount remaining was recorded as unextracted ash/g. Soluble ash was determined by subtracting unextracted ash from total ash.

Determination of amount of total extractive.—Weights of oven-dried tissue were determined from 1 g of each air-dried tissue. Samples were extracted successively with alcohol-benzene (1:2, v/v) and alcohol in a Soxhlet apparatus, then in a boiling water bath (1). Amount of total extractives was calculated as wt loss from unextracted tissues that were oven-dried.

Determination of total phenolic compounds.—One-g air-dried samples of each tissue were extracted with methanol for 8 hr in a Soxhlet apparatus. Each extract was brought to 50 ml and concentrated to 5 ml in a rotary evaporator. Extracts were centrifuged at approx 750 g for 10 min in an International clinical model centrifuge and decanted. Total phenolic compounds were determined on a 0.5-ml sample by the Folin-Ciocalteu total phenol method (9).

Determination of lignin and cellulose.—Lignin content was determined on extracted wood samples equivalent to 1 g oven-dried unextracted tissue by the 72% sulfuric acid method (2). Cellulose content was calculated by subtracting amounts of lignin and insoluble ash from sample weights of extractive-free tissue.

Determination of pH and specific gravity.—One-g samples of air-dried tissues ground to pass a 20-mesh screen were placed in 5 ml deionized water for 1 hr, and

the pH was measured on a Beckman pH meter (19). Approximately 4-cc sections of each tissue were placed in distilled water in graduated cylinders and repeatedly aspirated until refusal. Displacement volume was recorded. Tissue were oven-dried and sp gr was calculated as the ratio of oven-dried wt of tissue to displacement volume.

RESULTS AND DISCUSSION.—*Organisms isolated from columns of discolored and decayed tissue.*—Very few organisms were isolated from clear tissue. Bacteria and nonhymenomycetous fungi, mostly of the genera *Phialophora* and *Acrostaphylus*, were isolated from discolored tissue in advance of *F. connatus*. *Fomes connatus* was isolated in a narrow band of discolored tissue at the border of decayed and discolored tissue (Fig. 1). Bacteria, Actinomycetes, and nonhymenomycetous fungi, *Trichoderma viride* Pers. ex Fries and *Mortierella* sp., were isolated commonly from decayed tissue behind *F. connatus*. Nematodes (*Rhabdites* sp.) and black carpenter ants (*Camponotus* sp.) were common in tissue in an advanced state of decay. Microorganisms from these columns associated with *F. connatus* were found in a successional pattern as described for sugar maple (14, 17).

Characteristics and composition of clear, discolored and decayed sugar maple tissue.—The chemical composition of clear, discolored, and decayed tissues is given in Table 1. Specific gravity of clear tissue (0.6) did not change measurably in discolored tissue, but decreased to 0.25 in decayed tissue. These data indicate a wt loss of 42%/unit volume in decayed tissue. Chemical constituent values were adjusted for decayed tissues to account for the noted changes in sp gr, allowing comparisons on a constant tissue volume basis (Table 1). The pH of discolored (6.4) and decayed (6.6) tissues was significantly greater than that of clear tissues (5.5).

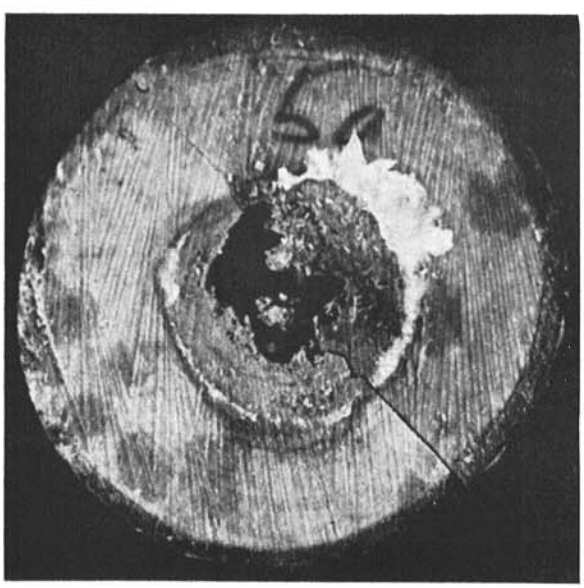

Fig. 1. Decay column cross section of sugar maple showing *Fomes connatus* in a thin band of discolored tissue at the decay border after 1 week's incubation at 25 C.

TABLE 1. Characteristics and composition of clear, discolored, and decayed sugar maple tissue

Constituent	Condition of tissue			
	Clear	Discolored	Decayed	Decayed[b]
	mg/g oven-dried weight[a]			
Total ash	3a	11b	54c	22
Soluble ash	1	4	22	9
Insoluble ash	2a	7a	32b	14
Total extractives	46a	47a	98b	41
Total phenolic compounds	7a	2b	0.3	0.1
Sulfuric acid lignin	144a	174b	184b	77
Cellulose	806a	767b	697b	290

[a] Different letters designate significant differences at the 5% level of significance.
[b] Decay values corrected for difference in specific gravity for comparisons based on weight per unit volume.

Ash.—Amount of total, insoluble, and soluble ash per unit volume increased significantly from 3, 2, and 1 mg, respectively, in clear tissue to 11, 7, and 4 mg in discolored tissue and 22, 14, and 9 mg in decayed tissue. The increase in total ash concentration from clear to decayed tissue was ×18 based on wt and ×8 based on volume. An increase in mineral content was shown to be associated with the processes of discoloration and decay in sugar maple (15).

These increases in soluble and bound mineral constituents could affect the utilization of other wood constituents in several ways. The concentration of inorganic salts can affect the swelling of cellulose micelles in the cell walls (18). Such swelling could affect the activity of cellulases and other wall-attacking enzymes. The mineral constituents can alter pH (8), which increased significantly from 5.5 in clear tissue to 6.4 in discolored and 6.6 in decayed tissue, and could affect the activity of enzymes. Changes in the concentration of an essential cofactor might also be important (11).

Total extractives.—Amount of total extractives per unit volume of clear tissue did not change significantly in discolored and decayed tissues. Although the amount of total extractives appeared relatively constant, there were changes in the composition of extractives during discoloration and decay.

One part of the total extractives which changed radically was that portion comprised of ash constituents. Soluble ash comprised 22% of the total extractives of decayed tissue as compared to 2% of clear tissue. Another part of the extractives which changed radically was the phenolic component. Total extractable phenolic compounds per unit volume decreased from 7 mg in clear to 2 mg in discolored tissue, and were negligible in decayed tissue. In clear tissue, phenolic compounds appeared to comprise 15% of the total extractives, whereas they comprised only 4% in discolored tissue. Total phenolic content was determined from methanolic extracts. A similar pattern of phenolic content was shown by results of extracts from the same tissues which had been extracted with ethanol:benzene (1:2, v/v).

The observed decrease in phenolic constituents may be important to the invasion of *F. connatus*. In vitro growth studies have shown that isolates of *Phialophora melinii* were able to grow at concentrations of certain phenolic compounds which did not permit the growth of *F. connatus*, and the ability of *P. melinii* to utilize and to alter those inhibitory compounds was demonstrated (16). Because of the high frequency of isolation of *Phialophora* sp. in advance of *F. connatus*, it is possible that alteration of phenolic constituents by *Phialophora* sp. or other nonhymenomycetous fungi allowed *F. connatus* to invade discolored tissues.

Lignin and cellulose.—Amount of lignin per unit volume increased significantly from 144 mg in clear to 174 mg in discolored tissue, and decreased significantly to 77 mg in decayed tissue. Amount of cellulose per unit volume decreased significantly from 806 mg in clear tissue to 769 in discolored and 290 mg in decayed tissue. The ratio of cellulose to lignin remained approximately 5:1 in all tissues.

The increase in lignin could be explained by the bacteria and nonhymenomycetous fungi attacking nonlignified cellulose in the discolored tissue. *Phialophora* and other Fungi Imperfecti cause soft-rot in wood products by attacking nonlignified cellulose in the cell walls (7). Several isolates of two species of *Phialophora* utilize cellobiose at rates equal to or greater than D-glucose (4). This decrease in cellulose could be too small to be measured by the sp gr method used in this experiment, and could be sufficient to account for an apparent increase in lignin concentration. Alteration in wall structure caused by nonhymenomycetous fungi and bacteria in advance of *F. connatus* may make the wall more susceptible to its attack. Discolored and decayed tissue differed by 56% in lignin/unit volume and 64% in cellulose/unit volume. There was no significant change in the lignin-to-cellulose ratio, which suggests that lignin and cellulose are utilized at a similar rate.

Another possible explanation of the noted increase in the amount of lignin is that lignin or ligninlike substances actually increased during the process of discoloration. Increases in the amount of lignin have been reported as a resistance mechanism (6). The colored substances formed during the discoloration process may act as sulfuric acid lignin.

Fomes connatus, which was isolated most frequently in the boundary zone between discolored and decayed tissue, is probably the primary organism which attacks the lignified cellulose of the cell wall. *Fomes connatus* appears to attack effectively both lignin and cellulose. However, microorganisms such as *T. viride*, found in the decayed tissues, can produce cellulases (11). While the primary role of *F. connatus* may be to degrade the lignin in cell walls, degradation of cellulose in advanced decay may be due to several extracellular cellulase systems.

LITERATURE CITED

1. American Society for Testing and Materials. 1969. Preparation of extractive free wood. ASTM Desig. D1105-56. ASTM Book of Standards, Pt. 16, Philadelphia, Pa.
2. American Society for Testing and Materials. 1969. Lignin in wood. ASTM Desig. D1106-56. ASTM Book of Standards, Pt. 16, Philadelphia, Pa.
3. Boyce, J. S. 1961. Forest pathology [3rd ed.] McGraw-Hill Book Co., N.Y. 572 p.
4. Brewer, D. 1959. Studies on slime accumulation in pulp and paper mills. II. Physiological studies of Phialophora fastigiata and P. richardsiae. Can. J. Bot. 37:339-343.
5. Cowling, E. B. 1961. Comparative biochemistry of sweetgum by white-rot and brown-rot fungi. USDA Forest Serv. Tech. Bull. 1258. 79 p.
6. Craft, C. C., & W. V. Audia. 1962. Phenolic substances associated with wound-barrier formation in vegetables. Bot. Gaz. 123:211-219.
7. Duncan, C. G. 1960. Wood-attacking capacities and physiology of soft-rot fungi. U.S. Forest Serv. Rep. FPL-2173. 28 p.
8. Good, H. M., P. M. Murray, & H. M. Dale. 1955. Studies on heartwood formation and staining in sugar maple Acer saccharum Marsh. Can. J. Bot. 33:31-44.
9. Horowitz, W. [ed.] 1960. Official methods of analysis of the association of official agricultural chemists. [9th ed.] Assoc. Official Agr. Chem., Washington, D.C. 832 p.
10. Nordin, V. J. 1954. Studies in forest pathology. XII. Decay in sugar maple in the Ottawa-Huron and Algoma extension forest region of Ontario. Can. J. Bot. 32:221-258.
11. Pew, J. C., & P. Weyna. 1962. Fine grinding, enzyme digestion, and the lignin-cellulose bond in wood. Tappi. 45:247-256.
12. Scheffer, T. C., & E. B. Cowling. 1966. Natural resistance of wood to microbial deterioration. Annu. Rev. Phytopathol. 4:147-170.
13. Shigo, A. L. 1965. The pattern of decays and discolorations in northern hardwoods. Phytopathology 55:648-652.
14. Shigo, A. L. 1967. Successions of organisms in discoloration and decay of wood, p. 237-299. *In* International Rev. Forestry Res. II. Academic Press, N.Y.
15. Shigo, A. L., & E. Sharon. 1970. Mapping columns of discolored and decayed tissues in sugar maple Acer saccharum. Phytopathology 60:232-237.
16. Shortle, W. C., T. A. Tattar, & A. E. Rich. 1971. Effects of some phenolic compounds on the growth of Phialophora melinii and Fomes connatus. Phytopathology 61:552-555.
17. Spaulding, P. 1951. Spongy white-rot of hardwoods. Tree Pest Leaflet No. 38, New England Sec., Soc. Amer. Forest. 3 p.
18. Stamm, A. J. 1934. Effect of inorganic salts upon the swelling of wood. U. S. Forest Serv. Rep. FPL-1156.
19. Stamm, A. J. 1961. A comparison of three methods for determining the pH of wood and paper. Forest Prod. J. 11:310-312.