Extractable Phenols in Clear, Discolored, and Decayed Woody Tissues and Bark of Sugar Maple and Red Maple

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ABSTRACT

Ethyl acetate-soluble fractions were prepared from hot water extracts of 10-g samples of clear, discolored, and decayed tissue of sugar maple, *Acer saccharum*, and red maple, *A. rubrum*. Extracts were chromatographed in two dimension cellulose thin-layer chromatography plates with butanol:acetic acid:water (6:1:2) and 7% acetic acid:0.03% sodium acetate. Total phenols were determined by the Folin-Ciocalteu method on methanol extracts of clear red maple tissue. Gallic acid and catechin were identified as the major phenols in clear tissue of both red maple and sugar maple. These phenols were absent from discolored and decayed tissue. Total phenols in clear red maple woody tissues were the same at the cambium and at the pith. The processes of discoloration and decay result in decreases in extractable phenols confined to clear unaffected tissue. The low level of phenols in discolored wood may permit the growth of decay fungi unable to grow at the phenol concentration occurring in clear tissue.

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Additional key words: Fomes connatus, Phialophora melinii, succession of microorganisms.

Phenolic compounds have been reported as the primary factor in the natural resistance of wood to deterioration (8). They affect the growth of decay fungi (7, 9) and canker fungi in culture (5). In living sugar maples (*Acer saccharum* Marsh.), total phenols decreased significantly from clear to discolored tissues, and only trace amounts were found in decayed tissues (13). There is also evidence that phenolic compounds in unaffected tissues of living trees provide a chemical basis for the succession of microorganisms in living trees (9).

The objective of this study was to determine the qualitative and quantitative changes in the extractable phenolic compounds of red and sugar maple as a result of the processes of discoloration and decay. We studied the extractable phenols in bark, sap, clear, discolored, and decayed tissues of red and sugar maple.

MATERIALS AND METHODS.—Ten sugar maple and red maple (*Acer rubrum* L.) trees, 8 to 15 cm in diam, 1.4 m aboveground bearing fruit bodies of *Fomes connatus* (Weinm.) Gill., were cut. Logs were cut transversely through the sporophores and at 10-cm intervals above and below the sporophores until the columns of decay and discoloration ended. The billets were split radially, and then each section was split longitudinally along the radial face. Samples of a relatively uniform age range (ca. four annual rings) were obtained.

Samples of clear, discolored, and decayed tissues and samples of bark of red and sugar maple were ground to pass a 20-mesh screen. Methanol extracts were prepared from 1-g air-dried samples of each tissue (13), total phenols were determined on these extracts by the Folin-Ciocalteu method (4), and the results were compared to Eastman gallic acid reagent standards.

Extraction, chromatography, and identification of phenolic compounds.—Ten grams of each tissue were added to 200 ml of deionized water and autoclaved at 15 psi for 20 min. Extracts were filtered through Whatman No. 40 cellulose paper and extracted with 100 ml of ethyl acetate by continuous shaking for 1 hr. The water layer was then removed in a separatory funnel, acidified to 1 N HCl, and placed in a boiling water bath for 1 hr. The acidified water layer was then extracted with ethyl acetate and separated.

Ethyl acetate extracts were concentrated in a rotary evaporator to 5 ml at 70-72 C. Portions (20-60 μliters) of these extracts were chromatographed on cellulose thin-layer chromatography (TLC) plates with butanol:acetic acid:water 6:1:2 in the first direction and 7% acetic acid:0.03% sodium acetate in the second.

After drying at room temperature, the plates were examined under ultraviolet light before and after exposure to NH_3 and compared to plates with standard phenols. Unknown and standard plates were then sprayed with 1% $FeCl_3$, ferric chloride-ferric cyanide, diazotized sulfanilic acid, or diazotized *p*-nitroaniline (10). Ultraviolet absorption spectra were determined on spots eluted from the chromatogram with 50% (v/v) ethanol on a Beckman DBG-recording spectrophotometer.

Enzyme extracts in 0.1 M potassium dihydrogen phosphate buffer (pH 7) were prepared from separate acetone powders of potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), and mushroom [*Agaricus bisporus* (Lange) Sing.], sprayed on the plates, then incubated overnight in a moist

TABLE 1. Total extractable phenols in clear, discolored, and decayed tissues, and bark of sugar maple and red maple

Tissue	Total phenols (mg/g)[a]	
	Red maple	Sugar maple
Bark	136 ± 10	147 ± 12
Clear	9 ± 2	8 ± 2
Discolored	2 ± 1	2 ± 1
Decayed	Trace	Trace
Xylem sap	0.08 (mg/ml)	0.07 (mg/ml)

[a] Mean of at least five observations. The 95% confidence limits are reported for each treatment mean.

chamber. This method is similar to that used by Waggoner & Dimond (14) to test for the presence of substrates of the *o*-diphenol oxidase enzymes.

RESULTS.—*Total extractable phenols.*—Amounts of extractable phenols were highest in the inner bark and outer bark of both red and sugar maple (Table 1). They remained in approximately the same amounts in clear woody tissues of both species regardless of age (Table 1). Xylem sap of both species collected daily during periods of positive sap pressure contained small amounts (0.04 mg/ml) total phenols. The amounts of phenols increased to 0.54 mg/ml when sap flow became limited near the end of positive sap pressure in April 1971.

In trees containing columns of discolored and decayed tissues, small amounts of phenols were found in these tissues in both species (Table 1). These results agree with those reported previously in similar tissues in sugar maple associated with *Fomes connatus* (13).

Identification of extractable phenols.—Examination of developed TLC chromatograms from water and acid-hydrolyzed extracts of bark and clear tissue of red and sugar maple under ultraviolet light and after application of detection reagents indicated the presence of a number of phenolic compounds (Fig. 1). Similar examination of chromatograms of extracts from xylem sap, discolored, or decayed tissue revealed very few phenols. A yellow fluorescent compound appeared in higher concentration in chromatograms of some discolored samples.

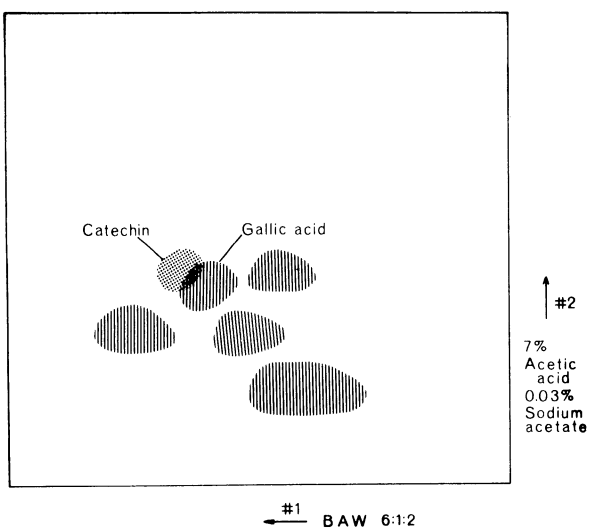

Fig. 1. Thin-layer chromatogram of major extractable phenols from both water and acid hydrolyzed extracts in clear tissue and bark of sugar and red maple. Two-way chromatograms were developed in butanol:acetic acid:water (BAW) (6:1:2) and 7% acetic acid 0.03% sodium acetate.

Detection reagents and ultraviolet spectral analyses revealed that the unknown phenols reacted the same as either the gallic acid or catechin standards. The unknown phenols were located on the 2-way TLC plates in the same position as the gallic acid and catechin standards, or close to them (Fig. 1). Haslam (2), in his report of the phenols in leaves of the Aceraceae, called the compounds galloylesters which were in the vicinity of gallic acid and catechin. The position of these compounds compares very closely with those galloylesters. The yellow fluorescent compound from discolored tissue did not react with any of the detection reagents, and yielded an ultraviolet spectrum which could not be identified.

Enzyme extracts from acetone powders in 0.1 M phosphate buffer of potato, tomato, and mushroon each reacted with the phenols of clear wood and bark tissue (Fig. 1) to destroy the fluorescence and produce light brown pigments. The yellow fluorescent compound previously described did not react with the extracts of the acetone powders.

DISCUSSION.—Both the outer and inner bark of red and sugar maples appeared to be large reservoirs of phenolic compounds. The bark is usually considered the first line of defense against infection and invasion by microorganisms in the living tree. The bark of these trees contained sufficient quantities of phenols to inhibit the growth of microorganisms isolated from discolored and decayed tissues in sugar maple (12). Phenols could represent a chemical barrier in the bark to the entrance of microorganisms into unwounded trees.

Canker fungi that enter through wounds avoid phenols in the bark by invading the sapwood directly through wounds. Hubbes (5, 6) found that *Hypoxylon pruinatum* could not grow in culture on extracts of aspen (*Populus tremuloides*) bark, and could not infect healthy aspen bark when inoculum was placed upon the surface of living trees, but caused cankers when placed in fresh wounds of these same trees. Inhibitory compounds such as phenols in the bark of living trees may determine the pattern of development of some cankers and canker rots.

Phenols were not accumulated in older clear woody tissues in stems of sugar and red maple. In trees lacking columns of discolored and decayed tissues, the amounts of extractable phenols in the cambial area were comparable to those in the pith area. Sugar and red maple do not produce a true heartwood as found in oaks (*Quercus* sp.). Heartwood formation is considered to involve translocation of extractives produced by the most active tissues at the cambium to older tissues in the xylem until toxic quantities of these compounds kill the oldest living

cells (3, 11). Red and sugar maple tissues have been found to contain live parenchyma cells which were 100 years old (1). There is chemical evidence that older woody tissues in these species do not contain any more natural resistance to decay than those most recently formed.

There is evidence phenols present in clear unaffected woody tissues are not present in discolored and decayed tissues of red and sugar maple. Gallic acid, catechin, and related phenols in clear tissues of these species were probably oxidized during the host-parasite interactions and were not detected in discolored or decayed tissues.

The major phenols of bark and clear tissues of red and sugar maple gave positive reactions upon treatment with the enzymes of tomato, potato, and mushroom tissue. These enzyme extracts were chosen because of the difficulty in obtaining enzyme preparations from the host and the large amount of polyphenol oxidase enzymes known to be present. It is possible these phenols are oxidized by polyphenol oxidase enzyme systems of both the host and invading microorganisms during the processes of discoloration.

Gallic acid was found to strongly inhibit the growth of *F. connatus*, a hymenomycetous fungus associated with discolored and decayed tissue of sugar and red maple (10). In this same study, *Phialophora melinii*, a nonhymenomycetous fungus commonly associated with *F. connatus* in discolored tissues, was able to alter gallic acid in culture and permit the growth of *F. connatus*. *Phialophora melinii* was the only fungas associated with *F. connatus* in sugar maple which substantially utilized gallic acid (12). It is possible that extractable phenols occurring in the woody tissues of red and sugar maple determine the initial invading microorganisms, which in turn affect the following microbial successions in discolored and decayed tissues. Wounds in sugar and red maple may be predisposed to the invasion of *P. melinii* due to the presence of gallic acid and galloylesters. *F. connatus* and *Polyporus glomeratus* are commonly isolated in discolored tissue behind *Phialophora melinii*.

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