Compartmentalization of Decay in Red Maple and Hybrid Poplar Trees

Walter C. Shortle

Research plant pathologist, Forest Service, U.S. Department of Agriculture, Northeastern Forest Experiment Station, Durham, NH 03824.

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ABSTRACT

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Samples of wood discolored as a result of drill-bit wounds, the contiguous sapwood, and the bright-colored marginal tissue between them were taken from red maple (*Acer rubrum*) and hybrid poplar (*Populus deltoides* × *P. trichocarpa*) trees. The wounds (1.5 cm diameter × 5 cm deep) that initiated discoloration were 1–4 yr old in maple and 1 yr old in poplar. The concentration of soluble dry matter and phenols was greater in wood in the marginal zones of both species than in sapwood or discolored wood. Phenols in the marginal zones differed from those of sapwood and discolored wood by their solubility in organic solvents, ultraviolet spectra, and chromatographic behavior. Soluble dry matter in the marginal zone, which was rich in phenols, inhibited the growth in vitro of two fungi that commonly decay living red maple trees. Zones of high phenolic content may limit the spread of microorganisms colonizing discolored wood and may account, in part, for compartmentalization of decay in trees.

Additional key words: phenols, wood discoloration.

Decay in living trees is limited to the wood present when the trees are injured (1). This decay is further limited to sapwood or heartwood that becomes discolored by the wound (9). A model system called compartmentalization of decay in trees was proposed to describe this phenomenon (10).

Formation of a phenol-enriched "reaction zone" that surrounds wounds in pine and spruce infected with *Fomes annosus* has been described (5–7). Phenols, as well as other compounds in these zones, inhibit growth of *F. annosus*, which suggests a dynamic mechanism of resistance that limits the spread of decay.

Formation of a similar zone at the margins of columns of discolored and decayed wood in red maple and hybrid poplar trees may account in part for compartmentalization of decay in these trees. This study was performed to determine if quantitative and qualitative differences exist among phenols in sapwood, discolored wood, and the bright-colored margin between them, which might explain the limited spread of fungi in wounded stems.

MATERIALS AND METHODS

Tree species studied were red maple, *Acer rubrum* L., and hybrid poplar, *Populus deltoides* Marsh. × *P. trichocarpa* Hook. The maples, 30–40 yr old, were growing in Bartlett or West Thornton, NH; the 25 yr old poplars were in Alfred, ME. All trees were wounded with a drill bit at 1, 1.5, and 2 m above ground at four locations (1.5 cm diameter × 5 cm deep) at each height. Wounds were 1 yr old (four maples from Bartlett and four poplars from Alfred, harvested in July) or 4 yr old (three maples from W. Thorton, harvested in December) and had been inflicted during the growing season. Trees were felled, and 3-cm disks were cut from bolts containing columns of discolored and decayed wood initiated by the drill bit wounds.

Samples of discolored wood, the contiguous sapwood, and the green-colored (maple) or orange-colored (poplar) marginal tissue between them were taken and treated as follows: (i) Shavings were extracted from fresh disks with a 1.6-mm drill bit and air-dried at 42 C (1 yr old maple wounds), (ii) 0.5 × 1.5 × 3 cm chips were split from fresh disks with a chisel and air-dried (1 yr old poplar wounds), and (iii) shavings were planed from 1.5 × 2 × 3 cm air-dried blocks split from fresh disks (4 yr old maple wounds). The air-dried samples were ground in a Wiley mill to pass a 425-μm mesh sieve.

Wood samples were taken from six additional red maple trees for bioassay of soluble dry matter. Drill wounds were 1–2 yr old. Blocks 1 × 2 × 4 cm were split tangentially from disks and freeze-dried. Shavings were planed from the blocks and ground to pass a 250-μm mesh sieve.

Determination of hot-water-soluble dry matter and total phenols. Duplicate 1-g samples (0.5 g for 6 yr old maple wounds) of air-dried tissue were weighed. One sample was oven-dried to constant weight at 104 C to determine the moisture correction factor. The other sample was extracted by constantly stirring with 50 ml of distilled water under reflux in a boiling water bath for 1 hr. The sample was filtered in a tared fritted glass crucible, porosity C. The filtrate was cooled for chemical analysis. The extracted wood was rinsed with 100 ml of hot water and dried to constant weight at 104 C. Soluble dry matter was calculated as loss in initial oven-dry weight.

Two milliliters of the filtrate were diluted five times with 95% ethanol. Total phenols were determined by the Folin-Ciocalteau method (2). The absorption spectrum of the diluted extract was obtained with a Bausch & Lomb Spectronic 200 UV spectrophotometer. The remaining filtrate was made acid to litmus and extracted three times with 20 ml of ethyl ether (Et_2O) and then with ethyl acetate (EtAc). Each organic fraction was washed, dried over Na_2SO_4, filtered, concentrated over steam, air-dried, dissolved in methanol, and scanned for its UV absorption spectrum. Compounds dissolved in the methanol were then separated by thin-layer chromatography.

Chromatograms were made primarily on precoated silica gel 60 plates (EM Laboratories) with benzene-methanol-acetic acid, 45:8:4; chloroform-methanol, 9:1; or toluene-ethyl acetate-formic acid, 5:4:1. Spots (10 μl) of extracts from sapwood, discolored wood, and marginal tissue were made on the same chromatogram so that direct comparisons of Rf values could be made. Spots on chromatograms were loacted by UV fluorescense and by spraying or dipping in ferric chloride-ferricyanide (FCF) reagent (13). Major spots, reacting with FCF reagent, were located on separate chromatograms by UV fluorescense or by marker strips sprayed with FCF reagent and eluted with methanol or 0.1 N ethanolic

(95%) HCl along with silica gel controls. UV absorption spectra of eluted compounds were determined in methanol or 0.1 N ethanolic (95%) KOH. Major spots also were compared to the following known compounds on the same chromatogram: gallic acid, tannic acid, catechin, salicin, cinnamic acid, *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid, and chlorogenic acid.

Gravimetric determination of phenolic, acidic, and neutral constitutents of red maple tissues. Duplicate, composite 5-g tissue samples from maples with 1 yr old wounds were preextracted with light petroleum ether and air-dried. These samples then were extracted with methanol in a Soxhlet apparatus for 4 hr at 4 cycles per hour. Methanol extractives were concentrated in vacuo, dissolved in water, and, after constant stirring for 1 hr, filtered, acidified, extracted twice with 25 ml of Et_2O, and then twice with 25 ml of EtAc. Organic layers were washed and extracted three times with 10% $NaHCO_3$, then with 10% NaOH. Basic extracts were immediately acidified with 6N H_2SO_4, reextracted with Et_2O or EtAc, washed, dried over Na_2SO_4, filtered, evaporated to dryness in tared aluminum weighing pans, and then oven-dried to constant weight at 104 C. Soluble dry matter in each fraction (acidic, phenolic, and neutral) was expressed as milligrams per gram of moisture-free wood.

Bioassays. Samples of freeze-dried tissues ground to pass a 250-μm mesh sieve (25 g of sapwood and discolored wood, 10 g of marginal tissue) were homogenized for 60 sec in 200 ml of distilled, deionized water in a stainless steel blender cup fitted with a Polytron head. The homegenate was prefiltered through four layers of cheesecloth and then filtered through filter paper under suction. The extract was then frozen and freeze-dried. The resulting powders were dissolved in deionized, distilled water at a rate of 9 mg of powder per milliliter. These solutions were filtered using 0.2-μ filters, and 5-ml portions of the clarified, sterile solutions were each transferred aseptically into sterile, 25-ml Erlenmeyer flasks each containing 5 ml of nutrient solution (mg/ml: 7.5 glucose, 1 yeast extract, 1 KH_2PO_4, and 0.5 $MgSO_4 \cdot 7H_2O$). Flasks were seeded in triplicate with 3-mm agar plugs from 14-day cultures of *Phialophora melinii* (Nannf.) Conant, *Polyporus versicolor* (L.), or *Fomes connatus* (Weinm.) Gill. (all isolates from red maple). Samples were taken from powder solutions to determine pH, total soluble dry matter (2 ml of solution dried at 104 C in tared aluminum pans), total phenols (2), and total carbohydrates (4). Flasks were incubated 14 days at 28 C and the mycelium was harvested on tared Whatman No. 1 filter paper under suction while rinsing with distilled, deionized water. Mycelium was oven-dried at 104 C and mean oven-dry weights were calculated.

Decay tests were conducted on 0.2 × 1 × 3 cm blocks of sapwood, marginal tissue, darkly discolored wood next to the margin, and less discolored wood in early stages of decay cut from freshly cut red maple disks. Three pairs of blocks of similar fresh weight were selected from each tissue. One block from each pair was oven-dried at 104 C to correct fresh weight to oven-dry weight. The other block was sterilized in a microwave oven (3 min at setting 4 of a Sears Microwave Oven Model 99651) and placed in a malt-yeast-agar chamber (8-oz French square bottle) containing a 1-wk culture of *P. versicolor*. Blocks were harvested at 6 wk and surface mycelium was removed. Blocks were oven-dried at 104 C and weight loss determined as a measure of decay.

RESULTS

The concentrations of phenols were significantly greater in the marginal zone (Fig. 1A, B) than in sapwood or discolored wood in both red maple and hybrid poplar (Table 1). Soluble dry matter also was significantly greater in the marginal zone, except in maple with 1 yr old wounds. Planing wood from air-dried blocks taken from trees with 4 yr old wounds gave a much purer sample of the narrow bands (0.5–1.5 mm) of green-colored wood than the drill bit, which cut into some sapwood and discolored wood. Wider bands, 1.5–5 mm of orange-colored wood in poplar were easier to separate from sapwood and discolored wood than from the green-colored wood of maple. Soluble dry matter and phenolic contents of discolored wood from older columns were significantly less than those of sapwood.

In maple, the phenolic fraction (substances soluble in NaOH but not in $NaHCO_3$) of organic substances was much greater in the marginal zone than in sapwood (Table 2). The solubility of phenols in the less polar solvent, ether, reached a maximum in discolored wood. In sapwood, phenols constituted about 30% of the organic substances; the remainder was mostly neutral substances. In the marginal zone, about 75% of the organic substances were phenols, and the remainder was acidic and neutral substances in a 1:2 ratio.

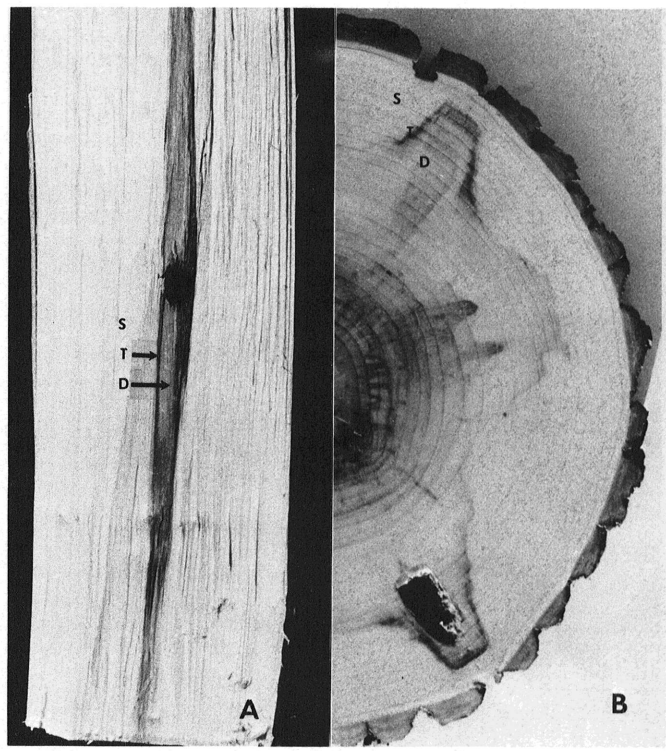

Fig. 1. A) Tangential view of column of wood discolored as a result of a drill-bit wound in red maple (S = sapwood; T = marginal zone, green; D = discolored wood). **B**) Transverse view of column of discolored wood initiated by drill-bit wounds in hybrid poplar (T = marginal zone, orange).

TABLE 1. Concentration of phenols in tissues of red maple and hybrid poplars

Species	Tissue		
	Sapwood	Marginal	Discolored
	mg phenol/g moisture-free wood[a]		
Red maple I[b]	8	13**	5
Red maple II[c]	16	35**	9**
Hybrid poplar[d]	4	16**	3
	mg soluble dry matter/g moisture-free wood		
Red maple I	58	75	54
Red maple II	49	79**	27**
Hybrid poplar	30	51**	30
	mg phenol/g soluble dry matter		
Red maple I	135	188*	103
Red maple II	334	447**	326
Hybrid poplar	122	311**	107

[a] Means significantly different from sapwood indicated by asterisks (* = $P < 0.05$; ** = $P < 0.01$).
[b] Means of four duplicate determinations per tissue sample from each of four trees. Samples from trees with 1 yr old wounds cut in summer.
[c] Means of three duplicate determinations per tissue sample from each of three trees. Samples from trees with 4 yr old wounds cut in winter.
[d] Means of four duplicate determinations per tissue sample from each of four trees.

In discolored wood, about 40% of the organic substances were phenols; the remainder was acidic and neutral substances in a 1:4 ratio.

No UV spectrum of red maple sapwood total extracts, organic fractions, or major spots reacting with FCF reagent had a maximum absorbance (λ max) between 300 and 370 nm. All UV spectra of red maple marginal tissue total extracts, organic fractions, and major spots reacting with FCF reagent had a λ max between 300 and 370 nm. Five major spots were consistently observed on chromatograms of extracts of marginal tissue, but substances eluted from these spots varied in retardation factor values, UV absorption maxima, and bathochromic shifts. Most spectra of red maple discolored wood total extracts, organic fractions, and major spots had weak absorption maxima in the 270–300 nm region, but occasionally bands occurred in the 320–360 nm region.

UV absorption spectra of total extracts and organic fractions of marginal tissue of hybrid poplar had a λ max at 278 nm, which did not occur in sapwood or discolored wood. Several readily oxidized spots from marginal tissue were observed on chromatograms, but these were not characterized further.

TABLE 2. Concentration of phenolic dry matter in ether and ethyl acetate fractions of methanol extracts of sapwood, marginal zone, and discolored wood of red maple

Fraction	Sapwood	Marginal	Discolored
	mg phenolic dry matter/g moisture-free wood[a]		
Ether fraction	0	1	1
Ethyl acetate fraction	2	8	2
	% of total phenols[b]		
Ether fraction	0	10	20
Ethyl acetate fraction	25	60	40

[a] Based on duplicate determination of extracts made on 5-g composite tissue samples of red maple I.
[b] Percentage based on total phenols for red maple I, Table 1.

TABLE 3. Growth of fungi on soluble dry matter[a] extracted from sapwood, discolored wood, and marginal tissue of red maple

Fungi	Growth (mg) on: Sapwood	Marginal wood	Discolored wood
Phialophora melinii	5	3*	3*
Polyporus versicolor	4	1*	1*
Fomes connatus	3	0*	1*

[a] 6 mg/ml oven-dry mycelium at 14 days. Mean of 3 observations; Asterisk (*) indicates mean differs significantly from sapwood mean. LSD = 1.55 ($P < 0.05$).

TABLE 4. Composition[a] and pH of extracts from three wood tissues used for assay of fungal growth

Factor	Extracts from: Sapwood	Marginal wood	Discolored wood
pH	6.0	6.6	7.0
Soluble dry matter (mg/ml)	6.0	5.5	5.5
Phenol (mg/ml)	0.5	2.0	0.6
Carbohydrate (mg/ml)	5.5	1.2	1.6
Other (mg/ml)	0	2.3	3.2

[a] Average of three determinations.

Fungi grew in nutrient solution (10 mg of dry matter per milliliter) without formation of soluble pigments—*P. melinii* (14 mg), *P. versicolor* (9 mg), and *F. connatus* (6 mg) at 14 days. All fungi grew significantly less in solutions of soluble dry matter from discolored wood and marginal tissue than from sapwood (Table 3), although each solution contained approximately the same total concentration of dry matter, 6 mg/ml (Table 4). *P. melinii* grew more than the decay fungi, *P. versicolor* and *F. connatus*, in solutions from all tissues. Although the marginal tissue solution was highest in phenol and lowest in carbohydrate (Table 4), growth was equally poor on dry matter from discolored wood (Table 3).

Greenish brown solutions from discolored wood and marginal tissues became dark brown during incubation with *P. melinii* and *F. connatus*. The same solutions became pale yellow (discolored wood) and light golden brown (marginal tissue) during incubation with *P. versicolor*. Nearly colorless solutions of sapwood became pale yellow during incubation with *P. versicolor* and darker shades of yellow-brown with *P. melinii* and *F. connatus*.

Weight loss of wood blocks caused by *P. versicolor* after 6 wk was as follows: sapwood 45%, marginal tissue 7%, darkly colored wood contiguous to marginal tissue 10%, lighter discolored wood in early stages of decay 23% (LSD = 17%, $P < 0.05$).

DISCUSSION

The brightly colored marginal zones between sapwood and discolored wood of red maple and hybrid poplar are rich in phenols, which differ from those in sapwood or discolored wood. The phenols of red maple were not gallic acid or catechin found in sapwood and removed from discolored wood (14), common hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic), or chlorogenic acid.

All three test fungi grew more poorly on water-soluble dry matter of marginal tissue and discolored wood, which were both sapwood at the time of wounding, than on dry matter of unaltered sapwood. *P. melinii*, a common pioneer colonizer of discolored wood in maple, grew better than either decayer of maple. This is consistent with earlier observations that *P. melinii* grew better than *F. connatus*, an important agent of decay in maple trees, on a variety of phenols at concentrations less than those of marginal tissues in this study (12). Wood decayed by *F. connatus* has virtually no phenols (15). In this study the phenol/carbohydrate (wt/wt) ratio of dry matter increased from 1:10 in sapwood to 1:3 in discolored wood to 2:1 in marginal tissue, indicating that conditions were unfavorable for decay fungi at the marginal tissue.

P. versicolor, common in early decay of red maple, readily decayed unaltered sapwood, but it caused little decay of marginal tissue or contiguous darkly discolored wood. Decay appeared to increase in partially decolorized, discolored wood. Decolorization of discolored wood is important to the growth of decay fungi (11). In this study, *P. versicolor* removed pigments from solutions where it grew poorly. Pigment loss was greater from discolored wood dry matter than from marginal tissue dry matter.

The increased soluble dry matter content, which differs in composition from sapwood or discolored wood in two unrelated tree species, indicates a dynamic defense mechanism operating within the wounded tree. Such a mechanism is consistent with: (i) the concept of compartmentalization (9,10), (ii) the general response of plant tissue to injury and infection by a shift in oxidative metabolism that favors accumulation of phenols (3,5), (iii) the specific response of pine and spruce to injury and infection by *F. annosus* (5,7,8), and (iv) the role of natural phenols in decay resistance (6).

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