Physiology and Biochemistry

An Ion Profile of Wounded Red Maple

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ABSTRACT

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Twelve red maple trees (*Acer rubrum*) with multiple drill wounds were
studied to determine the ion content of tissues associated with wounds.
Water extracts of sapwood, discolored wood, and the boundary layer
between them were analyzed for ion content by ion chromatography and
atomic absorption spectrophotometry. Total phenols, extract electrical
resistance, pH, and total soluble dry matter were also determined. There
were greater accumulations of K^+, Ca^{2+}, acetate, oxalate, formate, and
phenols in the boundary tissue than in sapwood. Discolored wood had
increased levels of K^+, acetate, malate, oxalate, and formate compared to
sapwood. The organic anions seem to occur as acids in the discolored wood
and as K-salts in the boundary tissue.

When a tree is wounded, a column of discolored and infected
wood begins to form and the exposed wood may be invaded by a
succession of microorganisms (9,10,13,14,23). Infections that may
form are walled off or compartmentalized in the tree by protective
layers of tissue (11,16), referred to here as column boundary layers.
The column boundary layer is related to walls 1, 2, and 3 of the
CODIT (Compartmentalization Of Decay In Trees) model (12),
but does not include wall 4, the barrier zone (7). The column
boundary in red maple has been previously described (4,15,20).

Electrical resistance has been used to detect early stages of decay
in trees (19,22). As wood becomes progressively less sound, the
resistance becomes lower, indicating increased ion concentrations
in the intercellular spaces (22). Potassium and hydrogen cations are
primarily responsible for this drop in resistance in the discolored
wood (1,3,8,17,18,22). Electroneutrality must be maintained; thus,
increased anion concentrations must accompany increased cation
concentrations.

The increased ion concentrations in the discolored wood may
indicate that shifts in metabolism occur when a tree responds to
wounding and infection. If this is so, an understanding of the
identity and concentrations of the ions present in sapwood,
discolored wood, and the column boundary layer would help to
identify what the changes are, as these ions would be the by-
products of reactions of the metabolic pathways utilized by the
wounded tree.

MATERIALS AND METHODS

Tissue samples. Twelve red maple trees with 15-mo-old drill wounds were cut in the Hubbard Brook Experimental Forest in August 1982. Each tree had 12 holes 5 cm deep and 1.4 cm in diameter. There were three holes 1 cm apart in a vertical line on each of four faces of the tree. A single bolt was cut from each tree, 60 cm above to 60 cm below the wounds. The 12 bolts were brought back to the laboratory and cut with a chain saw into disks 5 cm thick the next day. Electrical resistance measurements were made on the fresh disks by using a model OZ-67 Shigometer (Osmose Wood Preserving Co., Buffalo, NY). The method of Shortle and Ostrofsky (18) was used except that readings were taken randomly in the various zones rather than at 1-cm intervals. The electrical resistance of the sapwood and discolored wood was measured; resistance of column boundary layers was not measured because of their small size (<1 mm wide, Fig. 1). The disk immediately above and immediately below the sets of wounds were used to split out blocks containing sapwood, discolored wood, and boundary-layer tissue. Other disks were used to collect additional boundary-layer tissue. The blocks were placed in paper bags and oven-dried overnight at 55 C. The three different tissues were separated using a wood planer, and the chips were ground in a Wiley mill to pass a 420-μm sieve. Prior to analysis, powders of like tissue were pooled and stored in glass jars in a dark, dry cabinet at room temperature.

Preliminary characterization of tissues. Electrical resistance of wood extracts was measured by extracting 0.2 g of tissue in 10 ml of H_2O according to the method of Shortle and Ostrofsky (18).

To measure total soluble phenols, triplicate 20-mg samples of each tissue type were extracted in 4 ml of 76% ethanol at 70 C for 1 hr with vortexing for about 2 sec at 15 and 30 min. Total phenols were determined for the extracts by using the Folin-Ciocalteau method (5).

Absorbance was measured with a model 200 Bausch & Lomb Spectronic UV Spectrophotometer (Shimadzu Seisa Kusho, Ltd., Kyoto, Japan).

Triplicate 1-g, air-dried samples of each tissue type were extracted in 50 ml of boiling water under reflux with constant stirring for 1 hr. After extraction, the wood was filtered on fritted glass crucibles, rinsed, oven-dried, and used for elemental cation
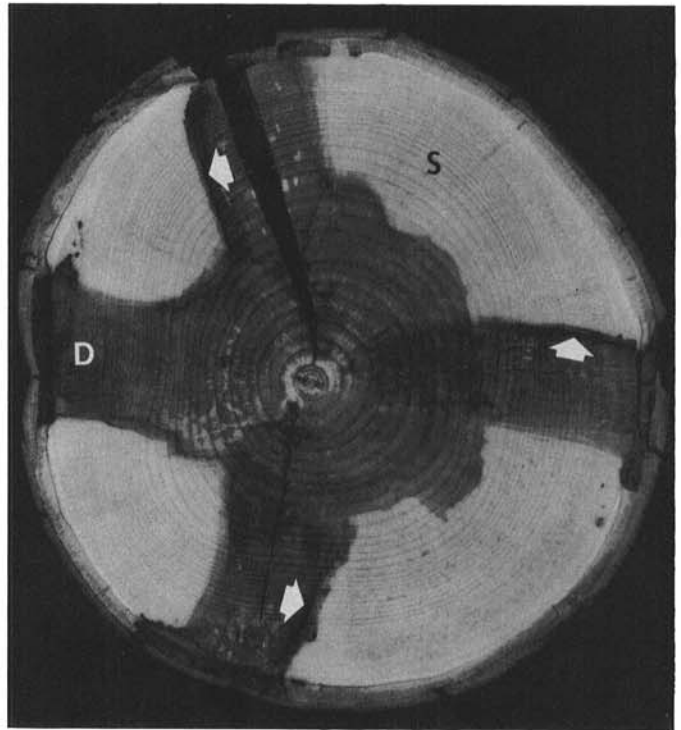

Fig. 1. Disk from wounded tree showing sapwood (S), column boundary layer (arrows), and discolored wood (D).

TABLE 1. Concentration of ions readily soluble in distilled, deionized water, found in red maple trees

Soluble ions	Mean concentration (μg/g)[a]			Mean concentration (meq)[b]		
	Sapwood	Column boundary layer	Discolored wood[c]	Sapwood	Column boundary layer	Discolored wood
Cations, monovalent						
Potassium	640	4,763*[d]	936*	0.33	2.00	0.50
Sodium	92	<1*	<1*	0.08	<0.01	<0.01
Hydrogen	<1	<1	<1	0.01	<0.01	0.01
Cations, divalent						
Calcium	<1	764*	15	<0.01	0.80	0.02
Magnesium	68	<1*	30*	<0.01	<0.01	<0.01
Manganese[e]	5	86*	2	<0.01	0.03	<0.01
Anions, inorganic						
Chloride	57	130	62	0.03	0.08	0.04
Sulfate	33	24	20	0.01	<0.01	<0.01
Phosphate	19	13*	13*	<0.01	<0.01	<0.01
Nitrate	3	17*	54*	<0.01	<0.01	0.06
Anions, organic						
Acetate	280	2,800*	2,300*	0.09	0.94	0.75
Malate	165	190	1,700*	0.04	0.06	0.50
Oxalate	43	750*	2,100*	0.02	0.34	0.92
Formate	120	230*	350*	0.05	0.10	0.15
Totals						
Cations, total	805	5,613	983	0.42	2.86	0.53
Anions, total	720	4,154	6,599	0.25	1.52	2.42
Anions, organic	608	3,970	6,450	0.20	1.44	2.32
All ions	1,525	9,767	7,582			
Dry matter[f]	21,000	42,000*	14,000*			

[a] Mean of triplicate samples in micrograms per gram of oven-dry tissue.

[b] Milliequivalents derived from same extracts used to determine micrograms of soluble ions per gram at a fixed rate of 1 g of tissue per 50 ml of H_2O held at 100 C for 1 hr with constant stirring under reflux.

[c] Discolored wood in early phase of column development with ER values generally equal to or greater than the sapwood values.

[d] Asterisk (*) indicates means that differ significantly, $P<0.05$, from mean values for sapwood.

[e] Manganese is reported as Mn^{2+}, but other forms are possible.

[f] Total dry matter soluble at fixed rate of extraction, footnote b.

analysis. Soluble dry matter determinations were made on 20-ml samples of each water extract.

Determination of soluble elemental cations. To determine the concentration of soluble elemental cations, triplicate 0.5-g samples of whole tissue and hot-water-extracted tissue (1 g/50 ml as described) were digested with a nitric/perchloric acid mixture, and the digests were analyzed for K, Na, Ca, Mn, and Mg with a model 306 Perkin-Elmer Atomic Absorption Spectrophotometer (Perkin-Elmer Analytical Instruments, Norwalk, CT). The concentration of elements in the whole tissue and in the extracted tissue was calculated as milligrams per gram of oven-dried wood by using a moisture correction factor to determine the oven-dry weight of the 0.5-g sample. The soluble elemental cation concentration was calculated by subtracting the concentration of insoluble element from the total concentration of the element in the sample.

The concentration of H^+ in the extract solution (1 g/50 ml) was determined with a pH electrode.

Determination of soluble elemental anions. Samples (1 g, air-dried weight) were extracted for 1 hr in 50 ml of boiling water under reflux with constant stirring. These extracts were analyzed by using a model 10 Dionex Ion Chromatograph (with anion concentrator, separator, and suppressor columns (Dionex Corp., Sunnyvale, CA). An aqueous solution of 3 mM $NaHCO_3$ and 2.4 mM Na_2CO_3 was used as an eluent for the analysis with a flow rate of 1.9 ml/min. A second aqueous eluent, which changed the elution order of the peaks, was 1 mM $NaOH$ and 2 mM Na_2CO_3, and was used only to confirm the identity of the peaks. Three extracts of each tissue were analyzed; two chromatograms were run for each extract. Identification was done by comparison with standard solutions that were run on the same day and at the same instrument settings. Samples were also amended with appropriate standards when it was discovered that the retention times of some ions were affected by the sample matrix. Quantitation was accomplished by preparing standard curves and comparing peak heights with those of solutions of known concentration. Standard curves were run daily for each ion determined to be present in the sample extracts.

Extracts prepared as above were also analyzed for weak organic acids with Dionex Ion Chromatography Exclusion (ICE) separator and suppressor columns in the ion chromatograph. An eluent of 0.05 mM HCl (aqueous) and a flow rate of 0.38 ml/min were used to achieve separation; identification and quantitation were done as described above. Confirmation of the identities of these peaks was done by cochromatography with a Bio-Rad TSK Gel IC-Anion-SW column (Bio-Rad Laboratories, Richmond, CA) and an aqueous eluent of 1 mM potassium acid phthalate with a flow rate of 0.77 ml/min.

RESULTS

The predominant water-soluble cation in all tissues was K^+, accounting for 80% (sapwood), 84% (column boundary layer), and

TABLE 2. Characteristics of tissue samples used for ion analysis

Character[a]	Sapwood	Boundary layer	Discolored wood
Soluble dry matter (mg/g dry wood)	21	42*[b]	14*
Total phenols (mg/g dry wood)	11	17*	8*
Extract ER[c] (kohms)	204	93*	235
Extract pH	5.0	6.4*	5.1

[a] Values are the means of three observations. Extracts for soluble dry matter and pH were made by boiling 1 g of tissue in 50 ml H_2O under reflux with constant stirring for 1 hr. Extacts for phenols were made by heating 20 mg of tissue in 4 ml of 76% ethanol for 1 hr with stirring at 15 and 30 min. Extracts for ER were made by heating 0.2 g of tissue in 10 ml of H_2O at 90 C for 1 hr with stirring at 15 and 30 min.
[b] Asterisks (*) indicate means that differ significantly, $P = 0.05$, from mean values for sapwood.
[c] ER = electrical resistance.

95% (discolored wood) of the total mass of cations and 79% (sapwood), 70% (column boundary layer), and 94% (discolored wood) of total cationic milliequivalents (Table 1). Na^+ was detectable only in sapwood, accounting for 11% total cation mass and 19% total cationic milliequivalents. Ca^{2+}, which was mostly insoluble, was partially soluble in the column boundary layer, accounting for 15% total cation mass and 28% total cationic milliequivalents. H^+ made no significant contribution to total mass or total milliequivalents in any of the tissues. H^+ concentrations were roughly equivalent in sapwood and discolored wood (pH 5) and decreased 10-fold in the column boundary layer (pH 6).

The predominant anions in solution were the organic anions acetate, malate, oxalate, and formate. These anions accounted for 84% (sapwood), 96% (column boundary layer), and 98% (discolored wood) of the total anionic mass and 80% (sapwood), 95% (column boundary layer), and 96% (discolored wood) of the total milliequivalents. The unresolved anions needed to balance total cationic milliequivalents, accounting for 40% (sapwood) and 47% (column boundary layer) of the needed total anionic milliequivalents were probably carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-). These common anions found in plant tissues could not be determined with the available ion chromatograph. Attempts to titrate carbonate and bicarbonate failed due to the buffering capacity of the extract, which behaved much like an acetate/acetic acid buffer.

The organic anions in discolored wood probably existed as the partially ionized organic acids (acetic, malic, oxalic, and formic), which would account for the apparent excess in total anionic milliequivalents compared to total cationic milliequivalents in that tissue.

Soluble acetate, oxalate, and formate, but not malate, concentrations increased significantly in the column boundary layer compared to the corresponding concentrations in sapwood (the tissue from which it was derived) after wounding (Table 1). In the column boundary layer, the acetate concentration was 10-fold higher and the oxalate concentration was 17-fold higher than in sapwood. In discolored wood, soluble malate concentration increased significantly along with those of acetate, oxalate, and formate: acetate was eightfold, oxalate was 49-fold, formate was threefold, and malate was 10-fold the sapwood concentration. The four organic acids in discolored wood accounted for almost half the total water-soluble dry matter extracted from discolored wood; however, these anions accounted for only about 3% of the soluble dry matter in sapwood and about 10% of the soluble dry matter in the column boundary layer (Table 1).

Inorganic anions were minor constituents of the total soluble dry matter, although chloride (all three tissues) and nitrate (discolored wood) made some contribution to total milliequivalents. Chloride increased significantly with respect to sapwood in the column boundary but not in discolored wood. Nitrate increased significantly in the column boundary layer and discolored wood, attaining its maximum concentration in discolored wood. There was no significant difference among tissues for sulfate. Phosphate exhibited a small but significant decrease in the column boundary layer and discolored wood with respect to sapwood.

K and Na were readily extracted from all three tissues with water, but Ca, Mg, and Mn were not. The concentrations of these water-insoluble forms, relative to those in sapwood, increased significantly in the column boundary layer but not in discolored wood. The concentration of manganese decreased significantly in discolored wood.

When H_2O was replaced with 0.1 M HCl in the extraction process, Ca, Mg, and Mn were readily extracted from all tissues. Ion chromatography of the HCl extract of the column boundary layer indicated no increase in oxalate, which eliminated calcium oxalate as a significant chelator of Ca in the column boundary layer.

When H_2O was replaced with 0.1 M KCl in the extraction process, Ca, Mg, and Mn were extracted nearly as well as with HCl from sapwood and discolored wood. However, only about half of the Ca and Mn was removed from column boundary layer by KCl.

DISCUSSION

The discolored wood sampled in this study was different from that sampled in earlier studies (3,4,8,9,13–15,20,22,23) because it was not the moist type of discolored wood found in association with already decayed wood. The wetter discolored wood of maple has lower tissue electrical resistance than sapwood (19,22), higher pH than sapwood (15), a K^+ concentration two- to fivefold that of sapwood (8), and a decreased total phenol concentration (15). The discolored wood that we sampled was associated with wounds only 15 mo old showing no visible decay. It was characterized by a tissue electrical resistance, pH, and total phenol concentration equivalent to sapwood (Table 2), and a K^+ concentration only 25% above that in sapwood. We concluded, therefore, that our samples of discolored wood were at an earlier stage of decay.

At this stage, we found a major change in the organic anions in the discolored wood. Four organic anions accounted for 46% of the total soluble dry matter compared to 3% in the sapwood. These anions seemed to exist as the weakly dissociated free acids rather than as the K-salts found in the column boundary layer. The most marked increase was in oxalic acid (50-fold that of the sapwood concentration), which can be highly toxic to both host and fungus cells in either the free acid or in the K-salt form, but not in the virtually insoluble Ca-salt form (24). Along with this increase in oxalate, acetate (which can inhibit some decay fungi [21]) and malate both increased tenfold compared to concentrations in healthy tissue.

Concentrations of organic anions and phenol also increased in the column boundary layer (Table 2). The high K^+ concentration and high pH indicate that the organic anions occur as K-salts rather than as acids. The K^+ concentration also indicates the presence of functioning membranes in this layer. The column boundary layer has a high concentration of Ca, only half of which is extractable with KCl. Of the two most common forms of Ca crystals in plants, calcium oxalate and $CaCO_3$ (2), oxalates were eliminated because 0.1 M HCl released all Ca from the tissue with no increase in oxalate in the extract solution. Thus, perhaps half the Ca in the boundary layer exists in $CaCO_3$, the remainder either on cell wall exchange sites or in organic complexes.

Increases of organic acids in the discolored wood and the column boundary layer indicate a shift in metabolism in response to wounding. Shifts in oxidative metabolism that cause primary metabolites to form inhibitory secondary metabolites and tissue-sealing materials have long been known as a key host response to expanding infections originating at tree wounds (6). The importance of such mechanisms that remove readily available food in favor of poisoning and plugging substances has been discussed for live wood systems (16). An oxidative metabolism shift, which resulted in the accumulation of organic acids rather than phenols, seems to have occurred in discolored maple wood. A shift in oxidative metabolism at the boundary layer between discolored wood and healthy sapwood involved the formation of both phenolic substances and organic acids.

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