

# CHEMICAL PROPERTIES ASSOCIATED WITH BACTERIAL WETWOOD IN RED OAKS

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(Received March 2000)

## ABSTRACT

Bacterial **wetwood** is a major cause of value loss in the red oak lumber industry in the United States. Red oak trees in Mississippi, South Carolina, and Florida were sampled and evaluated for certain chemical properties possibly associated with the **wetwood** condition. Specific variables investigated were **pH** and **concentrations** of methane, cations (**Na<sup>+</sup>**, **Ca<sup>++</sup>**, **K<sup>+</sup>**, and **Mg<sup>++</sup>**), nonstructural carbohydrates, and organic acids (acetate, propionate, and butyrate).

The degree of bacterial **wetwood** infection and development was greater in red oaks from Mississippi than from South Carolina as evidenced by increased concentrations of methane, total **Na<sup>+</sup>**, total **K<sup>+</sup>**, total **Ca<sup>++</sup>**, and by decreased concentrations of total sugar and reducing sugar. Of all the variables tested, methane concentration was the best indicator of **wetwood** in living red oak trees at all three locations. **pH** was not an indicator of **wetwood** in living trees or in green red oak lumber. Of the remaining variables tested, greater **concentrations** of acetic acid, total **K<sup>+</sup>**, and lesser concentrations of nonstructural carbohydrates characterized wetwood-affected trees, but their potential as **wetwood** indicators depends on **wetwood** severity, not its mere presence.

**Keywords:** Chemical properties, **wetwood**, red oaks, tree disease identification.

## INTRODUCTION

Red oaks are very important commercial trees in the United States. In 1991, more than 3.5 **MBF**, 36% of the total lumber in the U.S. hardwood market, was comprised of red oak (Murdoch 1992). The widespread occurrence of bacterially infected **wetwood** is an ongoing concern in the production and continued **uti-**

lization of quality red oak lumber. Since **wetwood**-affected oak is more prone to develop honeycomb, ring failure, and surface checking than is healthy oak (McMillen et al. 1979; Ward 1972), the hardwood industry suffers a great financial loss due to the reduction in quality of red oak lumber. This fact was brought to the attention of the Hardwood Research Council in 1983 (Murdoch 1992).

Since that time, concerns also have been raised about the identification of living **wetwood**-affected oak trees in the forest. **Mild**-drying schedules can be applied to **wetwood**-affected lumber to reduce drying defects if it is identified and separated from healthy lumber before drying. However, it is difficult to recognize the presence of **wetwood** in red oak boards on the green chain during the milling operation. At present, the only practical way to identify **wetwood** is to sniff the fresh **heartwood** to detect the characteristic sour odors of fatty acids, and other compounds produced by the bacterial populations associated with the disease. This technique is fallible and may lead to identification errors because the human nose rapidly suffers "overload" (Murdoch 1992).

There are still no consistently reliable methods of detecting the presence of **wetwood** in red oaks. An accurate **wetwood** detection system would greatly benefit the hardwood lumber industry. Chemical properties appear to be good potential indicators of **wetwood** since **wetwood** is always associated with changes in wood chemical properties. For example, **wetwood** **pH** usually differs from the **pH** of adjacent normal wood; it tends to be more acidic in conifers (Bauch et al. 1975; Hartley et al. 1961), and more alkaline in hardwoods (Carter 1945; Hartley et al. 1961). Moisture content is usually greater in **wetwood**-affected wood (Toole 1968) because the absorptive and moisture-holding capacity of **wetwood** is increased by bacterial slime and heavy deposition of **extractives** (Ward and Zeikus 1980). Concentrations of cations such as  $\text{Ca}^{++}$ ,  $\text{K}^+$ , and  $\text{Na}^+$  are often elevated in some tree species (Murdoch et al. 1987). Calcium carbonate crystals were detected in the **wetwood** of Japanese poplar (*Populus maximowiczii* Henry) (Fukazawa et al. 1985). The fermentative action of **wetwood** bacteria produces gas with pressures recorded up to  $4.22 \text{ Kg/cm}^2$  (60 lb per square inch) (Hamilton 1980). These high pressures often force accumulated gas and sap from the trunk through cracks in branch crotches, wounds made by removing branches, or through other

injuries. The flux is colorless to tan at **first**, but darkens upon exposure to the air, leaving a light gray encrustation on the bark (Stipes 1971). Bacterial-affected heartwood in oak is associated with unpleasant, sour, or rancid odors that are not characteristic of normal, uninfected heartwood. The fatty acids responsible for these odors are produced by anaerobic bacteria (Ward 1982; Ward and Zeikus 1980). The acid mixture consists of acetate with a strong vinegar odor, butyrate with an odor similar to rancid butter, **valerate** with a putrid odor, and caproate with a goat odor. Anaerobic bacteria isolated from bacterial oak will produce sour and rancid odors in laboratory cultures similar to the abnormal odors found in the oak **wetwood** (Ward 1982).

This investigation compared **pH** and concentrations of methane,  $\text{Na}^+$ ,  $\text{Ca}^{++}$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$ , nonstructural carbohydrates, and organic acids measured in **wetwood** to these variables measured in healthy wood of red oaks to determine their potential for use as possible predictors of **wetwood**.

#### MATERIALS AND METHODS

##### *Sites and sample trees*

Chemical variables were measured for sixty-two red oak trees in the Clemson Experimental Forest in **Pickens** County, South Carolina (SC), ten red oaks in the Delta National Forest in Sharkey Co., Mississippi (MS), and twenty red oaks on **Anderson-Tully** Co. land in Issaquena Co., MS. Fourteen oak trees were sampled in Leon, **Hillsborough**, and Sarasota counties, Florida (FL), but **only** for methane content. The number of trees associated with the measurement of each chemical variable is listed in Tables 1-5. The sample sites and tree species selected in SC and MS are described in detail in Xu et al. (2000). The tree species selected in FL were: water oak (*Quercus nigra* L.) (No. = 5), live oak (*Q. virginiana* Mill.) (No. = 3), laurel oak (*Q. hemisphaerica* Bartr.) (No. = 4), and swamp laurel oak (*Q. laurifolia* Michx.) (No. = 2). The sample trees were located in low and wet sites.

TABLE 1. Average methane concentrations as related to wetwood health status of red oak trees in South Carolina, Mississippi, and Florida.

Health status	South Carolina		Mississippi		Florida	
	n	Methane concentration (ppm)	n	Methane concentration (ppm)	n	Methane concentration (ppm)
WW <sup>2</sup>	25	432.9 a <sup>1</sup> , B <sup>1</sup>	17	735.5 a, A	6	577.1 a, B
NW	21	72.4 b, A	9	24.2 b, A	8	45.8 b, A

<sup>1</sup>Means with the same uppercase letter within each row, or with the same lowercase letter within each column, are not significantly different at  $P = 0.05$ .

<sup>2</sup> WW = wetwood; NW = normal wood.

### Sampling and statistical method

All trees were arbitrarily selected regardless of age, DBH, height, and other physical properties. Trees in SC were selected in February 1997, sampled for pH in July, sampled for methane in September, and sampled for other chemical parameters in July 1998. Sampling of trees in MS for pH and methane was in September 1997, and for other chemical parameters in July 1998. Sampling of trees in FL occurred in September 1997. Based on the odor of an increment core removed from the bole at 1.4 m above the ground, the sample trees were initially classified as wetwood infected (WW) or normal (NW). This technique is recognized as standard for identifying bacterial wetwood in the field (Murdoch 1992) and has been used in other studies (Verkasalo et al. 1993; Ross et al. 1992, 1994). In addition to the foul odor, bacterial wetwood may have a dark, water-soaked appearance that distinguishes it from healthy wood. Sample trees were cut down in order to obtain boards and wood tissue for this and a related study (Xu et al. 2000). While bacterial infections were not verified by culturing or visual inspection with a microscope, only the most obviously infected trees were Selected for study based on odor and appearance. Wetwood normally occurs in a cone-shaped region in the heartwood of infected trees, which makes it fairly easy to detect once a tree is down. Based on the odor of increment cores, it appeared that the degree of wetwood infection in MS trees was greater than that of SC wetwood trees. Analysis of variance and Duncan's Multiple Comparison Test ( $P = 0.05$ ) were used to analyze the data

to determine differences between WW and NW for pH and concentrations of methane, nonstructural carbohydrates, organic acids, and some nutrient minerals.

### Methane concentrations

Increment cores (12 cm long) were placed into screw-cap tubes (10 x 125 mm) immediately after being removed from the sample trees. Each tube was sealed with a rubber septum and an open-top closure, and placed on ice for transport to the lab. In the laboratory, samples were stored in a freezer at -5°C until they were analyzed. Samples were warmed to room temperature prior to analyzing them for methane concentrations using a gas chromatograph (Varian 3400) with a flame ionization detector and a packed column (3.3 mm Porapak 70-100 mesh). The injection amount was 1 ml, and the carrier gas was N<sub>2</sub> with a flow rate of 25 ml/min. Gas chromatograph temperatures were as follows: injector 150°C; column 50°C; and detector 250°C. Methane concentrations were calculated based on the areas beneath response peaks. The standard was 103 ppm methane in helium (National Specialty Gases, Research Triangle Park, NC). The final methane concentrations were calculated based on the areas of the methane peaks in samples compared to the areas of the methane peaks in standards.

### Total cation concentrations (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup>, and Mg<sup>++</sup>)

Wood samples, ground into flour able to pass a 0.5-mm sieve, were oven-dried at 103°C to a constant weight. Approximately 2

g of dried wood flour was reduced to ash in a muffle furnace (Model E555C-1, BLUE M Electric Co., Blue Island, IL) at 550°C for 4 hours. The ash was dissolved in 10 ml 6 N HCl and diluted into 1: 10 and 1: 100 solutions with 6 N HCl. These solutions were injected into an Atomic Absorption Spectrophotometer (Model 5000, Perkin-Elmer Co., Norwalk, CT) in order to measure total Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup>, and Mg<sup>++</sup>.

#### *Nonstructural carbohydrates*

Nonstructural carbohydrates (sugars and glucose) were extracted from wood samples ground to pass through a 0.5-mm sieve, and then dried in an oven. The extraction was done using 80% ethanol heated in a Soxhlet extraction apparatus for 6 hours. The resulting extraction solution was then assayed for total sugars using the phenol-sulfuric acid method (Dubois et al. 1956) and reducing sugars by Nelson's test (Clark 1964). The wood flour remaining after the extraction was then measured for starch content using a starch assay kit (SA-20, SIGMA). Sugar and reducing sugar concentrations for each sample were determined by averaging the results from three different dilutions of the same sample.

#### *Organic acid concentrations*

Wetwood sap was collected from fresh bolts cut from sample red oak trees at 1.4 m above the ground. Fluid was expressed using a hydraulic press capable of pressures up to 15 Mpa, and then centrifuged (DYNAC II centrifuge, Becton Dickinson, Sparks, MD) for 5.0 min at 10,000 rpm. Aliquots of 100 µL were injected into a high performance liquid chromatograph equipped with a Waters 490 Programmable Multiwavelength Detector and a Waters Spherisorb® 3µm ODS2 4.6 X 150 mm analytical column (Waters Corporation, Milford, MA). The mobile phase at 20°C was 0.1 N methanesulfonic acid (0.025M n-octylamine was added into 2 L 0.1 N methanesulfonic acid) at a flow rate of 1.5 ml/min. The geometric areas under sample response peaks

were used to quantify organic acid concentrations. A measurement standard consisted of 1 ml each of propionate and butyrate, and 0.5 ml of acetate, brought to 100 ml with the mobile phase.

#### *pH*

pH was measured in sap samples. To obtain sap, a fresh increment core was put into a polyethylene tube, and the tube containing the core was compressed with a hydraulic press. The pH of the expressed sap was measured with a pH meter (Accumet Model 910, Fisher Scientific Co., Pittsburgh, PA.).

### RESULTS

#### *Methane concentrations*

Methane concentrations were significantly greater in wetwood trees than in healthy trees in SC, MS, and FL (Table 1). The average methane concentration in MS wetwood trees (735.5 ppm) was significantly greater than that in SC (432.9 ppm) and FL (577.1 ppm) wetwood trees.

#### *Total cation concentrations*

The MS wetwood trees had significantly greater concentrations of total Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup>, and Mg<sup>++</sup> than did SC trees (Table 2). Concentrations of Na<sup>+</sup> and K<sup>+</sup> were also greater in the healthy wood of MS trees compared to that of SC trees. In MS trees, total Na<sup>+</sup>, K<sup>+</sup> and Mg<sup>++</sup> concentrations were significantly greater in wetwood trees (Na<sup>+</sup>: 25.7 ppm; K<sup>+</sup>: 1133.8 ppm and Mg<sup>++</sup>: 230.0 ppm) than in healthy trees (Na<sup>+</sup>: 14.2 ppm; K<sup>+</sup>: 368.8 ppm and Mg<sup>++</sup>: 115.4 ppm). In SC trees, total K<sup>+</sup> and Ca<sup>++</sup> concentrations were significantly greater in wetwood trees (K<sup>+</sup>: 366.7 ppm and Ca<sup>++</sup>: 700.5 ppm) than in healthy trees (K<sup>+</sup>: 243.2 ppm and Ca<sup>++</sup>: 485.7 ppm).

#### *Nonstructural carbohydrate concentrations*

Total sugar and reducing sugar concentrations were significantly less in wetwood and healthy trees in MS compared to wetwood and

TABLE 2. Average cation concentrations of South Carolina and Mississippi red oak trees as related to wetwood health status.

Health status	Mississippi		South Carolina		Mississippi		South Carolina	
	n	Mean (mg/g)	n	Mean (mg/g)	n	Mean (mg/g)	n	Mean (mg/g)
WW <sup>2</sup>	5	25.7 a <sup>1</sup> , A <sup>1</sup>	37	7.1 a, B	5	1133.8 a, A	366.7 a, B	700.5 a, B
NW	5	14.2 b, A	25	5.9 a, B	5	368.8 b, A	243.2 b, B	485.7 b, A

<sup>1</sup> Means with the same uppercase letter within each pair (SC versus MS) in each row, or with the same lowercase letter within each column, are not significantly different at P = 0.05.  
<sup>2</sup> WW = wetwood; NW = normal wood.

TABLE Total sugar, reducing sugar, and starch concentrations as related to wet-wood health status of red oak trees in South Carolina and Mississippi.

Health status	South Carolina		Mississippi	
	n	Mean (mg/g)	n	Mean (mg/g)
WW <sup>1</sup>	20	11.5 b <sup>1</sup> , A <sup>1</sup>	5	5.7 b, B
NW	12	14.8 a, A	5	9.6 a, B
ww	20	14.3 a, A	5	1.9 b, B
NW	12	14.3 a, A	5	6.0 a, B
WW	20	1.0 a, A	5	1.0 a, A
NW	12	1.0 a, A	5	1.0 a, A

<sup>1</sup> Means with the same uppercase letter within each row, or with the same lowercase letter within each variable by column, are not significantly different at P = 0.05.  
<sup>2</sup> WW = wetwood; NW = normal wood.

healthy trees in SC (Table 3). Total sugar and reducing sugar concentrations in SC and MS trees ranged from 1.3-3.6 times less in wet-wood-affected trees than in healthy trees. Starch concentrations did not differ between the health status of trees within a state, or between states.

**Organic acid concentrations**

Acetate concentrations were greater in wet-wood than in healthy wood for both MS and SC trees (Table 4) Of the three organic acids measured, acetate had the highest concentrations in every tree. However, little or no propionate was measured in MS or SC trees. Wet-wood and healthy trees sampled in MS had a greater concentration of acetate than did those in SC.

**pH**

The average pH of all samples ranged from 3.65 to 3.81 (Table 5). There were no significant differences in pH between healthy and wetwood trees. There were no differences in pH between SC and MS trees.

**DISCUSSION**

Methane is a product of methanogenic bacteria, which are strict anaerobes that obtain energy by converting CO<sub>2</sub>, H<sub>2</sub>, formate, methanol, acetate, and other compounds to either methane or methane and CO<sub>2</sub> (Prescott et al. 1990). Trees that contain methane are usually

TABLE 4. Organic acid concentrations in the sap South Carolina and Mississippi red oak trees as related to wetwood health status.

Health status	n		Acetate (mM)		Propionate (mM)		Butyrate (mM)	
	Mississippi	South Carolina	Mississippi	South Carolina	Mississippi	South Carolina	Mississippi	South Carolina
WW <sup>2</sup>	5	18	66.1 a <sup>1</sup> , A <sup>1</sup>	34.0 a, B	0.0 a, B	4.0 a, A	9.7 a, A	16.1 a, A
NW	4	14	30.3 b, A	13.5 b, B	0.0 a, B	1.9 a, A	5.0 a, A	5.8 b, A

<sup>1</sup> Means with the same uppercase letter within each pair (SC versus MS) in each row, or with the same lowercase letter within each column, are not significantly different at  $P = 0.05$ .

<sup>2</sup> W W = wetwood; N W = normal wood.

located on poorly drained soils, particularly in low-lying areas surrounding lakes and rivers (Zeikus and Ward 1974). The results of the present study suggest that methane is common in red oak trees, and that while the mere presence of methane might not indicate wetwood, a concentration that is six to thirty times greater than that of healthy trees indicates the presence of wetwood. Methane is relatively easy and quick to measure with a gas chromatograph. However, a disadvantage of using methane as an indicator of wetwood in lumber is that methane quickly volatilizes after the tree is cut.

Mineral elements are essential for tree growth. The essential elements supplied from soil include P, K, Ca, Mg, Cu, Zn, B, Fe, Mn, and Mo (Hocker 1979). These mineral elements are taken up by tree roots and accumulate in the tree. The elements may also return to the soil through secretions, by leaching from roots, and by annual litter fall (e.g., leaves, twigs, flowers, and fruits). Each living tree contains certain relative amounts of these essential elements. Our results show that Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup> and Mg<sup>++</sup> concentrations increased in wetwood-affected MS and SC trees, al-

though not all were significant differences. This phenomenon has been reported for wetwood in many tree species (including those in which higher concentrations of mineral nutrients were found in wetwood-infected heartwood, and in young trees (Fukazawa et al. 1985; Worrall and Parmeter 1982). Murdoch et al. (1987) suggested that the elevated levels of cations in wetwood resulted from the selective degradation of cell membranes by wetwood bacteria, leading to leakage of woody cell contents. Over time, this would lead to the accumulation of cations. It is believed that this concentration of cations accounts for an increase in osmotic potential in wetwood, which leads to the accumulation of water in wetwood-affected heartwood tissues (McLemore et al. 1999; Worrall and Parmeter 1982). Of the four cations measured in the present study, K<sup>+</sup> concentrations were the most different between healthy and wetwood trees in MS, which suggests that K<sup>+</sup> concentration could serve as an indicator of wetwood in MS red oaks. Concentration of Ca<sup>++</sup> may serve as a good indicator of wetwood in SC oaks. In general, cation concentrations could be good indicators of the severity of wetwood since the accumulation of cations seems to correlate with the formation of wetwood, especially in severely affected wood. An advantage of using cation concentrations as indicators of wetwood is that a minimal amount of sample is needed, and living trees could be nondestructively sampled by using increment cores.

Very little has been published regarding wetwood and nonstructural carbohydrates. Nonstructural carbohydrates are found in all

TABLE 5. Average pH as related to wetwood health status of red oak trees in South Carolina and Mississippi.

Health status	South Carolina		Mississippi	
	n	pH	n	pH
WW <sup>2</sup>	25	3.8 a <sup>1</sup> , A <sup>1</sup>	5	3.7 a, A
NW	16	3.7 a, A	5	3.6 a, A

<sup>1</sup> Means with the same uppercase letter within each row, or with the same lowercase letter within each column, are not significantly different at  $P = 0.05$ .

<sup>2</sup> W W = wetwood; N W = normal wood.

living plants. Plants store energy as starch, which is in a dynamic state since plants can convert starch to sugar and then change sugar back to starch. Therefore, starch concentrations vary with season and with a number of other environmental factors. Sugars and starch occur in the cytoplasm and can be easily accessed and metabolized by bacteria. Significant differences were detected in total sugar and reducing sugar concentrations in SC and MS trees with **wetwood** when compared to healthy trees. The lower concentrations of nonstructural carbohydrates in MS trees compared to those of SC trees suggest that MS **wetwood** trees had more severe infections compared to SC **wetwood** trees. Even if this study had detected differences in starch concentrations between **wetwood** and healthy wood, it is unlikely that starch concentrations would be a good predictor of **wetwood** since even healthy heartwood contains little starch. Total sugars and reducing sugars could confirm the presence of severe **wetwood**, but since this measurement is destructive and time-consuming, it probably is not a good predictor of **wetwood** in red oaks.

According to **McMillen** et al. (1979), the presence of rancid odors (from butyrate and valerate) indicates advanced or severe bacterial infections. A strong vinegar odor (from acetate) usually indicates early, and less severe, bacterial infection. The results of the present study do not agree with their research since we measured more acetate and less butyrate in MS and SC **wetwood** trees overall, and the severely infected trees in MS had less butyrate and more acetate than did the moderately **wetwood**-affected trees in SC. Acetate concentrations increased significantly in response to **wetwood** infection in both SC and MS trees, but only in SC trees was increased butyrate related to **wetwood** infection. Acetate concentration has the potential to be a good indicator of **wetwood** since it can, to a certain degree, reflect **wetwood** severity. The measurement of organic acids, collected from the sap of fresh increment cores could be a useful

nondestructive approach to detect **wetwood** in living trees.

Previous studies that compared the pH of healthy and **wetwood**-affected heartwood were not in agreement. **Schroeder** and **Kozlik** (1972) found that the pH values of **wetwood** and normal heartwood in western hemlock were identical. **Toole** (1968) found that **wetwood** in cottonwood had a higher pH compared to normal **sapwood**. **Wetwood** of other species can be either more alkaline or more acidic than healthy wood (**Ward** and **Pong** 1980). The present research found no differences in pH between healthy and **wetwood** trees, which suggests that pH is not a good indicator of **wetwood** in oaks.

#### CONCLUSIONS

This study suggests that the degree of bacterial **wetwood** infection and development as measured by a number of chemical variables is greater in MS trees than in SC trees. These results support the results of a related study using these same trees which defined some mechanical and drying properties of bacterially infected and healthy woods (**Xu** et al. 2000). Among all the chemical variables tested, the relative methane concentrations appear to be the best indicator of **wetwood** in red oak. Greater concentrations of total  $K^+$ ,  $Ca^{++}$ ,  $Mg^{++}$  and acetate, and concentrations of total and reducing sugars were also characteristic of certain **wetwood**-affected red oaks. However, these variables are less useful than methane concentrations, to indicate the presence of **wetwood** infections, because they were not associated with all **wetwood** infections.

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