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Wetwood formation as a host response in white fir

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Abstract

A bacterium associated with wetwood of white fir did not cause wetwood in inoculated trees, and wetwood formed while external water was excluded. Wetwood formed in response to various injuries, suggesting that it is a host response to parenchyma death.

1 Introduction

Wetwood is defined as "a type of heartwood in standing trees which has been internally infused with water" (WARD and PONG 1980). As such, it occupies the internal cylinder of nonliving wood in affected trees. Columns of wetwood, usually continuous with the central cylinder, also occur in association with branch traces (ETHERIDGE and MORIN 1962; LAGERBERG 1935; BAUCH et al. 1975), mechanical wounds (DAVIDSON et al. 1959; CAMPBELL and DAVIDSON 1941; HORNIBROOK 1950), insect attacks (JOHNSON and SHEA 1963; WICKMAN and SCHARPF 1972; OWEN and WILCOX 1982), and root and butt decay (COUTTS and RISHBETH 1977; LAGERBERG 1935; LINZON 1958; SCHMITZ and JACKSON 1927). Such tissues are also nonliving. Thus, wetwood in such tissues, which in some cases has been called "pathological wetwood" (BAUCH et al. 1978, 1979) or "wet pathological heartwood" (COUTTS and RISHBETH 1977), may be homologous with what is termed "protection wood" (HEPTING and BLAISDELL 1936; JORGENSEN 1962), "reaction wood" (SHAIN 1967, 1971), "pathological heartwood" (MCNABB et al. 1959) or "discolored sapwood" (HART and JOHNSON 1970).

As discussed in recent reviews (HARTLEY et al. 1961; WARD and PONG 1980), various theories are espoused by different authors as to the cause of wetwood. The association of wetwood with branch stubs and wounds has led to the conclusion that external water enters through such pathways and accumulates in wetwood (ETHERIDGE and MORIN 1962; LAGERBERG 1935) and to the proposal that wetwood is a disease associated with bacteria entering through the same pathways (CARTER 1945; SELISKAR 1952; MURDOCH and CAMPANA 1981). Although inoculations have been claimed to demonstrate a causal role for bacteria in wetwood (CARTER 1945; DAY 1924; DOWSON 1937; CRANDALL 1943; SELISKAR 1952), careful examination of such data and the limited number of tree species examined suggest

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that generalizations and assumptions of bacterial etiology of wetwood, at least in fir (*Abies* spp.) (BRILL et al. 1981; ULRICH 1981), may be unfounded. Data of COUTTS and RISHBETH (1977) suggest that wetwood in *A. grandis* may form in the absence of bacteria or external water.

In white fir (*A. concolor*) wetwood is formed in association with injuries, branch stubs, and wood decay as well as in the central cylinder of heartwood. A coryneform bacterium (WWB) has been consistently isolated in numbers as high as 10^6 per ml wetwood fluid and has been characterized by WILCOX and OLDFAM (1971, 1972). The studies reported here were designed to examine the role of wounds, *Heterobasidion annosum* infection, WWB, and ray parenchyma death in formation of wetwood in fir.

2 Materials and methods

2.1 Field observations

Patterns of wetwood development in naturally wounded and/or diseased trees were observed in various stands of the Lassen, Modoc and Stanislaus National Forests in the northern Sierra Nevada in California. Freshly cut stumps left by logging crews, trees uprooted in randomly selected plots in conjunction with another study, and trees felled in and adjacent to *Heterobasidion annosum* (Fr.) Brefeld (*Fomes annosus* [Fr.] Cooke) infection centers provided material for examination.

2.2 Induction of wetwood in trees

Wetwood response to various treatments was studied in trees in the Stanislaus National Forest. White firs with average d.b.h. of 14 cm were selected for extremes of growth rate based on morphological characters, including distance between internodes on the terminal, crown form, and smoothness of bark. As a result, ages were later found to vary from 17 to 129 years. Five treatments were systematically assigned three per tree to 10 fast-growing (mean radial growth rate = 5 mm/yr) and 10 slow-growing (mean radial growth rate = 1.4 mm/yr) trees, and one tree of each class received each treatment individually, giving a total of 30 trees. The treatments were: a. 0.01 M mercuric chloride was applied to test the effect of slow killing of the parenchyma (COUTTS and RISHBETH 1977); b. a freshly prepared aqueous turbid suspension of the WWB (WILCOX and OLDFAM 1971, 1972; kindly supplied by W. W. WILCOX, Univ. of Calif. Forest Products Laboratory, Richmond, CA, USA) was applied to assess its ability to cause wetwood; c. 0.1 M KCl ($\psi_s \approx -5$ bars) was included to test the effect of an osmoticum similar to that in wetwood (WORRALL and PARMETER 1982); d. sterile distilled water; and e. blank (no treatment). At 1.5 m above soil line, we shaved the bark smooth if it was rough, swabbed it and the drill bit with ethanol, and drilled 1 cm diameter holes angling down 20° from the horizontal to a depth of one-third tree diameter. Ten ml test solution was introduced, and holes were sealed with polyethylene sheeting and tree seal. After 3 months, 1 m stem lengths centered at the treatment were dissected by serial cross-section to determine vertical extent of wetwood and drying.

2.3 Wetwood formation in wounded trees

A second experiment in natural stands was designed to examine wetwood formation in response to wounds similar to those commonly inflicted on fir during logging. Wounds (10 cm wide \times 30 cm with lower edge 20 cm above soil line) were made by excising bark and scoring heavily with an axe on opposite sides of trees averaging 18 cm d.b.h. One wound per tree was sprayed with 3 ml conidial suspension of *Heterobasidion annosum*.

2.4 Effect of bacteria on wetwood and colonization by *Heterobasidion annosum*

A third experiment was designed to examine wetwood formation and *H. annosum* development in co-inoculations with the WWB. Bacteria were washed from 15-day-old YDCP plates with 0.1 M phosphate buffer (pH 6.4), washed twice by centrifugation and resuspension, then suspended in buffer at approximately 10^6 cells/ml. Bacterial suspension or sterile buffer was vacuum infiltrated into sterile dowels which had been split lengthwise into quarter sections (0.6×6 cm).

Heterobasidion annosum was incubated with similar sections for 3 months. Holes were drilled 1.5 m above soil line on opposite sides of white firs, 13–20 cm d.b.h., on the Stanislaus N. F. Each hole received a dowel section infested with *H. annosum* and a second dowel with WWB inoculum or buffer control.

2.5 Wetwood formation in inoculated seedlings

A fourth experiment was performed with 2-year-old fir seedlings in the greenhouse inoculated with isolates of *H. annosum* from pine or fir. Detailed methods are described elsewhere (WORRALL et al. in press). Briefly, incisions were made with sterile technique just above soil line, infested wedges or sterile control wedges were inserted, and the inoculation area was tightly wrapped with waterproof thermoplastic film.

3 Results

3.1 Field observations

Wetwood generally occurred as a clearly differentiated cylinder in the inner wood at the butt of sectioned trees. Occasionally only the outer heartwood was occupied by wetwood. In most cases a dry transition zone, 1–5 rings in width, was distinctly visible, especially on shaved sections. Apparent wetness, color, odor and the drier transition zone typical of wetwood were less pronounced in the upper bole. Lobes of wetwood extended from the central cylinder above and below branch traces, tapering to a point at their horizontal extension (Fig. 1A).

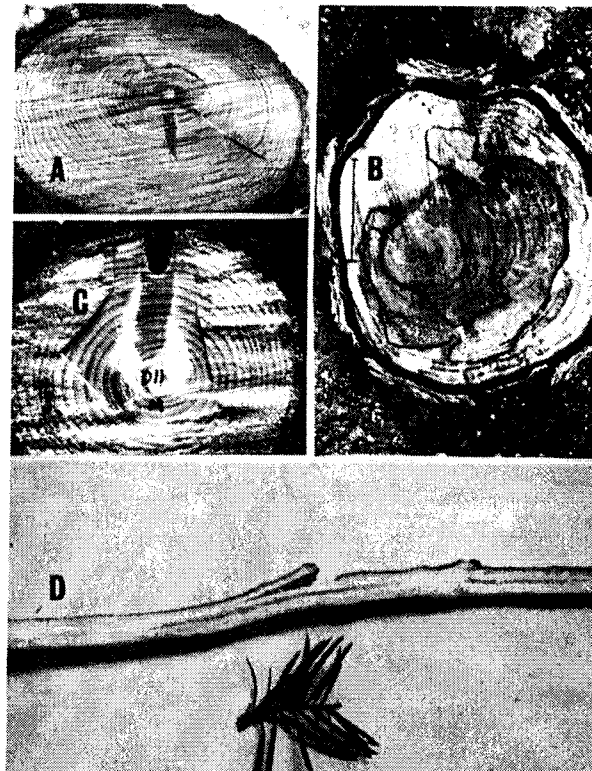
Such lobes also occurred in association with mechanical wounds and attacks by the bark beetle *Scolytus ventralis* (Coleoptera: Scolytidae), but terminated broadly where they met such lesions. Small lesions occasionally had wedges of wetwood internal to them which were unconnected with the central cylinder and were surrounded by normal sapwood.

Heterobasidion annosum caused decay columns which were wide and extensive at the root collar and tapered to incipient decay above the soil level. Such decayed areas were typically horseshoe-shaped and roughly occupied the transition zone (Fig. 1B). Wetwood was usually present in the wood external and internal to the decay, bulging outward in contrast to the undecayed side of the stem.

3.2 Induction of wetwood in trees

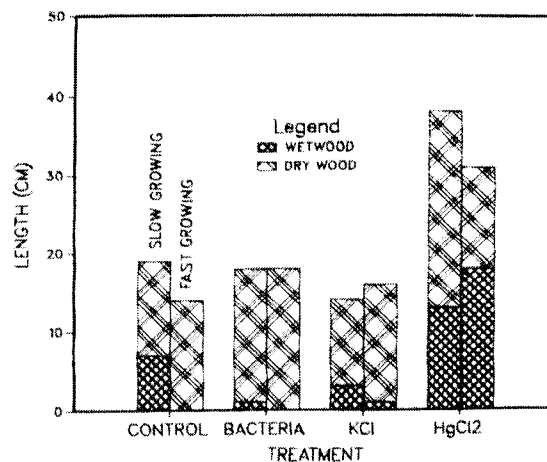
The treatment solutions tested for wetwood induction caused the formation of dry zones above and below the wound, within which wetwood columns were found in some cases (Fig. 1C, 2). Replications of the blank treatment were too few to analyze owing to interference from previous wounds or mistletoe infections. Large variation in lengths of dry zones and wetwood between trees was apparent. There was no significant difference between fast-growing and slow-growing trees for any treatment ($P = 0.05$) so they were combined for further analysis. The bacterial treatment resulted in less wetwood than the water control though this was not significant. Attempts to reisolate the bacterium failed.

Fig. 1. Wetwood and decay encountered in observations and experiments on wetwood formation in white fir. A. Wetwood in a healthy tree showing lobes associated with branch traces. B. Decay caused by *Heterobasidion annosum* and associated wetwood in a tree recently killed by the fungus. Obvious decay is outlined; wood internal to this is undecayed wetwood. C. Wetwood formed within a dry zone resulting from treatment with $HgCl_2$. No natural heartwood was present in this tree. D. Streak of wetwood in a seedling inoculated with *H. annosum*.



The KCl treatment also was not different from controls. Only $HgCl_2$ induced significantly longer dry zones ($P = 0.05$) and wetwood ($P = 0.1$) although several trees showed essentially no response. In view of the large variation between trees and the lack of pairing of controls and treatments on the same tree in this experimental design, it is felt that $P = 0.1$ is a reasonable level at which to reject the null hypothesis (mean length of wetwood for controls = mean length of wetwood for $HgCl_2$).

Fig. 2. Mean length (cm) of dry zones (DW) and wetwood (WW) formed in slow and fast growing white firs in 3 months in response to various treatments. Treatments induced drying of columns of wood within which wetwood formed to varying extents. Means for slow and fast growing trees are not significantly different. When growth rate classes were combined, only $HgCl_2$ resulted in significantly greater mean length of DW ($P = 0.05$) and WW ($P = 0.1$) than water controls



3.3 Wetwood formation in wounded trees

After three years, considerable wetwood was found in response to mechanical wounding with and without inoculation of *H. annosum* (Table 1). Of 9 trees examined, *H. annosum* was found only in two inoculation treatments and was also recovered from two uninoculated wounds. The effect of wounding was generally seen as a lobe of wetwood extending from and continuous with the central wetwood. This lobe extended almost to the surface at the wound site and was shorter radially in sections farther up and down from the wound. Great variation was observed among trees in length, apparent wetness and color of wetwood columns. Although in many trees inoculation was apparently unsuccessful and wetwood formation on both sides was equal, a paired t-test indicated that wetwood columns overall were significantly longer on inoculated wounds ($P = 0.05$).

Table 1

Length of wetwood development and *Heterobasidion annosum* colonization (cm) in trees which were mechanically wounded with and without spray-inoculation of *H. annosum* conidia

	Length of wetwood column ¹		Length of <i>H. annosum</i> colonization ¹	
	Inoculated	Uninoculated	Inoculated	Uninoculated
	100	0	0	0
	80	80	0	0
	120	120	0	80
	140	60	20	0
	100	40	0	0
	100	100	0	0
	0	0		120 ²
	140	140	20	0
	40	0	0	0
$\bar{x}^3 =$	91	60		

¹ Sampled at 20 cm increments.
² *Heterobasidion annosum* extensively colonized the heartwood of this tree on both sides.
³ Means for length of wetwood in response to inoculated and uninoculated wounds are significantly different ($P = 0.05$) with a paired t-test.

Table 2

Length¹ (cm) of wetwood development, *Heterobasidion annosum* colonization, and decay in six trees inoculated with *H. annosum* with (+) and without (-) the wetwood bacterium (WWB)

Means for +WWB and -WWB are not significantly different

Length of wetwood effect		Length of <i>H. annosum</i> colonization		Length of decay	
+WWB	-WWB	+WWB	-WWB	+WWB	-WWB
80	100		200 ²	20	60
200	160	0	0	0	0
80	60	80	--	40	20
120	100	140	120	100	80
180	140	180	60	100	100
100	100		180 ²	20	40

¹ Sampled at 20 cm increments.
² *H. annosum* extensively colonized the heartwood on both sides.