PROTECTION OF WOOD FROM DECAY FUNGI BY ACETYLATION—AN ULTRASTRUCTURAL AND CHEMICAL STUDY[1]

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ABSTRACT

At acetylated weight gains of 15% or above, ultrastructural evidence of wall decomposition was lacking and hyphal cells appeared to be "starved." Blockage of action of fungal catalysts appears to be the primary protection mode of the acetylation technique. The maximum acetylation treatments inhibited consumption of wall polymers and prevented bore-hole formation. Hyphal penetration of cell walls did not proceed by mechanical forces alone; rather, the process was dependent upon chemical action in advance of hyphal tips. A comparison of colonization habits and holocellulose consumption by decay fungi in acetylated woods suggests that the activity, synthesis, or both of lignin-degrading catalysts of the white-rotter is dependent on prior or simultaneous breakdown of carbohydrates.

Keywords: Fraxinus americana L., Pinus taeda L., Liriodendron tulipifera L., brown rot, white rot, acetylation, cell walls, bore-hole formation, hyphae, wood decay, Coriolus versicolor, Gleophyllum trabeum.

INTRODUCTION

Rowell (1975) defines chemical modification of wood as a chemical reaction between some reactive part of a wood component and a simple chemical reagent, with or without a catalyst, to form a covalent bond between the two. Hydroxyl groups on cellulose, hemicelluloses, and lignin are the most abundant reactive sites in wood; and covalent bonds of the carbon-oxygen-carbon type (ethers, acetals, and esters) are the ones of major importance.
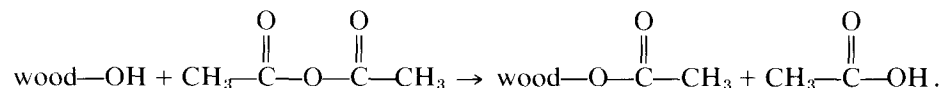
Wood modification techniques are currently receiving interest for the prevention of wood decay, since in some applications they may have advantages over the conventional broad spectrum-type preservatives. Because of their toxicity, conventional preservatives such as pentachlorophenol and creosote are the subject of growing environmental concerns. With wood modification, nontoxic compounds are fixed into the wood structure and the toxicity problems may be avoided.

Fungal enzymes, like most others, are highly specific, their mode of action depending on the substrate molecule, or a portion of it, fitting the active site of the enzyme in a lock-and-key type of relationship (Lehninger 1970). Through chemical modification, the substrate molecule's configuration is changed so that

it is excluded from active sites of enzymes, and degradative reactions cannot proceed. One such modification system, which has received considerable attention in this regard, is acetylation. Wood acetylated to weight percent gains (WPG) above 17 has been found to be resistant to decay by a variety of white-rot and brown-rot fungi (Tarkow et al. 1950; Goldstein et al. 1961; Ozolina and Svalbe 1966). Working with wood of lumber thickness, Goldstein and coworkers (1961) found uncatalyzed acetic anhydride in xylene at 100–130 C to be the optimum acetylation conditions:

$$\text{wood—OH} + \text{CH}_3\overset{\overset{\text{O}}{\|}}{\text{—C}}\text{—O}\overset{\overset{\text{O}}{\|}}{\text{—C}}\text{—CH}_3 \rightarrow \text{wood—O}\overset{\overset{\text{O}}{\|}}{\text{—C}}\text{—CH}_3 + \text{CH}_3\overset{\overset{\text{O}}{\|}}{\text{—C}}\text{—OH}.$$

All the weight gain in acetylation can be directly converted into units of hydroxyl groups blocked, since this technique is a single-site reaction (one acetyl per reacted hydroxyl group). The above acetylation system results in a good distribution of acetyl groups not only in cellulose and hemicelluloses, but in lignin as well (Rowell 1975). Thus, acetylated wood may be both an attractive and viable alternative to broad-spectrum preservatives where decay conditions are not severe or where environmental risks are concerned. This study, then, will evaluate the effects of decay fungi (chemical and ultrastructural) on woods acetylated to various levels of protection (WPG's) in an attempt to better understand the initial stages of cell-wall decomposition and the method of protection of an acetylation technique.

MATERIALS AND METHODS

Acetylation with acetic anhydride and acetyl content determinations

Blocks 1.0 cm × 1.0 cm × 0.5 cm (axial direction) were cut from the outermost sapwood of *Liriodendron tulipifera* L. (yellow poplar), *Pinus taeda* L. (loblolly pine), and *Fraxinus americana* L. (green ash), so that the three opposite faces were in transverse, tangential longitudinal, and radial longitudinal planes. The never-dried specimens were solvent-exchange dried, then oven-dried, weighed, and immersed in a solution consisting of 25% (V/V) acetic anhydride in xylene. A vacuum was drawn on this system for 30 min, after which the samples soaked in the acetylation media for 1 h at atmospheric pressure. The apparatus used was similar to that described in AWPA M10-74. Each block was immediately weighed and transferred to a 500-ml round-bottomed flask containing the above concentration of acetic anhydride in xylene. The solution was heated under reflux and samples were removed from the reaction at intervals of 1, 5, and 29 h. Following reaction, the blocks were washed in water until free acid was undetectable (three to four days), air-dried for seven to ten days and finally oven-dried and weighed. Selected samples were steam sterilized at 121 C for 30 min, transferred to soil-block decay chambers (AWPA M10-74) previously inoculated with either *Gleophyllum trabeum* (Pers. ex Fr.) Murr. (Madison 617) or *Coriolus versicolor* (L. ex Fr.) Quel (Madison 697), and incubated at 25 C and 70% relative humidity for a period of six weeks. After six weeks' decay, paired blocks were removed (some

used in EM studies), cleaned of surface mycelium, and dried to a constant weight. Weight losses due to decay were determined. In order to determine the relationship between WPG and degree of acetyl substitution, an acetyl determination was conducted by the transesterification method (Browning 1967).

Tribromoacetyl bromide synthesis, acetylation, and microdistribution

It is desirable to know which areas of the wood cell wall are being modified in order to assess more accurately the protective action of the acetylation technique. A search of the literature revealed that wood could be acetylated with tribromoacetyl bromide and that the tribromoacetyl groups introduced should be readily detectable in the SEM by EDXA (Energy Dispersive X-ray Analysis). This compound was synthesized by the method of Yocum and Joullie (1966). Acetylation proceeded when wood blocks were treated with a mixture of 25% (V/V) tribromoacetyl bromide in xylene and triethylamine (equimolar quantities with tribromoacetyl bromide) for 1 h at 55 C.

Blocks of the three wood species were prepared and vacuum impregnated with the above mixture in a manner identical to that in the previous section. Following reaction (1 hr at 55 C), the blocks were washed in water for five to seven days until free acid was undetectable, oven-dried, and WPG's calculated. Specimens with microtomed transverse faces were affixed on aluminum specimen holders, coated with carbon, and analyzed by EDXA on a JSM-2 scanning electron microscope operated at an accelerating voltage of 25 kV. Cell walls (all cellular types) were analyzed for Br at the S_1-S_2 and S_2-S_3 interface regions for a total of 200,000 X-ray counts each.

Since three bromines are present in each acetyl substitution, the percent acetyl add-on was calculated from the results of a bromine analysis (conducted on matched specimens by Mikroanalytisches Laboratorium am Inst. f. Physikalische Chemie, Vienna).

Electron microscopy of acetylated woods

Decayed blocks of acetylated wood were transferred directly from decay chambers to fixative and, with a razor blade, transverse and tangential longitudinal pieces (measuring 2 mm square) were cut from the blocks' centers. Samples were fixed in 6.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4 C for 15 h and postfixed in unbuffered aqueous 2% $KMNO_4$ at 4 C for 48 h. Acetylated samples (three WPG classes) of woods lacking fungal exposure were carried through the same procedures. Fixed material was embedded either in a methacrylate mixture (80% n-butyl and 20% methyl methacrylate plus 1% benzoyl peroxide) or epoxy and sectioned at a thickness of 600–900 Å. Methacrylate sections were collected on parlodoin and carbon supporting films and were either observed directly or following resin removal with xylene vapors. Additional sections were platinum shadowed (resin removed) or poststained with 2% $KMNO_4$ for 24 h. Only specimens from the low and medium acetylation levels were examined with a transmission electron microscope operated at an accelerating voltage of 80 kV.

Decayed acetylated blocks to be prepared for scanning electron microscopy were removed from culture bottles, excess surface mycelium was removed, and the blocks were allowed to air-dry. Blocks microtomed to provide smooth trans-
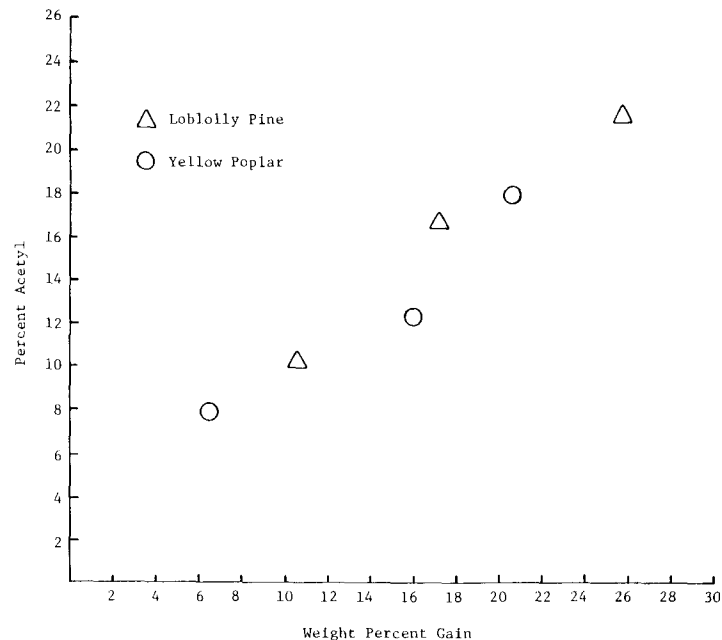
Fig. 1.   Relationship between WPG and acetyl content in acetylated (25% V/V acetic anhydride in xylene) loblolly pine and yellow poplar.

verse and tangential surfaces were affixed to specimen holders, coated with gold on a sputter coater, and examined in an SEM operated at an accelerating voltage of 25 kV.

Fungal viability in acetylated wood

Small pieces of brown-rotted and white-rotted wood were aseptically removed from the centers of decayed, acetylated blocks and plated out on 2% malt agar. Five specimens were used for each combination of species, acetylation level (WPG), and test organism, and allowed to incubate for one to two weeks. Viability was expressed as the number of plates in which the fungus grew from wood to the malt agar surface.

Utilization of carbohydrates

A carbohydrate analysis was conducted on nonacetylated decayed samples, decayed acetylated woods, and sound-wood controls. A slight modification of the method of Borchardt and Piper (1970) was employed using a gas chromatograph with a flow rate of 60 cc/min and operated isothermally at 195 C.

Colonization of acetylated and unmodified wood

Degree of colonization in all three levels of acetylated as well as nonacetylated wood was measured after six weeks' exposure to each decay fungus. Decayed wood was embedded in 100% n-butyl methacrylate (plus 1% W/V benzoyl per-oxide), sectioned at 14 μm on a sliding microtome, and stained for hyphal dif-

TABLE 1. *Relationship between reaction time (hours) and weight percent gain in woods given different acetylation treatments.*

Acetylation treatment levels	Reaction time (hrs.)	Weight percent gain		
		Poplar	Ash	Pine
Low	1	7.8	7.5	10.7
Medium	5	15.9	14.9	17.2
High	29	20.7	20.3	26.0

ferentiation with aqueous safranin O and picro aniline blue (Wilcox 1964). At least 500 of each cell type were examined from both transverse and tangential longitudinal sections by means of conventional light microscopy. Fungal colonization was expressed as percent of cells containing hyphae.

RESULTS AND DISCUSSION

Relationship between acetyl content and weight percent gain

Figure 1 illustrates the relationship between net weight gain and acetyl content in acetylated samples of poplar and pine. Weight percent gain in these two species is a reasonable estimation of actual acetyl content. Agreement between these two values was better in blocks of pine and for both species in the lower WPG categories. The surface-to-volume ratios in these samples were quite large and loss of extractives and soluble storage products may account for discrepancies between these two parameters.

Effect of acetylation on wood decay

The low acetylation level (Table 1) significantly reduced weight losses for all wood species because of decay from both the white-rotter and brown-rotter (Table 2 and Fig. 2). Note also that for protection against white rot, the lowest level of acetylation was nearly as efficient as the higher acetylation treatments. However, with the exception of ash, the medium and high acetylation levels provided some additional protection against brown rot.

In each acetylation category, there was far more uniformity in weight losses due to brown rot than white rot. This observation is consistent with the literature (Cowling 1961; Scheffer 1973), as white rot characteristically occurs in localized pockets or zones.

Among white-rotted samples, loblolly pine was least decayed, while ash displayed the greatest sensitivity to this organism. Since softwoods in service or in

TABLE 2. *The average percent weight loss and standard deviation (n = 11) for brown-rotted and white-rotted samples of acetylated yellow poplar, green ash and loblolly pine.*

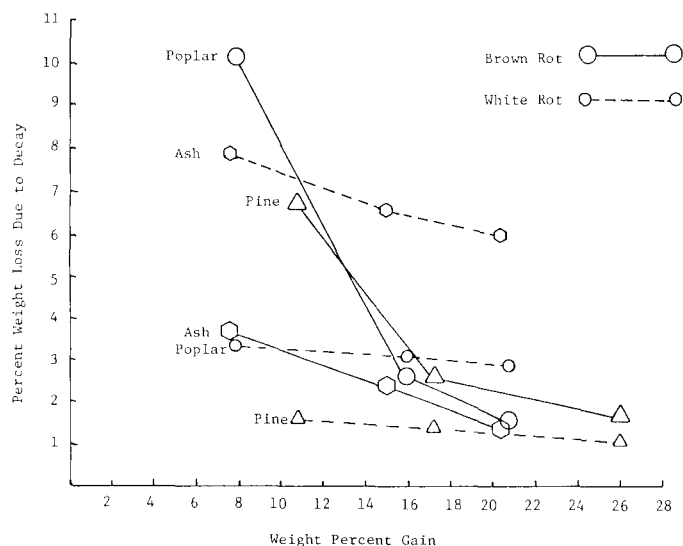| Treatments	% Weight loss (Standard Deviation)					
	Brown rot			White rot		
	Poplar	Ash	Pine	Poplar	Ash	Pine
Nonacetylated	66.8	63.7	61.0	33.6	40.8	28.0
Low acetylation	10.3 (.55)	3.7 (.30)	6.7 (.33)	3.3 (.39)	7.9 (.25)	1.6 (.27)
Medium acetylation	2.6 (.31)	2.4 (.34)	2.6 (.33)	3.1 (.35)	6.6 (.25)	1.4 (.32)
High acetylation	1.6 (.27)	1.4 (.41)	1.7 (.28)	2.9 (.22)	6.0 (.34)	1.1 (.16)

Fig. 2. Relationship between weight percent gain (WPG), due to acetylation (25% V/V acetic anhydride in xylene), and weight loss, due to decay in samples of ash, yellow poplar, and loblolly pine.

test are more susceptible to brown rot than white rot (Scheffer 1973), it is not surprising that weight losses were consistently lower in loblolly pine test blocks.

Distribution of tribromoacetates across cell walls

EDXA analysis (Table 3) indicated that bromine was distributed throughout the entire secondary wall in woods acetylated with tribromoacetyl bromide. Hence, there are several reasons to assume that all secondary wall layers are likewise modified, to some extent, in woods acetylated with acetic anhydride in xylene (the conventional system used in this study). First of all, acetyl substitutions in the tribromoacetyl bromide treated samples (Table 4) ranged from only 0.6% (ash) to 1.3% (loblolly pine) acetyl add-on, while the minimum acetyl add-on in anhydride-treated blocks was about 7% to 10% and ranged to over 20% (Table 1 and Fig. 1). Second, diffusion of acetic anhydride molecules into wood cell walls should be superior to that of tribromoacetyl bromide, since sterically,

Table 3. EDXA analysis of wood acetylated with tribromoacetyl bromide (25% V/V in xylene and triethylamine) indicating location of tribromacetates in cell walls (X-ray counts).

Cell type	Poplar		Ash		Pine	
	S_1-S_2	S_2-S_3	S_1-S_2	S_2-S_3	S_1-S_2	S_2-S_3
Earlywood tracheids	—	—	—	—	750	550
Latewood tracheids	—	—	—	—	450	1,150
Rays	525	1,850	725	1,825	750	1,733
Fibers	342	533	300	375	—	—
Vessels	433	1,333	250	900	—	—
Axial parenchyma	400	975	250	1,300	—	—

TABLE 4. *Relationship between weight percent gain (WPG) and acetyl add-on in wood acetylated with tribromoacetyl bromide (25% V/V in xylene and triethylamine).*

Species	Average WPG	% Br	% Acetyl add-on
Poplar	2.4	0.98	1.15
Ash	2.4*	0.54	0.63
Pine	1.7	1.14	1.33

* This value may be high due to inadequate washing and removal of unreacted tribromoacetyl bromide.

molecules of the former are considerably smaller than those of the latter (vacuum impregnation conditions, prior to reaction, were the same for both systems).

With the exception of pine earlywood tracheids, bromine counts were at a maximum in the S_2-S_3 interface region (Table 3) of cell walls. These results are not surprising, since this area is more accessible to the acetylation media occupying cell lumina than external wall layers. Thus, a diffusion gradient across cell walls may exist, so that cellulose triacetates and other formed esters (acetylation with acetic anhydride) are concentrated in the inner regions of the secondary wall and, with increasing WPG's, esterification of more external areas ($S_2 - S_1$) occurs.

Effect of acetylation on fungal viability

The protective action of the acetylation technique is apparently not fungitoxic, since in most instances each fungus grew out of the wood to the agar surface in culture plates (Table 5). In only one instance did either fungus completely fail to

TABLE 5. *Surface growth and viability of brown-rot and white-rot fungal hyphae in wood acetylated to various levels.*

Species	Acetylation levels	Test organism	Viability[1] (%)	Surface growth
Poplar	Low	White-rot fungus	100	Sparse
	Medium		100	Sparse
	High		100	Very sparse
Ash	Low	White-rot fungus	100	Sparse
	Medium		100	Sparse
	High		100	Very sparse
Pine	Low	White-rot fungus	100	Sparse
	Medium		40	Sparse
	High		0	Very sparse
Poplar	Low	Brown-rot fungus	100	Moderate to abundant
	Medium		60	Moderate to abundant
	High		40	Moderate to abundant
Ash	Low	Brown-rot fungus	100	Moderate to abundant
	Medium		100	Moderate to abundant
	High		100	Moderate to abundant
Pine	Low	Brown-rot fungus	100	Moderate to abundant
	Medium		100	Moderate to abundant
	High		60	Moderate to abundant

[1] Viability is expressed as the % of plated pieces (from block centers) producing fungal growth on 2% malt agar.
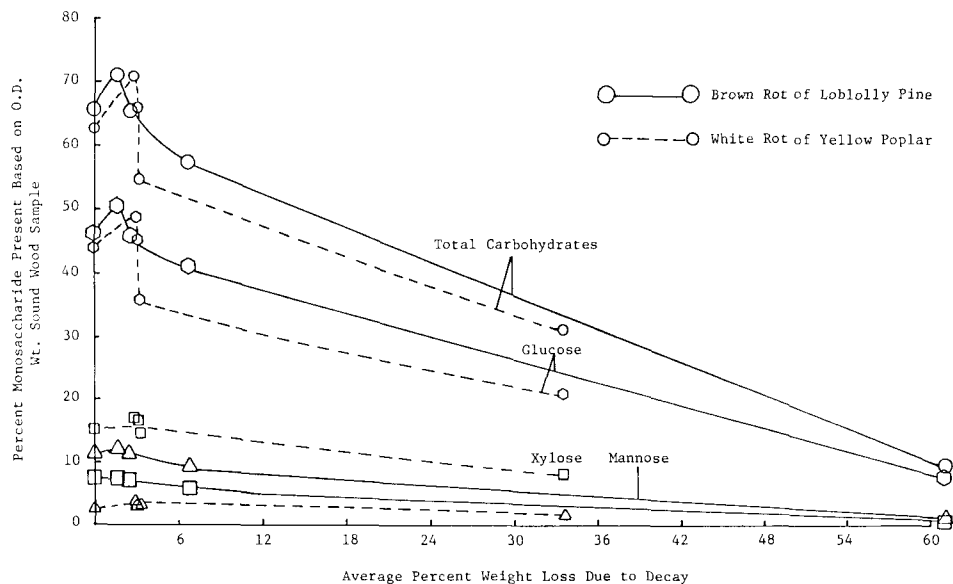
FIG. 3.   Gas chromatographic analysis of carbohydrates in brown-rotted loblolly pine and white-rotted yellow poplar.

grow out of acetylated wood: the white-rotter was inactive in pine at the maximum acetylation level, and its viability was somewhat diminished in the medium treatment level of the same wood. Viability of the brown-rot fungus was inhibited only in poplar with medium and high levels of acetylation and in pine with the highest treatment level.

Influence of acetylation on carbohydrate consumption

Both fungi degraded holocellulose in the same manner (Fig. 3 and Tables 6 and 7): considerable depolymerization and consumption occurred in nonacetylated samples (about 3 times more in pine than poplar), but with increasing acetylation

TABLE 6.   *Gas chromatographic analysis of carbohydrates in sound wood, decayed nonacetylated and acetylated wood samples (%).*

Species	Sample variables	Weight loss (%)	Arabi-nose	Xylose	Man-nose	Galac-tose	Glu-cose	Total carbo-hydrate
Poplar	Sound wood	—	0.8	15.0	2.6	0.6	43.6	62.5
	Decayed, nonacetylated	33.6	0.5	8.1	1.3	0.1	20.7	30.8
(white)	Decayed, low acetylations	3.3	1.1	14.3	3.2	0.4	35.6	54.6
rot)	Decayed, medium acetylations	3.1	1.0	16.4	3.0	0.3	44.9	65.6
	Decayed, high acetylations	2.9	1.1	16.7	3.8	0.5	48.4	70.6
Pine	Sound wood	—	1.6	6.9	10.8	0.5	46.1	65.9
	Decayed, nonacetylated	61.0	0.2	0.9	0.3	0.04	7.7	9.6
(brown	Decayed, low actylations	6.7	1.3	5.8	9.1	0.4	40.9	57.4
rot)	Decayed, medium acetylations	2.6	1.6	7.0	11.0	0.6	45.8	65.9
	Decayed, high acetylations	1.7	1.5	7.1	11.6	0.5	50.1	70.8

TABLE 7. *Change in percent carbohydrates, due to decay, expressed as a percent of the original levels in sound wood samples.*

Species	Sample variables	Weight loss (%)	Arabi-nose	Xylose	Man-nose	Galac-tose	Glu-cose	Total carbo-hydrate
Poplar	Decayed, nonacetylated	33.6	−31.3	−46.3	−48.5	−75.6	−52.5	−50.8
	Decayed, low acetylations	3.3	+51.5	−5.1	+24.3	−36.4	−18.3	−12.7
(white	Decayed, medium acetylations	3.1	+36.0	+9.1	+14.0	−42.6	+3.0	+4.9
rot)	Decayed, high acetylations	2.9	+50.0	+11.2	+45.0	−2.9	+11.2	+13.0
Pine	Decayed, nonacetylated	61.0	−87.6	−86.0	−92.2	−92.8	−83.4	−85.4
	Decayed, low acetylations	6.7	−21.5	−16.0	−16.0	−20.7	−11.3	−14.8
(brown	Decayed, medium acetylations	2.6	−3.0	+1.7	+1.7	+19.8	−0.7	+0.1
rot)	Decayed, high acetylations	1.7	−4.7	+3.2	+7.5	−13.0	+8.8	+7.5

+ Denotes increase (above original levels of sound wood).
− Denotes decrease (below original levels of sound wood).

total percent carbohydrates remained near sound wood levels in the medium and high treatment levels. Weight losses were lowered considerably by the minimum acetylations, but total carbohydrates were still depleted by 13% for poplar and 15% for pine. However, in the highest acetylation treatments, total percent carbohydrates were higher (7% for pine and 13% for poplar) than sound wood levels, indicating preferential removal of some other constituents. Interestingly, at each level of acetylation, total percent carbohydrates present (Tables 6 and 7) were very similar in both types of decayed wood. Curves for the individual monosaccharides follow these same general trends.

In both types of decay, holocellulose contents were very similar at each level of acetylation; however, the low treatment level was nearly as effective as higher ones in reducing weight losses due to white rot. Thus, the activity, synthesis, or both of lignin-degrading catalysts may in part be dependent on prior or simultaneous breakdown of carbohydrates. These breakdown products may (1) provide metabolic energy for the production of extracellular oxygenases and other fungal catalysts, or (2) may be involved in gene regulation and protein synthesis of enzymes, or (3) both. Esterification may also render free lignin hydroxyls unavailable to fungal secretions. These hydroxyl groups, however, are very infrequent in lignin macromolecules; thus their contribution to the protective action of acetylation should be minor.

Nonglucose sugars were proportionally more extensively degraded than glucose in brown-rotted, nonacetylated pine (Table 7). These findings were similar for the acetylated wood samples, although gas chromatographic results were erratic for the medium treatment level. In contrast, cellulose depolymerization exceeded that for hemicellulose polymers in nonacetylated and acetylated white-rotted poplar, since with few exceptions utilization of glucose was greater than nonglucose monomers. These results are consistent with literature on studies considering the order of removal of glucose vs. nonglucose sugars by different decay fungi (Boutelje et al. 1971; Kirk and Highley 1973).

Colonization of acetylated wood

In the nonacetylated woods, hyphal colonization of all cellular elements was nearly complete after just six weeks' exposure to decay fungi (Table 8). Acety-
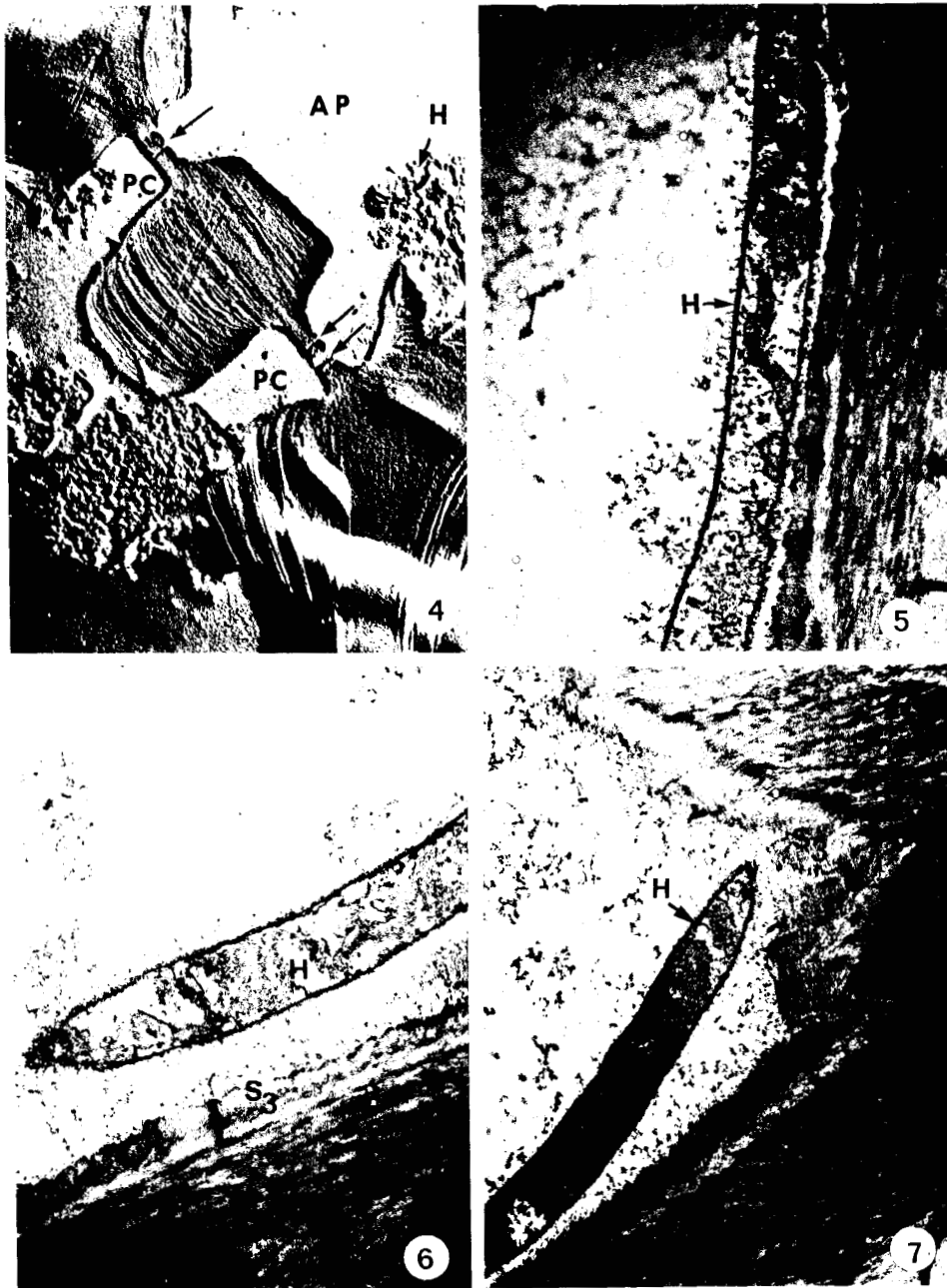
FIG. 4.   Degradation of pitting between ray (R) and axial parenchyma cells (AP) in ash, accompanied by enlargement of pit canals (PC) and plasmodesmatal connections (arrows) through pit membranes. White rot of ash; low acetylation (about 7.5 WPG); tangential section; Pt shadowed (20°); ×15,400.

FIG. 5.   A lumen-situated hypha (H) in contact with a pine tracheid wall. White rot of loblolly pine; medium acetylation (about 17 WPG); tangential section; KMNO$_4$ stained; ×23,400.

FIG. 6.   Hyphal cell (H) occupying a pine tracheid lumen; the S$_3$ is slightly degraded with no

TABLE 8. *Fungal colonization (percent of cells containing hyphae) of nonacetylated wood and wood at different levels of acetylation after six weeks' incubation.*

Type decay	Acetylation levels	Poplar			Ash			Pine		
		Fibers	Vessels	Rays	Fibers	Vessels	Rays	EW Trach.	LW Trach.	Rays
White rot	Nonacetylated	96.7	100.0	81.8	95.6	100.0	82.4	97.1	97.6	83.0
	Low	40.6	96.4	43.9	25.6	86.9	38.8	70.2	54.3	40.3
	Medium	16.2	53.8	10.8	13.0	47.2	18.3	30.6	9.8	13.2
	High	8.4	49.6	7.4	5.8	29.0	8.6	18.0	8.6	8.2
Brown rot	Nonacetylated	97.1	100.0	96.0	95.2	100.0	93.2	96.6	97.1	97.1
	Low	63.9	98.8	56.2	64.8	91.7	59.6	52.7	59.8	30.6
	Medium	18.0	65.4	13.0	13.6	54.0	17.4	23.0	11.6	9.0
	High	13.8	24.4	11.2	10.4	15.4	14.8	11.0	5.8	9.8

EW Trach. denotes earlywood tracheid.
LW Trach. denotes latewood tracheid.

lation profoundly affects colonization of wood; even the minimum levels of protection significantly reduced hyphal counts for both fungi. Although weight losses (from decay) were most noticeably affected by the lowest level acetylations, the largest differences in colonization generally occurred between the low and medium treatment levels. Over 70% fewer hardwood fibers contained brown-rot fungal hyphae, while counts for both fungi were approximately 80% less in latewood tracheids (medium acetylation level). Fungal colonization did not decrease as rapidly in cells with larger lumina, that is, hardwood vessels and earlywood tracheids. These cellular elements not only possess wide lumina, but large copious pitting and other openings (perforations in vessels) through which hyphae can more readily penetrate and colonize.

In contrast to the above situation, as the level of acetylation treatment increased from medium to high, colonization by brown-rot fungal hyphae was most adversely affected in the main conducting elements. The number of hardwood vessels containing hyphae was more than 60% and 70% less for poplar and ash, respectively, while counts were about 50% lower among pine tracheids (both earlywood and latewood). Hyphal numbers in rays were little affected (reductions of about 15% for all species) and only slightly more so in hardwood fibers.

Although higher levels of substitution inhibited carbohydrate consumption and decay (weight losses), colonization occurred in all cellular elements. To a certain extent, hyphae probably penetrate the wood structure through pit-pairs, fed by an energy source other than wood cell-wall substance. Since hyphae are able to translocate materials for considerable distances via cytoplasmic streaming (Ainsworth 1965), the soil substrate in culture bottles and storage products in ray parenchyma may supply this food. Decay fungi are also very efficient in recycling nitrogen (Cowling 1970) from older portions of hyphae to new zones where it

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apparent S_2 decomposition. White rot of loblolly pine; medium acetylation (about 17 WPG); tangential section; $KMNO_4$ stained; ×19,800.

FIG. 7.   Lumen at the tip of a pine tracheid. The tracheid wall in advance of the hyphal (H) tip is entirely intact. White rot of loblolly pine; medium acetylation (about 17 WPG); tangential section; $KMNO_4$ stained; ×13,200.
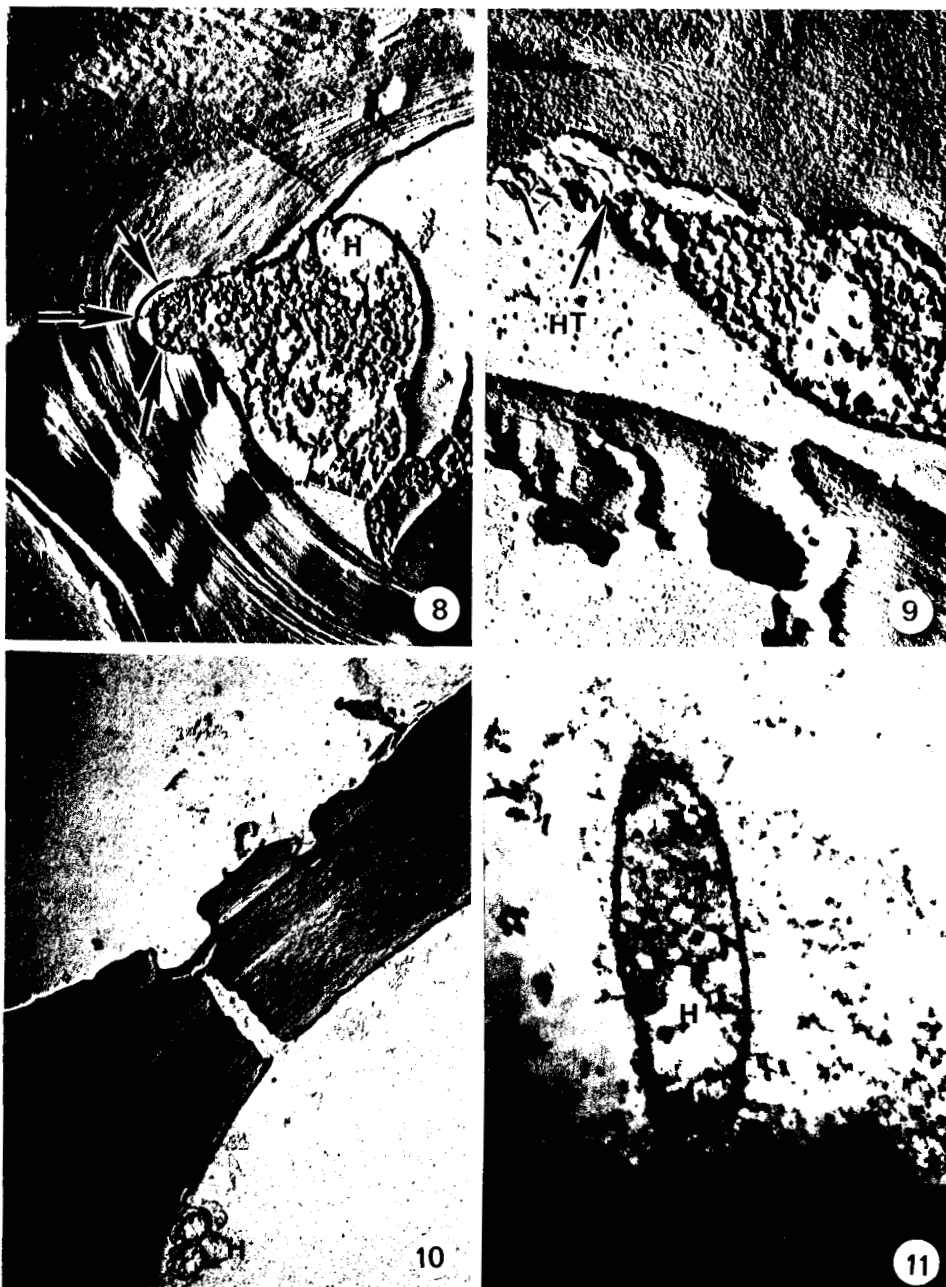
FIG. 8.   Bore-hole production in an ash fiber wall, without dissolution of surrounding wall material. Hyphal cell (H) shows signs of lysis. White rot of ash; low acetylation (about 7.5 WPG); cross section; Pt shadowed (20°); ×13,200.

FIG. 9.   Decomposition of an ash ray, along innermost secondary wall, is limited to an area in advance of the hyphal tip (HT). White rot of ash; low acetylation (about 7.5 WPG); cross section; Pt shadowed (20°); ×30,800.

FIG. 10.   Lumen hyphae (H) contacting an ash vessel wall. The laminar structure was preserved, erosion troughs were lacking, and cell-wall degradation was not evident. White rot of ash; low acetylation (about 7.5 WPG); cross section; Pt shadowed (20°); ×1,500.

could supplement such nutrients as starch and vitamins remaining in the sapwood.

In acetylated wood, the degree of dimensional stability, along with decay resistance, is proportional to WPG (Goldstein et al. 1961); in fact, the reduction in swelling at 20 to 25 WPG is about 70%. Therefore, Rowell (1975) feels this exclusion of cell-wall water is one of the most significant factors in the mechanism of protection, against fungi, by a variety of wood modification systems. If wood-in-service remains sufficiently dry (below FSP), decay normally will not occur, irrespective of chemical modification or preservative treatments. However, acetylated woods exposed to high moisture conditions, as in soil-block decay chambers, should contain free water in cell lumina and even at higher acetylation levels may therefore allow some colonization and growth (Table 8). The white-rotter used in this study attacks exposed cell-wall surfaces, causing an irregular decomposition of cell walls, progressing from the S_3 to the compound middle lamella (Bravery et al. 1974). So, at high moisture conditions (as in soil-block chambers), enough free water should be present in cell lumina or on lumen walls for white-rot fungal hyphae to initiate decomposition of the S_3 surface. Despite the presence of free water, weight-loss data (Table 2) and TEM observations of highly acetylated cell walls did not reveal cell-wall degradation. Therefore, in the acetylation of wood to prevent decay, the stereochemical changes that take place are far more important than its effect on cell-wall equilibrium moisture content.

Microscopy and ultrastructure of decay in acetylated wood

Similar patterns of cell-wall decomposition were noted for each type of decay fungus in the acetylated woods. Bore holes were produced by hyphae of either fungus only at minimum acetylation levels. Hyphal penetration of ray cell walls to adjacent ray cells and other cell types was common, but bore-hole formation between all longitudinal cell types was much more sporadic. In ray parenchyma pitting, plasmodesmatal connections (Fig. 4) were occasionally enlarged (minimum acetylations), while at all levels of protection, localized degradation of ray cell walls was often affected by hyphae inhabiting ray intercellular spaces.

Figures 5 and 6 depict lumen situated hyphae adjacent to the cell wall. Note the slight degradation of the S_3 layer and the intact S_2 layer. Most bore-hole and some lumen-hyphae underwent lysis, typified by loss of cell contents and usually accompanied by cellular debris (Figs. 4 and 8). Hyphal disruption of this nature may be attributed to residual acetic acid in amorphous regions of cell walls or perhaps to slight uptake or acetyls from wall substance in contact with hyphal tips.

The diffuse zone of catalytic activity (gelatinous sheath) normally associated with active hyphal tips was lacking in acetylated woods (Fig. 7), and bore holes were never larger than contained hyphae (low acetylation level). Removal of the interfibrillar matrix was restricted to the immediate area in advance of hyphal tips (Figs. 8 and 9), and cell-wall material surrounding hyphae in bore holes was completely intact (Fig. 8). But clearing of the cell-wall microfibrillar structure and

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Fig. 11.   Longitudinal view of the cell tip area of a pine tracheid. A lumen-situation hypha (H) is present, but no dissolution of the tracheid wall occurred. White rot of loblolly pine; medium acetylation (about 17 WPG); tangential section; KMNO₄ stained; ×26,400.

hence penetration of hyphal tips was completely prevented (Fig. 7) by WPG's of 15 or more (medium and high acetylations). Thus, bore holes apparently were not produced solely by mechanical forces as was originally suggested by Cartwright (1930). Rather, at least a partial metabolism of cell-wall constituents by fungal catalysts in advance of tips is required for penetration by these fungi.

That extracellular secretions of either fungus were unable to effect dissolution of wood cell walls at appreciable distances from producing hyphae was exemplified by a lack of submicroscopic voids in any wall layers, a lack of separation of wall lamellae, and retention of original contrast in wall layering (Fig. 10). Lumen hyphae in contact with cell walls did not form erosion troughs of dissolution (Figs. 10 and 11); however, in white-rotted poplar with the lowest acetylation, walls of ray and axial parenchyma cells were somewhat thinned. Neither fungus had much effect on wood acetylated to WPG's of 15 and above; degradation was limited to infrequent lysis of the S_3 layer in hardwood fibers and pine tracheids. It is hypothesized then, that in the higher acetylations, WPG's of 15 and more, not only has production and liberation of fungal secretions been reduced (fungal cells appeared "starved" and tips were devoid of gelatinous sheaths) but their affinity for the substrate was probably blocked.

CONCLUSIONS

The following conclusions emphasize the major results from chemical and ultrastructural analysis of the decayed, acetylated woods:

1. Inhibition of action of cell-wall dissolving catalysts of decay fungi is the primary mode of protection of the acetylation technique. In the higher acetylation treatments, cell-wall degradation and clearing of the microfibrillar structure were lacking, and hyphal penetration of wood cell walls was not observed. The acetylations were not fungitoxic; however, hyphal cells lacked internal organization, indicating that they were "starving" in the presence of plenty.

2. The synthesis, activity, or both, of lignin-degrading catalysts of the white-rot fungus may be dependent on prior or simultaneous breakdown of carbohydrates. Weight losses, due to white rot, varied only slightly from low to high acetylations, even though the patterns of holocellulose utilization and colonization were very similar to those of brown-rotted woods. Apparently with the blockage of cellulase activity, significant lignin consumption is prevented.

3. Results of the carbohydrate analysis indicated that in both the acetylated and nonacetylated woods, hemicelluloses were more extensively depleted than cellulose by the brown-rotter; this situation was reversed in the white-rotted woods.

4. In the higher level acetylation treatments, removal of wall polymers by fungal catalysts was inhibited and bore holes were lacking, suggesting that penetration of acetylated wood cell walls by these fungi was not possible on the basis of mechanical forces alone.

REFERENCES

AINSWORTH, G. C. 1965. The fungal cell. *In* The fungi, vol. 1, Academic Press, New York. 748 pp.
AMERICAN WOOD PRESERVER'S ASSOCIATION. 1974. Standard method of testing wood preservatives by laboratory soil block cultures. Std. Method M10-74.

BORCHARDT, L. G., AND PIPER, C. V. 1970. A gas chromatographic method for carbohydrates as alditol-acetates. Tappi 53:257–260.

BOUTELJE, J., K. E. ERIKSSON, AND B. N. HALLMARK. 1971. Specific enzymic hydrolysis of the xylan in a spruce holocellulose. Sven. Papperstidn. 74(2):32–37.

BRAVERY, A. F., N. PARAMESWARAN, AND W. LIESE. 1974. Electron microscopic investigations on the effect of tri-n-butyl tin oxide on the decay of beechwood by *Polystictus versicolor*. Part 1. Observations and solvent-treated control material. Material und Organismen 9(2):133–144.

BROWNING, B. L. 1967. Methods of wood chemistry, vol. 2. Interscience Publications. 882 pp.

CARTWRIGHT, K. ST. G. 1930. A decay of sitka spruce caused by *Trametes serialis*. Bull. For. Res. (Lond.) 4.

CHOU, C. K., R. D. PRESTON, AND M. P. LEVI. 1974. Fungitoxic action of a copper-chromium-arsenate wood preservative. Phytopathology 64:335–341.

COWLING, E. B. 1961. Comparative biochemistry of the decay of sweetgum sapwood by white-rot and brown-rot fungi. USDA For. Serv. Tech. Bull. No. 1258.

—————. 1970. Nitrogen in forest trees and its role in wood deterioration. Acta Universit. Up. 164: 1–19.

GOLDSTEIN, I. S., E. B. JEROSKI, A. E. LUND, J. F. NIELSON, AND J. W. WEAVER. 1961. Acetylation of wood in lumber thickness. For. Prod. J. 11:363–370.

KIRK, T. K., AND T. L. HIGHLEY. 1973. Quantitative changes in structural components of conifer woods during decay by white-rot and brown-rot fungi. Phytopathology 63(11):1338–1342.

LEHNINGER, A. L. 1970. Biochemistry. Worth Publishers, Inc., NY 83 pp.

OZOLINA, I. O., AND K. P. SVALBE. 1966. Acetylation of wood and biological testing of acetylated samples. Latv. PSR Zinat. Akad. Vestis 56–69.

ROWELL, R. M. 1975. Chemical modification of wood: advantages and disadvantages. Proc. AWPA 71:41–51.

SCHEFFER, T. C. 1973. Microbiological degradation and the causal organisms. Pages 31–106 *in* D. D. Nicholas, ed. Wood deterioration and its prevention by preservative treatments, vol. 1. Syracuse University Press, NY.

TARKOW, H. A., A. J. STAMM, AND E. C. O. ERICKSSON. 1950. Acetylated wood. USDA For. Serv. FPL Rep. No. 1593.

WILCOX, W. W. 1964. Preparation of decayed wood for microscopical examination. USDA For. Serv. Res. Note FPL-056.

YOCUM, R. H., AND M. M. JOULLIE. 1966. Polar, steric, and solvent effects in the cleavage reactions of trichloro- and tribromoacetates with primary and secondary amines. J. Org. Chem. 31(11):3823–3827.