Changes in the Chemical Composition of Wood Caused by Six Soft-Rot Fungi

W. E. Eslyn, T. K. Kirk, and M. J. Effland

Forest Products Laboratory, U.S. Department of Agriculture, Forest Service, Madison, Wisconsin 53705.
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ABSTRACT

Standardized blocks of alder, poplar, and pine wood were decayed by six soft-rot fungi (*Graphium* sp., *Monodictys* sp., *Paecilomyces* sp., *Papulospora* sp., *Thielavia terrestris*, and *Allescheria* sp.), all of which had been isolated from pulp chip storage piles. Samples of the woods at different weight losses were analyzed for lignin, glucan (cellulose), xylan, and mannan (hemicelluloses) to allow calculation of the depletion in these major components caused by the fungi. Carbohydrates were depleted faster than lignin in the alder and poplar, cellulose usually faster than the major hemicellulose (xylan). Lignin, which was analyzed by the "sulfuric acid" method, was depleted by all the fungi. Pine was not decayed significantly by three of the fungi, and only to low weight losses (15% or less) by the other three (*Paecilomyces* sp., *Papulospora* sp., and *Thielavia terrestris*). Analysis of blocks that were decayed showed that lignin was depleted faster than the cellulose or the hemicelluloses by *Paecilomyces* sp. and *Allescheria* sp.

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Of the three major types of fungal decay of wood (soft-rot, brown-rot, and white-rot) the least studied is soft-rot, which is caused by numerous members of the Fungi Imperfecti and Ascomycetes (1). Research has been especially deficient in elucidating the chemistry of the soft-rot of wood. In fact, the chemical effects of only one fungus, *Chaetomium globosum,* on one kind of wood, European beech (*Fagus sylvatica*), have been investigated (5). *Chaetomium globosum* causes extensive loss in weight of beech, depleting the cellulose and pentosans (mainly xylan) considerably faster than the lignin (7, 9, 10). Working with isolated polysaccharides rather than whole wood, Keilich et al. (4) and Takahashi and Nishimoto (11) found that *C. globosum* was capable of degrading xylans and mannans, the former being degraded at a slightly greater rate than the latter.

Soft-rot fungi, including *Chaetomium globosum,* have been isolated in large numbers from pulp chip storage piles in recent years (2). In laboratory decay studies, these isolates caused considerable loss of wood substance, particularly in hardwood chips, thereby emphasizing the importance of soft-rot fungi as destructive organisms. However, it has not been determined whether all or most soft-rot fungi cause chemical changes similar to those caused by *Chaetomium globosum,* or whether for example, some attack the lignin, the cellulose, or one of the hemicelluloses preferentially. Such information is needed for a full assessment of the destructive capabilities of these organisms, particularly those known to be prevalent in chip storage piles and capable of causing substantial weight losses in wood. The purpose of this research, therefore, was to determine quantitatively the changes in the major structural components of wood during decay by several wood-chip-inhabiting soft-rot fungi.

MATERIALS AND METHODS.—*Wood samples and decay.*—Outer xylem blocks, 25.4 × 12.5 × 6.3 mm (1 × 1/2 × 1/4 in.), with the 6.3 mm dimension in the fiber direction, were cut from single boards of red alder (*Alnus rubra* Bong.), balsam poplar (*Populus balsamifera* L.), and western white pine (*Pinus monticola* Dougl.). The blocks were numbered, conditioned to constant weight at 27 C and 70% RH, and then weighed. Eight replicate blocks from each wood species were strung together, sterilized, and then subjected to decay by the serial-block method (3). Following 12 weeks of incubation at 27 C for mesophilic test fungi, and at 45 C for thermophilic test fungi, the blocks were removed, reconditioned, weighed, and their weight losses calculated. The small size of the blocks, coupled with their relatively large cross-sectional area, permitted rapid penetration by the test fungi and uniform decay throughout the blocks.

The test fungi used were all isolated from wood chip storage piles, and had been shown in previous laboratory tests to be capable of causing appreciable weight losses in wood. Mesophilic isolates included *Graphium* sp. (ME-BC-11), *Monodictys* sp. (ME-GC-18), and *Papulospora* sp. (ME-PC-19). Thermophiles included were *Paecilomyces* sp. (ME-T-BC-10), *Thielavia terrestris* (Apinis) Mallock & Cain = *Allescheria terrestris* (H-63-1), and *Allescheria* sp. (ME-T-BC-9).

The decayed blocks of each species were roughly grouped according to weight losses caused by each test fungus, and blocks of desired weight loss were removed for individual chemical analysis. (The variation in weight loss within a wood species by the same decay fungus reflects primarily the intentional variation in the moisture contents among blocks in a test (3).) A block of poplar and one of pine, well decayed by each of the test fungi, were sectioned transversely and tangentially and examined microscopically to determine whether each of the test fungi caused typical soft-rot in both hardwoods and softwoods.

Analyses.—Decay and control blocks were ground to pass a 0.50-mm (40-mesh) screen and the meal dried thoroughly at 45 C under high vacuum. The samples were analyzed, without extraction, for "sulfuric acid lignin" by an adaptation of a published procedure (12) and for total reducing sugars in acid hydrolysates (determined colorimetrically as glucose) (8). Relative amounts of glucose, xylose, and mannose in the hydrolysates were determined colorimetrically after paper chromatographic separation (8). From these values, the glucan, xylan, mannan, and lignin, and the losses of each during decay were calculated (6).

Angiosperm wood is composed mainly of cellulose, a hemicellulose of the *O*-acetyl-4-*O*-methylglucuronoxylan type (approximately 75% xylan) and lignin, plus a smaller

TABLE 1. Analytical data for sound woods[a]

Wood	Hemicelluloses: Lignin (%)	Glucan (%)	Mannan (%)	Xylan (%)	Other[b] (%)
Balsam poplar	22.3	46.0	4.1	16.6	11.0
Red alder	25.4	40.8	2.3	20.0	11.5
White pine	27.5	40.8	15.8	7.0	8.9

[a]The analytical methods used here were developed to give accurate values with various wood samples (8). To estimate the precision of the methods, 12 replicate analyses were made over a period of time for a homogeneous sample of aspen wood, with statistical results as follows [data given in the following order: component analyzed, sample mean, sample standard deviation, confidence interval for the true mean ($P = 0.05$)]: glucan, 50.76, 0.31, 50.57-50.95; mannan, 2.36, 0.07, 2.31-2.41; xylan, 18.92, 0.33, 18.71-19.13; and lignin, 18.19, 0.32, 17.99-18.39, respectively.

[b]Includes extractives, acid-soluble lignin, acetyl, uronic acids, etc.

percentage of a hemicellulose of the glucomannan type (about 65% mannan) (13). Thus, determination of glucan gives an estimate of cellulose with a small error due to glucomannan, xylan gives an estimate of the major hemicellulose, and mannan provides an estimate of the minor hemicellulose.

In gymnosperm wood, the major hemicellulose is of the galactogluco-mannan type, which is about 70% mannan, with an arabino-4-*O*-methyl-glucuronoxylan (about 65% xylan) as a minor hemicellulose (13).

RESULTS.—All test fungi caused the formation of spindle-shaped cavities in the secondary cell walls of both poplar and pine wood blocks. The term "soft-rot" was applied by Savory (9) to the type of rot characterized by the development of hyphae within the cell walls and the formation there of the type of cavity described.

The percent contributions of the major structural polymers in the red alder, balsam poplar, and white pine wood are given in Table 1. By subtracting the total percent of major components (glucan + xylan + mannan + lignin) from 100%, the percentage of "other" materials is obtained. These include extractives, acid-soluble lignin, acetyl, inorganic components, and uronic acid. During decay of alder and poplar by all fungi, this percentage of "other" materials increased when expressed on the basis of the decayed samples. The maximum amount of "other" materials reached 16% (at a 41% weight loss in alder and at a 28% weight loss in poplar, decayed by *Paecilomyces* sp.). With the pine, which was decayed only to 15% weight loss or less, the percentage of "other" materials decreased. The increase in the case of the hardwoods reflects: (i) failure of the fungi to deplete inorganic components and perhaps certain extractives, and (ii) formation of additional materials such as acid-soluble degraded lignin and fungus products that did not analyze as one of the major wood components. These "other" materials are not considered further, since they do not affect our conclusions concerning depletion of the major components.

The analytical values for the decayed woods are summarized in Tables 2 (alder), 3 (poplar), and 4 (pine) as percent losses in lignin, glucan, mannan, and xylan.

Alder and poplar.—The major attack by the fungi was directed toward the cellulose (glucan) and the major hemicellulose (xylan). Glucan was depleted faster than the other components by all of the fungi on red alder (Table 2); however, on poplar, glucan and xylan were catabolized at similar rates (Table 3).

Depletion of mannan from the hardwoods was quite variable, partly because it is a minor component (Table 1) and the analysis for it can be significantly influenced by small amounts of other materials such as sugars of fungal origin. With *Graphium* sp. on alder, there was an

TABLE 2. Loss of major structural components from red alder wood test blocks decayed to various extents by six soft-rot fungi

Fungus	Extent of decay[a] (%)	Structural component loss[b] (%): Lignin	Glucan	Xylan	Mannan
Mesophiles:					
Graphium sp.	11	−5	31	17	−60
	26	20	34	34	−17
	34	18	49	45	−16
Monodictys sp.	9	2	14	7	24
	23	9	32	30	35
Papulospora sp.	10	9	15	6	14
	17	12	23	18	18
Thermophiles:					
Paecilomyces sp.	15	11	21	25	28
	25	13	37	23	20
	41	19	60	44	22
Thielavia terrestris	7	9	10	1	19
	28	17	40	29	21
Allescheria sp.	15	12	21	14	3
	30	16	44	31	23

[a]Percent of total original weight lost by test block.

[b]These data are based on a single determination of each component in each test block, and are expressed on the basis of the original amount of each component in the sound wood. The methods used have proven to give reproducible values (see footnote "a", Table 1).

apparent increase in mannan especially during initial decay. This surprising result was investigated by analyzing the hydrolysates for mannose by a paper chromatographic procedure which separates all five wood sugars (glucose, mannose, xylose, arabinose, and galactose). This procedure is more diagnostic for mannose, and the results confirmed the original data. Thus, on alder *Graphium* sp. evidently produced mannose or another reducing sugar which was inseparable from mannose in our chromatographic systems. In contrast, *Paecilomyces* sp. and *T. terrestris* caused a substantial depletion of the mannan in initial decay of both woods.

Lignin was depleted from both woods by all of the fungi, but considerably more slowly than were the carbohydrate components (Tables 2 and 3). *Graphium* sp. on alder, *Monodictys* sp. on both woods, and *Papulospora* sp. on poplar, depleted the lignin only slowly at first, but with the exception of *Papulospora* sp. on poplar, more rapidly later.

Pine.—The fungi caused only low weight losses in pine, compared to alder and poplar; three of the fungi caused only insignificant decay. Because of this it was possible to determine only the initial changes in composition caused by three fungi (Table 4). Results, however, showed that in pine the lignin is depleted more rapidly in proportion to the other components than with the same fungi on the alder or poplar. *T. terrestris* and *Paecilomyces* sp. preferentially attacked the lignin in pine at the weight losses indicated (Table 4).

DISCUSSION.—These results with alder and poplar are similar to the results obtained with the soft-rot fungus *C. globosum* on beech (7, 9, 10). A few variations are seen in the relative rates of removal of the components, but overall it is clear the *C. globosum* and the six soft-rot fungi examined here all have similar effects on hardwoods. Attack is heaviest on the cellulose and the (major) hemicellulose.

Levi and Preston (7) showed that *C. globosum* was similar to brown-rot fungi in its effect on lignin (causing

TABLE 3. Loss of major structural components from balsam poplar wood test blocks decayed to various extents by six soft-rot fungi

Fungus	Extent of decay[a] (%)	Structural component loss[b] (%)			
		Lignin	Glucan	Xylan	Mannan
Mesophiles:					
Graphium sp.	16	10	20	25	12
	22	15	27	34	16
Monodictys sp.	10	0	8	12	19
	22	13	27	26	38
Papulospora sp.	10	0	14	17	14
	21	4	27	29	25
Thermophiles:					
Paecilomyces sp.	14	10	15	35	28
	28	11	41	37	30
Thielavia terrestris	10	6	11	12	29
	23	13	25	27	43
Allescheria sp.	11	6	15	17	22
	28	9	32	29	30

[a]Percent of total original weight lost by test block.
[b]These data are based on a single determination of each component in each test block and are expressed on the basis of the original amount of each component in the sound wood. The methods used have proven to give reproducible values (see footnote "a", Table 1).

TABLE 4. Loss of major structural components from white pine wood test blocks decayed to various extents by three soft-rot fungi

Fungus	Extent of decay[a] (%)	Structural component loss[b] (%)			
		Lignin	Glucan	Xylan	Mannan
Mesophile:					
Papulospora sp.	15	12	18	18	13
Thermophiles:					
Paecilomyces sp.	10	14	7	8	6
Thielavia terrestris	7	14	3	9	−3

[a]Percent of total original weight lost by test block.
[b]These data are based on a single determination of each component in each test block and are expressed on the basis of the original amount of each component in the sound wood. The methods used have proven to give reproducible values (see footnote "a", Table 1).

primarily demethylation) while it was similar to white-rot fungi in its effect on the alkali-soluble fraction of wood. Seifert (10) classified the decomposition of beech wood cellulose and lignin by this fungus as intermediate between that of brown- and white-rot fungi. Generally, white-rot fungi deplete lignin as fast as, or faster than, the polysaccharides (5). In contrast, brown-rot fungi cause a progressive degradation, but not depletion, of the lignin (5). In the present study, all the soft-rot fungi utilized the carbohydrates of alder and poplar ahead of the lignin, and thus their action was similar to that of brown-rot fungi. However, on pine both *Paecilomyces* sp. and *T. terrestris* removed lignin faster than carbohydrates, as is more characteristic of white-rot fungi.

In measuring lignin by the "sulfuric acid" method (12), we have not determined the actual extent of degradation of the lignin. This relatively crude analysis for lignin is reproducible, but not specific or fully accurate. The remaining lignin should be characterized; Levi and Preston (7) presented evidence that the lignin remaining in soft-rotted beech is partly degraded; it has a lower methoxyl content and is more acid-soluble than the lignin in sound wood.

Soft-rot fungi do not attack softwoods as rapidly or as extensively as they do hardwoods. This was confirmed in the present study. The more rapid decay of lignin relative to the carbohydrates in pine and the lower overall weight losses may be related. If it is assumed that the lignin is a greater hindrance to soft-rot fungi in conifers than it is in hardwoods, then the carbohydrates could not be depleted substantially faster than lignin.

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