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## Changes in Cell Wall Components of White Pine and Maple by White-Rot Fungi

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### INTRODUCTION

Few detailed studies have been made of the relative rates of removal of the structural components of wood (cellulose, hemicelluloses, and lignin) during decay by white-rot fungi. Kretsberg et al. (1971) showed that the total pentosans are destroyed faster than the cellulose, and the lignin more slowly than cellulose or pentosans, during the decay of spruce by the white-rot fungus Trametes trogii. Kirk and Highley (1973) found that the relative rates of removal of lignin and the other components by three white-rot fungi in conifer woods decayed in soil-block tests varied during the decay process. Their results suggested that removal of glucomannan may precede removal of cellulose as found in brown-rots, but that additional wood and fungus combinations are needed before it can be established whether this is a valid generalization for white-rots. For hardwoods, such detailed analysis seem to have been done only by Cowling (1961) who found that Coriolus versicolor removed lignin and carbohydrates at about the same rate in sweetgum. Removal of glucan, mannan, and xylan, however, was not determined until 25% weight loss.

The selective removal of cell wall components by white-rot fungi may be changed by nutritional factors that influence fungus physiology. For example, Blanchette et al. (1985) found that Ganoderma applanatum and Ischnoderma resinorum selectively delignified wood in nature, but in

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laboratory soil-block tests, all cell wall components were removed. Factors that affect cellulose, hemicellulose, and lignin degradation need to be identified. One such factor appears to be nitrogen. Low nitrogen levels enhanced degradation of lignin model compounds by P. chrysosporium (Kirk et al., 1978). Later studies showed that low nitrogen induced formation of H<sub>2</sub>O<sub>2</sub> and ligninase in P. chrysosporium. On the other hand, high carbohydrate levels repressed carbohydrate and lignin degrading enzymes of P. chrysosporium (Ericksson, 1978; Kelly and Reddy, 1986).

It is important to establish if certain cell wall components are preferentially removed during decay and to determine if cultural parameters, such as nitrogen and carbohydrate levels, govern selectivity of removal. For example, if hemicellulose and/or lignin utilization are essential early steps in the decay process, blocking these steps will stop the whole decay process. The specific purposes of obtaining these data are (1) to elaborate on the changes in individual cell wall components during decay by selected white-rot fungi in hardwoods and soft woods, and (2) to determine the effect of exogenous nutrients on removal of cell wall constituents.

#### **MATERIALS AND METHODS**

##### Wood Samples and Decay Tests

Sapwood blocks 6.35 by 6.35 by 3.18 mm (1/4 by 1/4 by 1/8 in., the small dimension in the fiber direction) were cut from western white pine (Pinus monticola Dougl.) and maple (Acer spp.). Small test blocks were used rather than large blocks because small blocks provide more uniform decay throughout, particularly in the early stages. The blocks were numbered, conditioned to constant weight at 27°C and 70% relative humidity, and then weighed. Blocks were decayed by the American Society for Testing and Materials Standard soil-block method (ASTM, 1971) and an agar-block method. The soil-block method involves contact of the test wood with actively growing fungus on wood in soil contact, which provides a source of nutrients for the fungi, thus enhancing decay. The agar-block tests used in this study were designed to provide favorable conditions for decay, but also to prevent contamination of the block by foreign nutrient material and

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leaching of degradation products from blocks. With this method, the test fungi were grown on Whatman<sup>2</sup> No. 1 filter paper strips placed over a nutrient-agar medium (Highley, 1973a). After the fungi covered the filter paper, the strips were removed and placed on triangular-shaped glass rods over 1.5% water agar containing no additional nutrients, 10% glucose, 0.5% NH<sub>4</sub>NO<sub>3</sub>, or 10% glucose plus 0.5% NH<sub>4</sub>NO<sub>3</sub> in 8-oz French square bottles. In both soil-block and agar-block chambers, 20 blocks per bottle were decayed at different lengths of time to obtain samples in various stages of decay. Following incubation, the blocks were removed, reconditioned, weighed, and their weight losses calculated. Noninoculated blocks served as controls.

#### Fungal Culture

Blocks were decayed by the following white-rot fungi: Phanerochaete chrysosporium Burds. (ME-461), Coriolus versicolor (L.ex Fr.) (MAD-6471, Irpex lacteus (Fr.:Fr.)Fr. (HHB-7328-sp.), Bjerkandera adusta (Willd.:Fr.) Karst. (L-15359-sp.), and Phlebia brevispora Nakas. in Nakasone et Eslyn (HHB-7030-sp.).

#### Analytical Analysis

Sound and decayed wood 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 for Klason lignin using previously described methods (Effland, 1977). Relative amounts of glucose, xylose, and mannose in acid hydrolysates were determined using high-pressure liquid chromatography as described by Petterson et al. (1984). From these values the glucan, xylan, and mannan, and losses of each during decay were calculated (Springer, 1966).

Cellulose (~41%), hemicelluloses (~26%), and lignin (~29%) are the principal components of conifer wood. The two principal hemicelluloses are (1) a galactoglucomannan (~16%), which is about 70% mannan, and (2) an arabino-4-0-methylglucuronoxylan (~10%), which is about 65% xylan (Timmell, 1967). Thus, the measured amount of glucan is an estimate of cellulose content with a small error due to glucomannan; the amount of mannan is an estimate of the major hemicellulose, and the amount of xylan an estimate of the minor hemicellulose.

In angiosperm wood, 0-acetyl-methylglucuronoxylan is the major hemicellulose (~25%), which is about 75% xylan. The minor amount is of the glucomannan type (~3%), which is about 65% mannan.

#### **RESULTS**

The analytical values for the wood decayed over soil or water-agar are

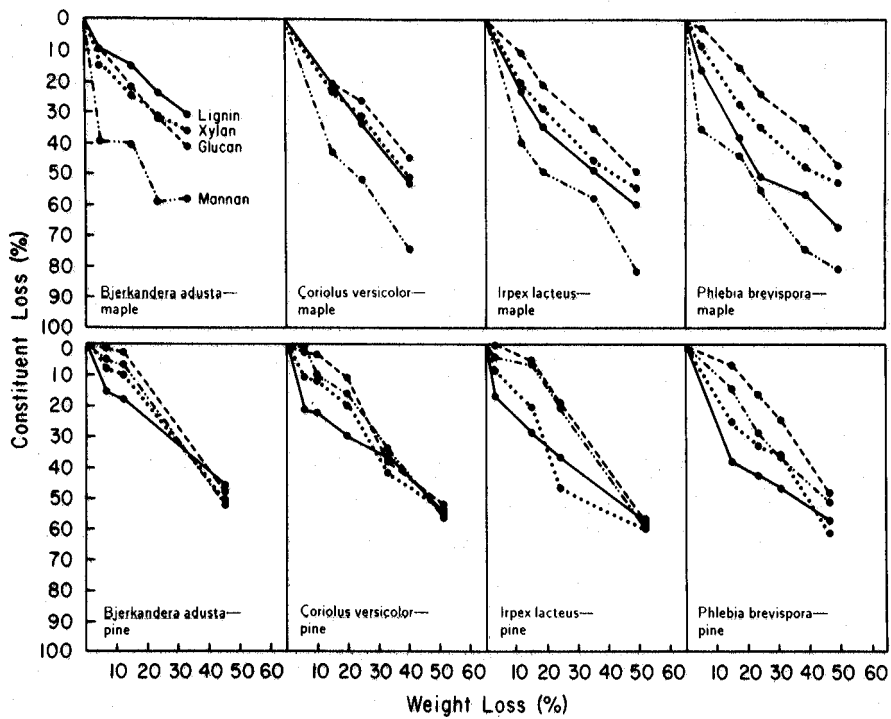


Figure 1. Progressive Loss of Major Structural Components in Pine and Maple Decayed by Four White-Rot Fungi by the Soil-Block Method. At Least 20 Blocks of Similar Weight Loss Were Combined for Analysis. Data are Based on a Single Determination of Each Component in the Combined Blocks and are Expressed on the Basis of the Original Amount of Each Component in the Sound Wood. The Analytical Methods have Proven to Give Reproducible Values (Moore and Johnson, 1967).

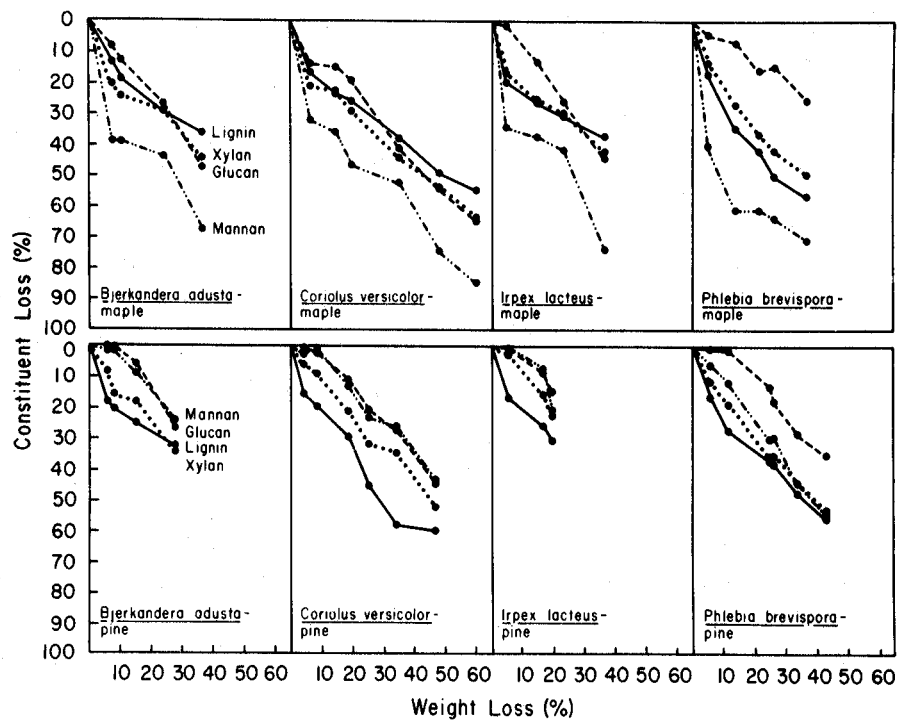


Figure 2. Progressive Loss of Major Structural Components in Pine and Maple Decayed by Four White-Rot Fungi by the Agar-Block Method. See Figure 1 for Explanation of Data.

illustrated graphically in Figures 1 and 2 as loss in lignin, glucan, mannan, and xylan, with results expressed as a percentage of the original amounts of each.

On the same wood species, the relative rate of removal of the major structural components was similar for all the white-rotfungi. Likewise, the relative rate of removal of components in wood decayed over soil was similar to that decayed over water-agar. Removal of components did differ with the wood species. In white pine, at the lower weight losses, lignin was removed faster than the carbohydrate components. As decay progressed, amount of lignin removal was generally comparable to the other components. In maple, lignin removal usually progressed at a rate similar to or slightly faster than xylan and glucan. Mannan was removed at a substantially faster rate in maple than all the other components by all the fungi.

Table 1 compares the loss in weight of maple blocks exposed over water-agar to that obtained with glucose or  $\text{NH}_4\text{NO}_3$  added to the medium. Ten percent glucose caused marked inhibition of decay by all the fungi and completely stopped decay of *Ph. brevispora* and *B. adusta* over the 24-month incubation period. Ammonium nitrate (0.5%) had little effect on the decay rate of the fungi except for *Ph. brevispora* where decay decreased. Glucose and  $\text{NH}_4\text{NO}_3$  together decreased the rate of decay about the same as glucose alone.

The effect of glucose and  $\text{NH}_4\text{NO}_3$  on removal of cell wall components in maple by the white-rotfungi is given in Table 2. Where decay occurred, glucose stopped cellulose utilization and accelerated lignin utilization. With  $\text{NH}_4\text{NO}_3$  in the medium, removal of cell wall components from maple differed little from blocks exposed over water agar. Similarly, glucose and  $\text{NH}_4\text{NO}_3$  together had little effect on removal of cell wall components where decay occurred.

#### DISCUSSION

The relative rates of removal of the major structural components in pine and maple among the white-rotfungi were similar. However, the fungi did remove cell wall constituents at a different rate in white pine than in maple. Differences in removal of cell wall constituents in different woods by the same white-rotfungus have been observed in other studies (Kirk and Highley, 1973; Kirk and Moore, 1972). Brown-rotfungi, on the other hand, remove cell wall components in wood at about the same rate in different wood species (Highley, 1987) and remove mannan substantially faster than the other cell wall components. Similarly, the white-rotters in this study preferentially removed mannan in maple, However, lignin was removed faster

Table 1. Effect of Glucose and  $\text{NH}_4\text{NO}_3$  on Weight Loss by White-Rot Fungi in Maple<sup>a</sup>.

Time (weeks)	Weight loss (%)															
	<u>C. versicolor</u>				<u>Ph. brevisporia</u>				<u>Irpex lacteus</u>				<u>B. adustus</u>			
	W-A	Gl	N	Gl+N	W-A	Gl	N	Gl+N	W-A	Gl	N	Gl+N	W-A	Gl	N	Gl+N
2	6	0	7	0	0	0	0	0	0	0	0	0	3	0	0	0
4	11	0	17	0	9	0	0	0	6	0	5	0	5	0	5	0
8	48	4	30	0	22	0	0	0	23	0	16	0	15	0	12	0
10	49	10	41	8	28	0	9	0	25	0	35	0	16	0	15	0
16	-	11	-	12	-	0	15	0	-	0	37	0	-	0	30	4
24	-	12	-	18	-	0	17	0	-	14	41	5	-	0	36	11

<sup>a</sup>W-A, 1.5% agar in water medium  
 Gl, 10% glucose added to agar medium  
 N, 0.5%  $\text{NH}_4\text{NO}_3$  added to agar medium

Table 2. Effect of Glucose and  $\text{NH}_4\text{NO}_3$  on Loss of Major Structural Components from Maple Decayed by White-Rot Fungi.<sup>a</sup>

Fungus, nutrient	Time	Loss(%)				
		Total weight	Lignin	Glucose	Mannan	Xylan
<u>C. versicolor</u>						
Glucose	10	6	8	0	3	7
	16	10	34	0	7	14
	24	17	44	0	10	28
Nitrogen	2	6	7	3	10	8
	4	17	21	17	20	18
	8	28	31	22	40	27
	16	40	45	53	63	46
Glucose + Nitrogen	10	5	3	0	4	4
	12	8	7	7	7	7
	24	18	21	15	10	18
	24	25	32	19	15	28
<u>Irpex lacteus</u>						
Glucose	24	9	44	0	13	19
	24	15	51	0	13	25
Nitrogen	4	8	8	8	20	8
	8	16	14	23	37	17
	10	24	21	35	50	25
	16	35	29	45	53	37
	24	46	49	52	50	48
Glucose + Nitrogen	24	5	3	5	0	6
	24	7	6	6	9	7
<u>Bjerkandera adustus</u>						
Glucose	24	0	0	0	0	0
Nitrogen	4	5	5	6	0	7
	8	10	12	8	3	5
	10	15	16	17	20	14
	12	23	23	26	27	20
	16	33	30	40	50	30
	24	44	39	49	63	38
Glucose + Nitrogen	16	6	0	4	13	4
	24	11	19	8	13	13
	24	15	23	15	17	13
<u>Phelbia brevispora</u>						
Glucose	24	0	0	0	0	0
Nitrogen	16	9	6	2	3	8
	24	15	20	6	10	28
	24	17	37	5	20	28
Glucose + Nitrogen	24	0	0	0	0	0

<sup>a</sup>Blocks were exposed to decay fungi over an agar medium with 10% glucose, 0.5%  $\text{NH}_4\text{NO}_3$ , or 10% glucose plus 0.5  $\text{NH}_4\text{NO}_3$ .

in white pine than the other components. In another study (Highley, 1982), C. versicolor removed carbohydrates faster than lignin from western hemlock, white spruce, and southern pine early in the decay process. As decay progressed, lignin and carbohydrates were removed at about the same rate. In this study, the white-rotters removed the hemicellulose faster than cellulose in both maple and white pine. As with brown-rotters (Highley, 1987), removal of cell wall constituents by the white rotters was similar when decayed by the soil-block or the low nutrient agar-block method.

The ability of white-rotters to degrade cell-wall components of wood is dependent upon production of several extracellular enzymes. Lignin degradation is dependent upon a number of enzymes collectively called ligninases, and carbohydrates are degraded by cellulase and hemicellulase complexes. Nitrogen supply plays a crucial regulatory role in wood decay in that high nitrogen levels may inhibit formation of ligninolytic enzymes and lignin degradation by white-rot fungi (Kirk, et al., 1978). On the other hand, nitrogen levels are reported to have a variable effect on cellulose decomposition by fungi (Park, 1976). Some fungi decomposed cellulose more slowly with low nitrogen availability, while others decomposed cellulose more slowly with increased nitrogen concentrations (Park, 1976). High nitrogen levels have been found to stimulate carbohydrate breakdown by some wood decay fungi (Levi and Cowling, 1969; Reid, 1983). Dill and Kraepelin (1986) found that adding nitrogen sources resulted in rapid and almost complete decay of the cellulose in Palo padrido. In the present study, high nitrogen levels in the medium did not affect rate of decay or lignin or carbohydrate utilization.

Glucose in the medium drastically reduced the rate of decay in maple by all of the white-rotters. Petterson and Cowling (1964) investigated the resistance of Sitka spruce and southern pine woods to attack by white-rot fungi. They demonstrated that the resistance of the wood was reduced by impregnating it with a 1% solution of glucose. In another study, Darbyshire et al. (1969) found that high glucose levels in the medium decreased decay rate in apple wood by Trametes (=Coriolus) versicolor. Most likely the reduction in wood decay by white-rotters associated with high sugar levels is related to failure to produce enzymes responsible for carbohydrate breakdown. Simple sugars have been found to repress production of both cellulases and hemicellulases by white-rot fungi (Ericksson and Goodell, 1974; Highley, 1973b, 1976). Where decay in maple occurred in the presence of glucose, removal of cellulose was completely inhibited. Lignin degradation in maple was accelerated over the

glucose medium even though glucose is reported repress ligninolytic enzymes of white-rot fungi (Kelly and Reddy, 1986).

With respect to susceptibility of wood to decay by white-rot fungi, our results suggest that if a continuous and relatively high level of simple sugar could be maintained in wood, decay would be inhibited. This, of course, would be very impractical method of wood protection. However, if nonmetabolizable sugar analogs could be found that repress decay, it might offer an opportunity to protect wood in an environmentally safe manner.

#### SUMMARY

Miniature blocks of white pine and maple wood were decayed by four white-rot fungi: Cariolus versicolor, Irpex lacteus, Bjerkander adusta, and Phlebia brevispora. The blocks were decayed over a soil medium, a low-nutrient agar medium, and an agar medium supplemented with 10% glucose, 0.5 NH<sub>4</sub>NO<sub>3</sub> or 10% glucose plus 0.5 NH<sub>4</sub>NO<sub>3</sub>. Quantitative changes in lignin, glucan, mannan, and xylan during decay were determined. On the same wood, white-rot removal of the cell components was similar. Removal of components in white pine differed from that in maple. In white pine lignin was removed faster than the carbohydrates. In maple, mannan was removed substantially faster than the other components. Glucose caused a marked inhibition of decay, while NH<sub>4</sub>NO<sub>3</sub> had little effect. Glucose stopped cellulose utilization and accelerated lignin utilization. NH<sub>4</sub>NO<sub>3</sub> had little effect on relative rate of removal of cell wall components.

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