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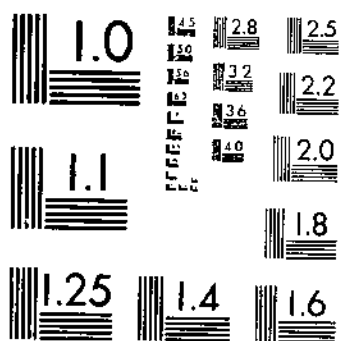
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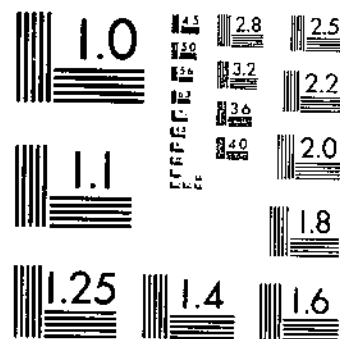
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COMPARATIVE BIOCHEMISTRY OF THE DECAY OF SWEETGUM SAPWOOD BY WHITE ROT
COWLING, E. B. 1 OF 1

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Comparative Biochemistry of the Decay of Sweetgum Sapwood by White-Rot and Brown-Rot Fungi

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COMPARATIVE BIOCHEMISTRY OF THE DECAY OF SWEETGUM BY WHITE-ROT AND BROWN-ROT FUNGI

Introduction

The biochemistry of wood deterioration is a neglected but potentially fruitful field of inquiry. Despite the biological and economic importance of this field, remarkably little fundamental research has been done. The mechanisms by which wood-destroying fungi depolymerize wood constituents, the nature of the degradation products they assimilate, and the biochemical pathways by which they metabolize the carbohydrates and lignin of wood are all essentially unknown. Each of these fundamental aspects of the decay process, however, can be studied by biochemical methods that have been applied successfully to the study of digestion, assimilation, and intermediary metabolism of other organisms.

One of the most intriguing fundamental problems in the field of wood deterioration is presented by the striking differences between the two major types of wood decay—white rot and brown rot. They may be distinguished by differences in the color, solubility, strength, dimensional stability, pulping properties, and chemical composition of the decayed wood. Clearly, these distinguishing features are the result of differences in the enzymatic effects of the decay fungi. Only occasionally have attempts been made, however, to interpret these distinguishing features in terms of differences in the enzymatic capacities of the causal organisms. Development of this correlation would provide information basic to more adequate understanding of the biochemistry of the decay process.

The first step necessary in the development of this correlation is to determine the physical and chemical nature of changes that take place in wood during both types of decay. The results of these determinations may then provide working hypotheses for research to characterize the extracellular enzymes of the decay organisms. Comparison of these enzymes and their mechanism of action may finally provide a fundamental explanation for differences in the two types of decay.

The purpose of the research described in this bulletin was to determine and compare the progressive changes in certain chemical and physical properties of wood during decay by representative white- and brown-rot fungi. Indirect evidence indicating the nature and relative rates of enzymatic effects on wood during both types of decay was obtained from particle-size distribution, solubility, composition, degree of polymerization, hygroscopicity, X-ray diffraction, and histological analyses of wood in progressive stages of decay.

The biological importance of wood deterioration provides considerable impetus for biochemical studies of the decay process. As a result of their unique ability to degrade the heavily lignified tissues of perennial plants, wood-destroying fungi play a virtually irreplaceable role in the carbon cycle. They rid forest lands of plant debris and thereby add organic matter to the soil and return to the atmosphere several billion metric tons of carbon each year (81).¹ Greater knowledge of the biochemistry of the decay process would increase present understanding of this important phenomenon in nature.

The wood-destroying fungi are also responsible for a considerable drain on the timber resources of the world. The "growth impact" of heart rot and other decay fungi that attack living trees in the United States has been estimated at 15 billion board feet of useable wood each year (24)—an amount greatly in excess of that of all other destructive agencies combined. The cost of materials used to replace wood decayed in service has been estimated at \$300 million annually (41). Greater fundamental knowledge of the decay process would be helpful in minimizing these losses by aiding in the development of more effective preservative treatments for wood products. It may also assist in the development of methods of controlling decay in living trees.

A third justification for fundamental research in the field of wood deterioration is the increasing use of wood-destroying organisms and isolated microbial enzymes in studies of lignin and cellulose structure (50, 55) and wood anatomy (3, 4, 43). The specificity of microbial enzymes, and the very mild conditions under which their reactions proceed, make them potentially ideal reagents for delicate studies of structure (61). More complete characterization of the enzymes of wood-destroying fungi, and their effects during decay, would allow these methods to be applied with greater precision and confidence.

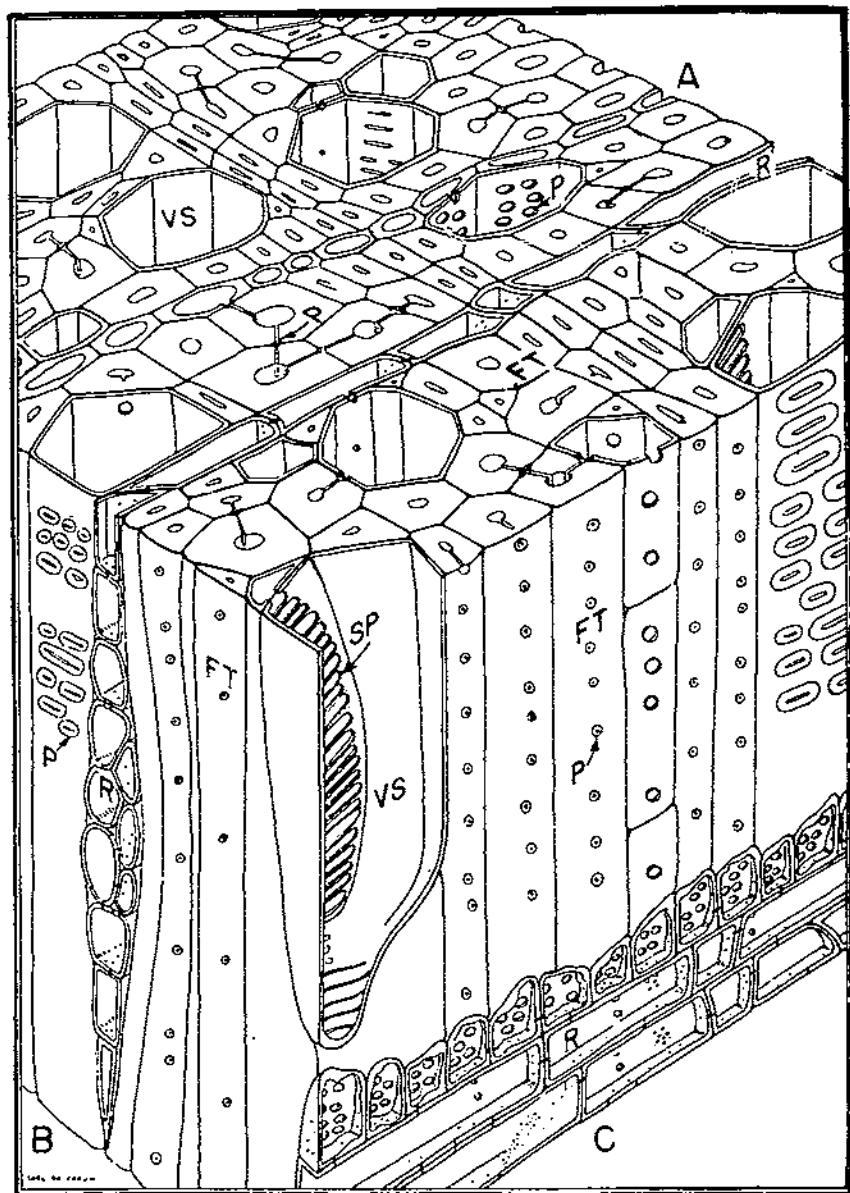
Orientation

Interpretation of the experimental work of this study requires some knowledge of the cellular structure of the wood of angiosperms, the molecular architecture and nature of relationships among its cell-wall constituents, and the nature of fungal enzymes and their action. These subjects are discussed briefly in this bulletin.

Wood Structure

Figure 1 illustrates the minute anatomy of sweetgum sapwood (*Liquidambar styraciflua* L.), the typical angiosperm used in the experimental phases of this study. The very thick-walled fiber tracheid cells (FT), the more thin-walled vessel segments (VS), and the ray cells (R) make up the bulk of the wood substance. It is the deterioration of the walls of these cells that accounts for the alteration of wood and of many of its useful properties during decay.

¹ Italic numbers in parentheses refer to Literature Cited, p. 75

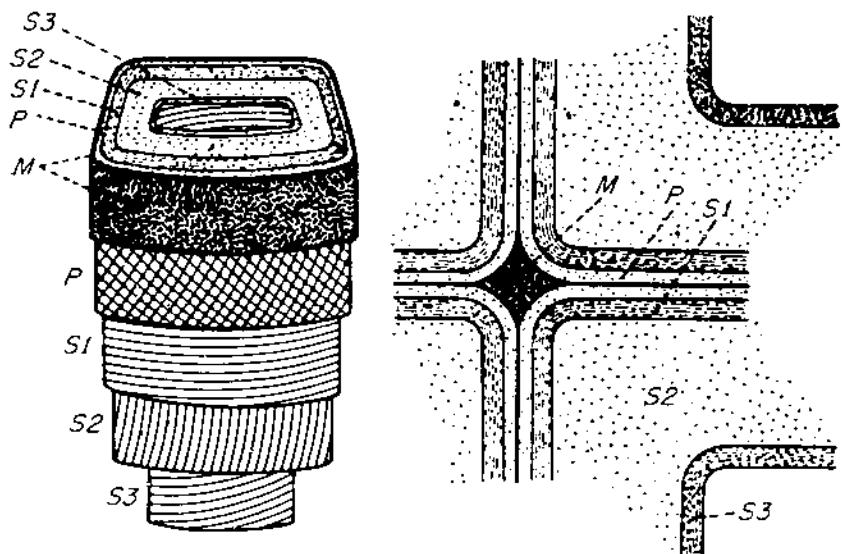


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FIGURE 1.—Minute anatomy of the wood of the typical angiosperm *Liquidambar styraciflua* L.: Each type of wood cell is depicted in transverse (A), tangential (B) and radial (C) view; the walls of fiber tracheid cells (FT), vessel segments (VS), and the wood ray cells (R) comprise the bulk of the wood substance; scalariform plates (SP) and pit pairs (P) between adjacent cells permit movement of fluids from one cell to another. (From Textbook of Wood Technology (6), by permission of McGraw-Hill Book Co.) Copyright, 1949.

The adjacent walls of contiguous fiber tracheid and vessel cells have a multilayered structure (fig. 2). The true intercellular substance or middle lamella (M) and the adjacent primary walls (P) comprise the so-called compound middle lamella. Lining the lumens of each of these wood cells are thick secondary walls, usually composed of three layers, designated S1, S2, and S3. The S1 and S3 layers usually are quite thin; the S2 layer is of variable thickness, but it usually makes up the bulk of the secondary wall substance. These layers may be distinguished, as shown in figure 2, by the angle of orientation of the microfibrils within them.

Within each layer of the secondary wall, the cellulose and other cell-wall constituents are aggregated into linear bundles called microfibrils. The microfibrils are distinct entities in that few cellulose molecules, if any, cross over from one microfibril to another (58).² These aggregates are shown at A in figure 3. Within each microfibril, the linear molecules of cellulose are bound laterally by hydrogen bonds and Van der Waal forces into a linear micellar structure. As shown at B (fig. 3), the cellulose molecules are associated in various degrees of parallelism. Regions that contain highly oriented molecules are called crystallites or micelles; those in which the cellulose molecules are more ran-



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FIGURE 2.—The various layers of contiguous wood cell walls. The true intercellular substance or middle lamella (M) and adjacent primary walls (P) together comprise the compound middle lamella; the secondary walls are composed of outer (S1), middle (S2), and inner (S3) layers. (From *Chemistry of Wood* (26), by permission of Academic Press.) Copyright, 1949.

²Also Kalmes, O. The distribution of constituents across the wall of unbleached spruce sulfite fibers. (Unpublished doctoral thesis. Copy on file at Inst. Paper Chem., Appleton, Wis.)

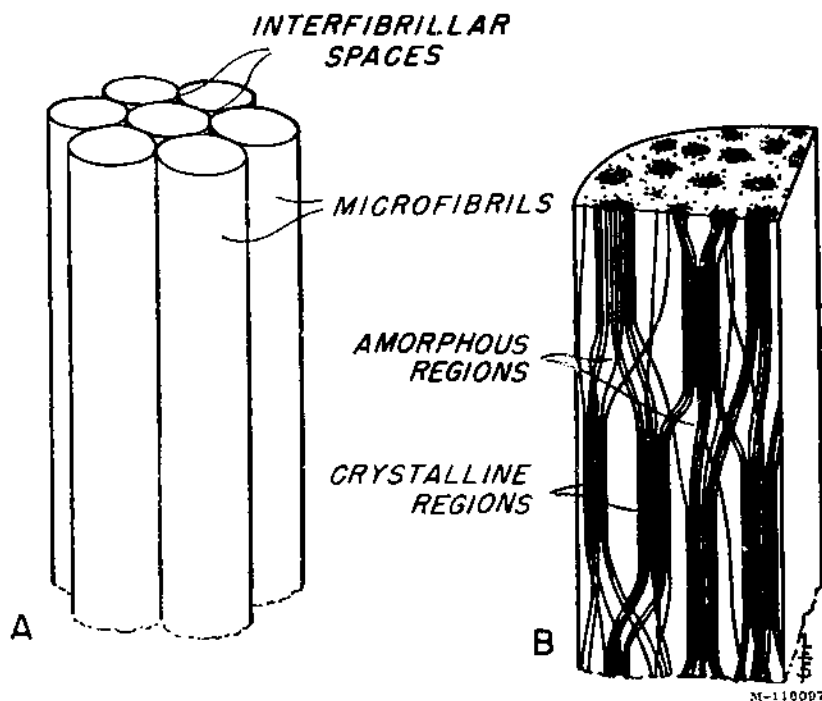


FIGURE 3.—The gross structure (A) and internal structure (B) of the microfibrils that compose the various layers of the secondary walls of wood cells. The linear cellulose molecules, depicted as lines within the sectional view of the microfibril at B, are associated in various degrees of parallelism. Regions that contain highly ordered molecules are called crystalline regions; those of more random orientation are called amorphous regions. (From Textbook of Wood Technology (6), by permission of McGraw-Hill Book Co.) Copyright, 1951.

domly oriented are called amorphous regions. These regions are interspersed with one another and are not discrete or uniform in size; the cellulose molecules grade gradually from the crystalline to the amorphous phase. As illustrated at B (fig. 3), the cellulose molecules are in general much longer than individual crystallites. They may therefore traverse several crystalline and amorphous regions.

Cell-Wall Constituents

The chemical constituents of wood include cellulose, hemicelluloses, lignin, extraneous materials, and a small amount of inorganic matter. Unfortunately, sharp lines of demarcation between any of these constituents cannot be drawn. The amounts reported in table 1, however, indicate the approximate composition of the sweetgum sapwood used in this study. When not otherwise cited, the following information regarding the nature, structure, and distribution of cell-wall constituents is taken from Wise and Jahn (86).

TABLE 1.—*Composition of sweetgum sapwood as revealed by two common methods of wood analysis, based on weight of the wood*

| Constituent | Amount present | Total |
|------------------------------|----------------|---------|
| Method I: | Percent | Percent |
| Ash..... | 0.5 | 26.5 |
| Total extractives..... | 4.0 | |
| Sulfuric acid lignin..... | 22.0 | |
| Holocellulose: | | |
| Alpha-cellulose..... | 46.5 | 73.5 |
| Beta-cellulose..... | .5 | |
| Gamma-cellulose..... | 26.5 | |
| | | 100.0 |
| Method II: | | |
| Ash..... | .5 | 26.5 |
| Total extractives..... | 4.0 | |
| Sulfuric acid lignin..... | 22.0 | |
| Hexosans: | | |
| Glucan (true cellulose)..... | 50.0 | 53.5 |
| Mannan..... | 2.5 | |
| Galactan..... | 1.0 | |
| Pentosans: | | |
| Xylan..... | 19.0 | 20.0 |
| Araban..... | 1.0 | |
| | | 100.0 |

The extraneous constituents of wood are a heterogeneous group of substances extractable in neutral solvents, such as ethanol, ethanol-benzene, and water. They include waxes, fats, essential oils, tannins, resins, soluble saccharides (gums), and proteinaceous materials.

The mineral constituents of wood include a wide variety of elements that are found in varying concentration among individual trees.

Lignin is an encrusting material of wood cell walls. It is known only as the product of certain empirical isolation procedures, and thus is poorly defined structurally. However, it is known to be a complex, 3-dimensional polymer of phenyl propane units (25, 86).

The total carbohydrate fraction of wood is known as holocellulose. It is isolated by extracting a sample of wood meal to remove extraneous materials, and then applying a succession of alternate chlorination and monoethanolamine extraction treatments to remove lignin. The isolated holocellulose may be divided into two fractions: Alpha-cellulose, which remains insoluble upon treatment of holocellulose with 17.5 percent aqueous sodium hydroxide, and the hemicelluloses, which are soluble in this sol-

vent. The hemicelluloses in turn consist of two fractions: Beta-cellulose, which precipitates upon acidification of the alkaline solution, and gamma-cellulose, which remains soluble in the acidified solution.

These three basic fractions of holocellulose differ mainly in the number and type of sugar units that compose their individual molecules. Alpha-cellulose is composed largely of polymers of glucose, and ranges in degree of polymerization² (DP) from about 200 to 3,000 or more. The beta- and gamma-celluloses include polymers of each of the five basic sugar units that make up the carbohydrates of wood. Beta-cellulose includes polymers that range in degree of polymerization from about 15 to 200; the DP of gamma-cellulose is less than 15. As shown in table 1, the amount of beta-cellulose present in sound wood is small in comparison with the amounts of alpha- and gamma-cellulose present.

The total carbohydrates of wood may also be identified in terms of their component monosaccharides. The five monomeric sugar units found in wood include the hexoses—glucose, mannose, and galactose—and the pentoses—xylose and arabinose. These monosaccharides are linked by *B*-glycosidic bonds into substantially linear polymers termed the glucans or true cellulose, mannans, galactans, xylans, and arabans. In the wood of angiosperms, the bulk of the total carbohydrate is made up of glucans and xylans. The other sugar polymers are present in very much lower concentrations.

Structure of the Wood Carbohydrates

The cellulose of wood, in common with that of cotton, is a linear polymer of d-glucose units linked by 1, 4-*B*-glucosidic bonds. These linear molecules are bound laterally by hydrogen bonds or Van der Waal forces into a linear, micellar structure whose component molecules are associated in various degrees of parallelism (fig. 3).

As mentioned earlier, the hemicelluloses of wood consist of relatively short polymers of the five basic sugar units that comprise the carbohydrate fraction of wood. Although it was earlier believed that each sugar polymer contained only a single type of monosaccharide unit, more recent evidence supports the view that two or more types of sugar units may exist in a given hemicellulose molecule (86). Thus, it is appropriate to speak of gluco-mannans, arabo-galactans, and arabo-xylans as well as the traditional glucans, mannans, galactans, arabans, and xylans.

Polymers of uronic acids of the basic sugar units also are included among the hemicelluloses. Both the true and polyuronide hemicelluloses are linear, essentially unbranched polymers containing *B*-glycosidic links.

Distribution of Constituents Within Wood Cell Walls

The accessibility of various cell-wall constituents to the extra-

² Degree of polymerization refers to the number of sugar units in a polysaccharide molecule.

cellular enzymes of decay fungi is determined by their distribution within the cell wall and the nature of structural relationships among them. A more detailed consideration of these factors in relation to cellulolytic enzyme activity is given by Cowling (18).

Lignin is concentrated in the compound middle lamella of wood cells and diminishes in concentration toward the lumen. In the wood of angiosperms, the secondary walls are believed to contain very little lignin and to consist almost entirely of carbohydrates (37, 38, 73). The secondary walls of angiosperms, however, have a definite affinity for the phloroglucinol hydrochloride and Mäule staining reagents (27). Pew (54) has shown that these reagents react specifically with the coniferyl aldehyde groups and syringyl nuclei of lignin. He has shown also that aspen wood meal has almost complete resistance to digestion by isolated cellulolytic enzymes, whereas holocellulose prepared from this same wood was almost completely digestible in comparable enzyme preparations. These observations suggest at least partial lignification of the secondary walls of angiosperms.

Within the secondary walls of wood cells, lignin is concentrated in the spaces between the microfibrils and in the amorphous regions between cellulose crystallites (31, 38, 84).⁴ Although a lignin-carbohydrate chemical bond has been postulated, present evidence suggests that the association is largely physical in nature—that the lignin and carbohydrates of wood form a mutually interpenetrating system of polymers (31). This intimate physical association has been considered responsible for the resistance of wood to microbial deterioration except by fungi that have enzyme systems capable of depolymerizing lignin as well as the carbohydrates of wood (18, 32, 43). Lignin apparently prevents the cellulolytic enzymes of many organisms from contacting a sufficient number of glucosidic links in the cellulose to permit significant hydrolysis.

The crystalline regions of wood cell walls consist almost wholly of true cellulose (glucans). Because of the very close packing and strong forces of attraction between cellulose molecules in these regions, crystalline cellulose is more resistant to enzymatic hydrolysis than is the more accessible amorphous cellulose (51, 82).

The hemicelluloses, lignin, and extraneous and mineral constituents of wood occupy the amorphous regions between cellulose crystallites. When moisture free, these regions contain a very small amount of void space. When moisture is adsorbed, however, the wood structure swells and a considerable capillary structure is created within the cell walls. The dimensions of these so-called transient cell-wall capillaries in the wood of angiosperms are not known with certainty. According to Stamm (personal communication), however, only a few of these capillaries are of sufficient size to accommodate molecules of the order of enzyme dimensions (greater than 20,000 in molecular weight). Thus, the extracellular enzymes of wood-destroying fungi probably gain access to constituents within the microfibrils of the

⁴ See footnote 2, p. 4

wood cell walls mostly by enlarging the transient cell-wall capillaries.

The Nature of Fungal Enzymes and Their Action

The particular enzymes produced by wood-destroying fungi determine the nature of effects they have on the substrates upon which they live. Some knowledge of the nature of enzymes and the mechanisms by which they act will therefore be useful in interpreting the chemical changes induced in wood during decay.

Enzymes are water-soluble protein molecules of high molecular weight (20,000 to 500,000). They may or may not also contain nonprotein prosthetic groups. They are active in very low concentrations and catalyze a specific type of chemical reaction (often on a particular type of chemical compound) without being permanently altered in the process. Actual contact between an enzyme and its substrate is considered necessary for catalysis. Some enzymes are secreted by the cells in which they are formed and act extracellularly; others are active within the cells of the organism. In the decay of wood, both types are necessary.

The fungal hyphae, which inhabit the cells of wood, secrete extracellular enzymes that diffuse through a film of water that coats the cell lumens. These enzymes catalyze the depolymerization⁵ of the various cell-wall constituents into water-soluble molecular fragments. These fragments then diffuse back to the fungal hyphae, where they are assimilated and further metabolized to provide the energy and substances needed by the fungus for its continued growth and development (59).

Two possible mechanisms of enzymatic depolymerization of structural polysaccharides, such as the carbohydrates of wood, have been suggested (52): (1) The polysaccharide chains could be cleaved at random along their length, so that relatively large polymeric fragments result. Alpha-amylase depolymerizes starch by this mechanism to give amylopectins. (2) The chains could be cleaved from their ends so that relatively short fragments result. Beta-amylase acts by this mechanism to give maltose from starch.

The mechanism by which cellulose is depolymerized by fungal cellulases has been an object of considerable controversy. Recently, however, Norkrans and Ranby (52) have very clearly demonstrated that the isolated cellulases of several fungi, including the white-rot fungus, *Collybia velutipes* (Curt. ex Fries) Quél., act by a random mechanism. This conclusion has been supported, although less vigorously, for the cellulolytic enzymes of other fungi. No studies of the mechanism of action of the hemicellulose-splitting enzymes of fungi are known to the present author. Hemicellulases with both random and endwise mechanisms of cleavage have been reported in marine algae, however (22).

⁵The term depolymerization has been used in this bulletin to denote cleavage of cell-wall constituents into smaller molecular fragments. It has not been established that enzymatic cleavage of all the polymeric constituents of wood takes place between, rather than within, monomer units.

Review of Differences Between the White- and Brown-Rot Types of Wood Decay

Three general types of wood decay are commonly recognized—white rot, brown rot, and soft rot. The last type has only recently been shown to be of importance (66) and will be considered again only in figure 5 of this bulletin. The white-rot and brown-rot types of decay are the most important forms of wood deterioration (11). Various distinguishing features of the two major types of decay are discussed under the following subheadings.

Differences in Color

As implied by their names, the white- and brown-rot types of decay can be distinguished by the color of the decayed wood. A brown color has been attributed to the lignin-enriched residue left by brown-rot fungi as a result of preferential utilization of the wood carbohydrates (11). A bleached appearance is characteristic of white-rotted wood, and it has been attributed to the utilization or modification of certain (as yet unidentified) chromogenic materials in the wood (67). It is not indicative of preferential utilization of lignin, as has often been presumed (11).

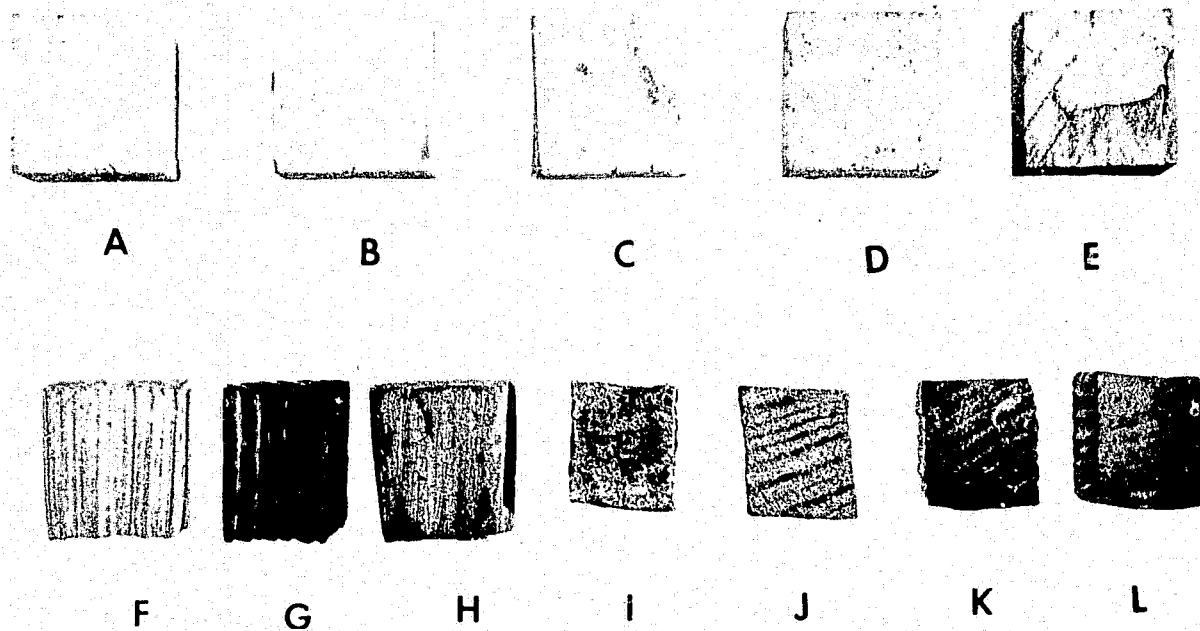
Mixtures of decay organisms can cause ambiguous gradations of color. At the Forest Products Laboratory both brown- and white-rot fungi have been isolated from wood bleached in a manner typical of the white-rot type of decay. Blocks typically browned as the result of extensive decay by the brown-rot fungus, *Poria monticola*, have subsequently been bleached by the white-rot fungus, *Polyporus versicolor*.

Dark-colored "zone lines" are a characteristic feature of decay caused by certain white-rot fungi in the wood of angiosperms. They have only rarely been reported in brown-rotted wood (30). Rhoads (63) has shown that these thin black lines consist of dark-colored hyphae and certain associated humic substances.

Differences in Dimensional Stability

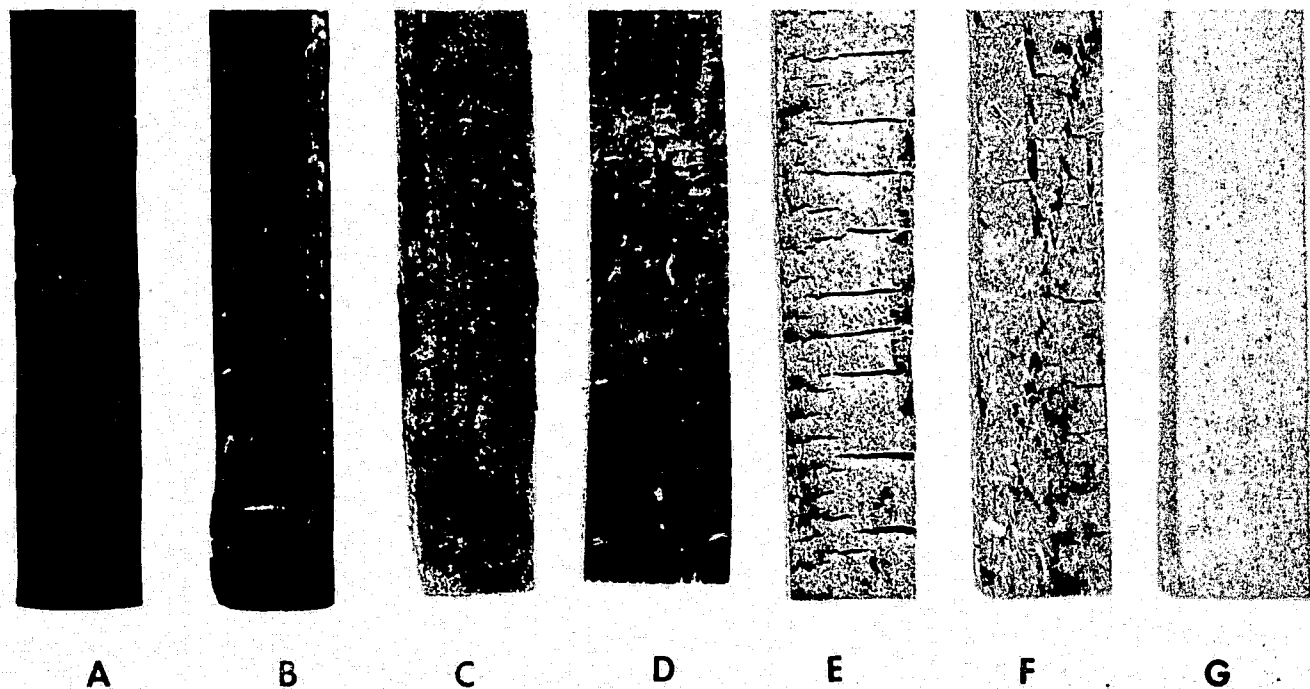
Brown-rotted wood tends to shrink abnormally when dried, whereas white-rotted wood shows normal shrinkage properties in almost all stages of decay. This distinction has been reported by Buro (7) and Scheffer (67), and is illustrated in figure 4. Buro (7) also observed an increase in longitudinal swelling capacity in brown-rotted wood, but he found none in specimens decayed by several white-rot fungi.

This abnormal longitudinal swelling and shrinkage of brown-rotted wood gives rise to a characteristic cubical pattern of checking that is not shown by white-rotted wood. This same pattern of checking becomes apparent, as shown in figure 5, when the wood is pyrolyzed, burned, subjected to gamma radiation, and hydrolyzed by strong acids, as well as when the wood is decayed by both soft-rot and brown-rot fungi. Each of these diverse treatments would appear to cause a similar change in the swelling and shrinkage properties of wood. Since these properties are attributed primarily to the cellulose of wood (71), some effect on this



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FIGURE 4.—Differences in dimensional stability of wood blocks decayed by white- and brown-rot fungi. All blocks were originally cubes of the same size and shape. Blocks A through E, sweetgum sapwood decayed by various white-rot fungi; F through L, southern yellow pine sapwood decayed by various brown-rot fungi. Decay organisms and percent weight losses for each block: A, *Polyporus gilvus* (Schw.) Fries, 48; B, *Polyporus tulipiferus* (Schw.) Overh., 64; C, *Polyporus versicolor* L. ex Fries, 66; D, *Fomes applanatus* (Pers. ex Wallr.) Gill, 60; E, *Fomes rimosus* Berk., 32; F, *Lentinus lepideus* Fries, 45; G, *Contiophora puteana* (Schum. ex Fries) Karst., 57; H, *Daedalea quercina* L. ex Fries, 55; I, *Lenzites trabea* Pers. ex Fries, 67; J, *Poria monticola* Murr., 61; K, *Poria cocos* (Schw.) Wolf, 66; L, *Poria incrassata* (Berk. & Curtis) Burt., 65.



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FIGURE 5.—These wood sticks illustrate the surface appearance characteristic of various forms of wood deterioration. Note the cubical pattern of checking on all sticks except G. Treatments applied to each specimen were as follows: A, pyrolysis; B, combustion; C, hydrolysis with concentrated sulfuric acid; D, irradiation with gamma rays from cobalt⁶⁰; E, decay by soft-rot fungi; F, decay by brown-rot fungi; G, decay by white-rot fungi.

constituent apparently is held in common by each of these treatments, but not by decay of the white-rot type.

Differences in Microscopical Evidence of Deterioration

Differences in microscopical features of white- and brown-rotted wood have been reviewed by Meier (43). In both types of decay, bore holes are formed by the fungi in passing from cell to cell. In white-rotted wood, however, a gradual thinning of the cell walls also occurs from the lumen toward the middle lamella.

From a microscopical study of bore-hole formation, Proctor (56) concluded that the holes result from enzymatic dissolution of the wood substance rather than by mechanical forces imposed by advancing hyphal tips. This explanation implies either secretion of extracellular enzymes from hyphal tips, or some mechanism for concentrating enzymatic activity at the interface between a hyphal tip and the wood cell wall. It further implies that the enzymes of the decay organisms can catalyze the dissolution of all cell-wall constituents.

When first formed, bore holes are only about as large as the penetrating hyphae (0.5 to 1.0 micron in diameter). They may later enlarge considerably and coalesce to form irregular, rounded passageways 2 to 10 microns in diameter (30, 85). The enlargement of bore holes implies lateral secretion or general diffusibility of extracellular enzymes that are active on all cell-wall constituents.

The volume of wood substance removed during bore-hole formation is wholly inadequate to account for the weight lost by wood during decay by brown-rot fungi. This apparent discrepancy was explained in an electron micrographic study by Meier (43). He showed that the apparently intact secondary walls of spruce wood decayed by brown-rot fungi actually contained only a delicate but coherent lattice, presumably consisting of lignin that remained after selective removal of the wood carbohydrates. The brown-rot fungi thus appear to possess a readily diffusible enzyme system in addition to one adopted to the formation of bore holes.

The general thinning of wood cell walls by white-rot fungi suggests that the extracellular enzymes of these organisms are readily diffusible from the hyphae and act primarily on the internal surfaces of wood cells (67). This pattern of dissolution of the cell-wall substance readily accounts for the utilization of the wood carbohydrates, which are in greatest abundance in the secondary walls of wood cells. However, it fails to explain adequately the rapid utilization of lignin in the early stages of decay, reported by Scheffer (67), Campbell (10), and others, since this constituent is concentrated in the compound middle lamella.

Differences in Strength Properties

The scattered literature on strength properties of wood decayed by white- and brown-rot fungi has been reviewed by Hartley (28). He concluded that, at comparable stages of decay, as measured by weight loss, brown-rot fungi reduce the strength of wood more than do white-rot fungi. This distinction is also evident in han-

dling decayed blocks of the two types. Brown-rotted wood is extremely friable, whereas white-rotted wood tends to be tough and elastic rather than brittle.

In a more recent paper, Kennedy (35) confirmed the above generalization with the two fungi used as test organisms in this study. He also demonstrated a very close correlation between the extent of strength reduction and the solubility of several wood species decayed by *Poria monticola* Murr. in 1 percent sodium hydroxide.

Pechmann and Schaile (53) have suggested that differences in rate of strength reduction between the two major types of wood decay may be due to a more rapid depolymerization of the cellulose of wood by brown-rot fungi. Although experimental proof is lacking, wood physicists have assumed that cellulose of high degree of polymerization is the constituent of wood primarily responsible for its strength (86). Thus, differences in the strength properties of wood decayed by white- and brown-rot fungi may very likely be due to differences in the nature of their effects on cellulose.

Differences in the Yield and Quality of Pulp Products

Sheridan (70) and Martin (42) have reported that the yields of wood pulp from white-rotted wood are not greatly different from the yields obtained from sound wood, when calculated on a weight basis. Pulp yields from brown-rotted wood, however, were very low. The quality of pulp products made from white-rotted wood was nearly as high as for pulps from normal woods, but brown-rotted wood gave pulp products of very low quality.

Differences in Chemical Composition

An important difference in chemical effects of white- and brown-rot fungi is the ability of the former to metabolize both the carbohydrates and lignin of wood, whereas the latter primarily utilize the carbohydrates. Prior to the work of Hawley and co-workers (29) it has been presumed, from the bleached appearance of white-rotted wood and its failure to give typical lignin staining reactions (with phloroglucinol hydrochloride, safranin, and so forth), that lignin was preferentially utilized by white-rot fungi (11). This was considered especially certain in the case of the white-pocket type of decay in which the fibrous material in the decayed pockets was essentially pure white. Although this misconception persists in the literature even today, it was shown in 1927 and has been confirmed repeatedly (8, 9, 10, 12, 13, 23, 42, 67) that white-rot fungi derive most of their nourishment from the cellulose and other polysaccharides of wood.

Analyses of the fibrous material removed from the white pockets in a sample of oak decayed by *Stereum hirsutum* showed that it contained 12.5 percent lignin compared with 21.5 percent in the sound wood (9). A comprehensive study of Douglas-fir wood decayed by the white-pocket rot organism, *Pomes pini*, showed that the ratio of lignin to holocellulose in the wood fell only from 0.40 in the sound wood to 0.37 for samples taken from the "centers of extremely decayed logs" (42). Thus, even the fungi that cause

the white-pocket type of decay have been shown to obtain most of their nourishment by utilization of the carbohydrates of wood.

Wood decayed by brown-rot fungi contains a higher proportion of lignin than does sound wood. By analyzing both sound wood and that in progressive stages of brown rot, however, Bray and Andrews (5) were able for the first time to express analytical results on the basis of the original sound wood. In this way, they demonstrated that the lignin content remained fairly constant or decreased slightly rather than apparently increasing as decay progressed. The slight loss of lignin they observed was accounted for almost wholly by loss in methoxyl groups.

Since lignin has been shown not to be utilized to an appreciable extent by brown-rot fungi, many researchers have presumed that it remained unchanged during decay as well, being merely laid bare by the removal of carbohydrates from the wood (43, 49, 50). This assumption has persisted in the literature despite the following evidence to the contrary: (1) demonstrable loss of methoxyl groups from lignin even in the early stages of decay (2, 5); (2) changes in the solubility of lignin in ethanol, methyl cellosolve, and dilute aqueous sodium hydroxide (55); (3) changes in ultraviolet absorption properties (40, 55); and (4) differences in elementary composition (40).

Differences in Alkali Solubility

Differences in the solubility of white- and brown-rotted wood in 1 percent aqueous sodium hydroxide have been shown to provide a reliable chemical means for distinguishing the two types of decay (11). The solubility of white-rotted wood is only slightly greater than that of sound wood. Brown-rotted wood, however, is much more soluble than sound wood.

The increase in alkali solubility of brown-rotted wood has been attributed largely to carbohydrate degradation products by Campbell (11), Kennedy (35), and Pechmann and Schaile (53). Campbell (11) justified this assumption by stating (apparently without experimental evidence) that "in the early stages of brown rot, the alkali solubility of the lignin in decayed wood is only slightly greater than that of the lignin in sound wood."

Differences in Oxidase Reactions of the Causal Organisms

Differences in oxidase reactions provide one of the most useful cultural means for distinguishing the white- and brown-rot fungi. This test involves the formation of a colored zone in agar containing a polyphenolic material, in the presence of a fungus culture that secretes an extracellular polyphenol oxidase of the laccase type (47). White-rot fungi secrete this enzyme and give pronounced diffusion zones; brown-rot fungi give negative reactions. The test is about 95 percent efficient in separating the two types of fungi (19, 48).

The enzyme responsible for the oxidase reaction, laccase, has been presumed to account for the ability of white-rot fungi to utilize lignin. The following circumstantial evidence supports this view: (1) The enzyme is almost invariably found in wood-de-

stroying organisms that are able to utilize lignin and only rarely detected in organisms unable to utilize this material. (2) Lignin is a phenolic material and is readily oxidizable. Its removal from wood in the pulping processes and in the determination of the total carbohydrate content of wood is dependent upon this property. (3) Oxygen uptake has been demonstrated by Braun's "native" lignin in the presence of culture filtrates of *Polyporus versicolor*, in which polyphenol oxidase activity also was demonstrable (20).

The metabolism of a complex substance such as lignin undoubtedly involves several enzymatic steps. An organism lacking one or more of the enzymes that catalyze the transformations involved in each step would be unable to utilize the substance unless alternate metabolic pathways were available to it.

If laccase is involved in lignin metabolism, as suggested by the circumstantial evidence described above, lack of this enzyme in brown-rot fungi could account for their inability to utilize lignin. This is pure conjecture, however, since none of the enzymatic steps involved in lignin metabolism are known at present. Evidence suggesting that laccase may not be involved in lignin metabolism is presented by the fact that the oxidative reaction products of polyphenol oxidase activity are quinones (83) which, unless subsequently transformed to other products, will spontaneously polymerize to give dark-colored melanic pigments. Such pigments are observed in white-rotted wood only in occasional and sparse zone lines. Actually, the wood is usually bleached rather than darkened, as is common in other plant tissues in which melanic pigments accumulate as the result of polyphenol oxidase activity (82).

For these reasons, it appears desirable to maintain an objective view of the possible role of laccase in lignin metabolism until more is known concerning the metabolic steps and enzymes involved.

Differences in Host-Wood Distribution of the Causal Organisms

A final distinction between white- and brown-rot fungi is their apparent host-wood preferences. White-rot fungi are most often associated with decays of angiosperms, and brown-rot fungi with those of gymnosperms (28).⁶ Table 2 contains the results of comparative studies by Duncan (21) and the present author, which show a distinctly more limited capacity of several white-rot fungi to cause decay in nondurable woods of two gymnosperms than in that of an equally nondurable angiosperm.

Some evidence as to the generality of this apparent host preference in nature was obtained from a survey of the mycological literature and the isolation records for the culture collection of the Division of Forest Disease Research of the U. S. Forest Service. The results are shown in table 3. Among the white-rot fungi

⁶ This correlation apparently does not hold for the ground-contact type of exposure involved in stake tests of preservatives, as reported by Zabel and Moore (87). Their identifications of the type of decay, however, were based on macroscopical appearance of decayed stakes, rather than isolation and identification of the decay organisms.

TABLE 2.—Average percent loss in weight induced by various white-rot fungi in the wood of sweetgum and spruce or southern yellow pine

| Fungus species | Isolate designation of the Division of Forest Disease Research | Average weight loss after 8 weeks incubation | |
|---|--|--|----------|
| | | Spruce | Sweetgum |
| | | Percent | Percent |
| <i>Corticium galactinum</i> (Fr.) Burt. | MD 223..... | 0.3 | 0.8 |
| <i>C. sp.</i> (No. 1)..... | MD 46..... | .1 | 1.2 |
| <i>Peniophora mollis</i> (Bres.) Bourd and Galz. (?)..... | MD 22..... | .4 | .4 |
| <i>P. sp.</i> (No. 1)..... | MD 192..... | .7 | 4.9 |
| <i>Pleurotus ostreatus</i> (Jacq.) Quéf. | MD 19..... | .7 | 7.1 |
| <i>Polyporus adustus</i> (Willd.) Fr..... | MD 194..... | .8 | 14.6 |
| <i>P. anceps</i> Peck..... | 784-S..... | 7.9 | 25.6 |
| <i>P. abietinus</i> (Dicks.) Fr..... | Col. 51-53..... | 1.6 | 4.6 |
| <i>P. versicolor</i> (L.) Fr..... | Mad. 697..... | .0 | 19.3 |
| <i>Poria subacida</i> (Peck) Sacc..... | 4844..... | 2.5 | 14.7 |
| <i>Odontia spathulata</i> (Fr.) Litsch..... | MD 201..... | 1.0 | 3.7 |
| <i>Vararia investiens</i> (Schw.) Karst. (?) | MD 47..... | 1.6 | .9 |
| Unknown E..... | 5096-23..... | .5 | 3.8 |
| Unknown F..... | MD 305..... | .4 | 2.5 |

| Fungus species | Isolate designation of the Division of Forest Disease Research | Average weight loss after 12 weeks incubation | |
|---|--|---|----------|
| | | Southern yellow pine | Sweetgum |
| | | Percent | Percent |
| <i>Schizophyllum commune</i> Fr..... | Mad. 619..... | 1.1 | 1.9 |
| <i>Polyporus tulipiferae</i> (Schw.) Fr..... | Mad. 517..... | 20.6 | 56.3 |
| <i>Odontia spathulata</i> (Fr.) Litsch..... | 5096-21..... | 10.4 | 29.3 |
| <i>Polyporus gilvus</i> (Schw.) Fr..... | 71316..... | 18.1 | 53.2 |
| <i>P. zonatis</i> Berk. (?)..... | 4856..... | 12.7 | 24.2 |
| <i>Fomes applanatus</i> (Pers. ex Wallr.) Gill..... | Mad. 708..... | 33.2 | 60.6 |
| <i>Polyporus versicolor</i> (L.) Fr..... | Mad. 697..... | 21.3 | 61.9 |

in three of the four major families of wood-destroying fungi, a distinct preference for the wood of angiosperms is apparent. Among the brown-rot organisms, the reported preference for gymnosperms is less pronounced. These results agree with those of Nobles (48) for a more limited number of organisms in the Polyporaceae. Of the 52 fungi in this family that were reported more than occasionally on gymnosperms, 25 are known to cause the distinct white-pocket type of decay. If these fungi are removed from consideration in table 3, the percentage of white-rot fungi found predominantly on angiosperms becomes 85 percent of the total in this family.

Inhibitory extraneous materials have not been demonstrated in sufficient abundance in the sapwood of southern yellow pine or the equally nondurable heartwood of spruce to account for their apparent resistance to decay by white-rot fungi, as shown in table 2. Thus, a difference in physico-chemical structure between these woods and sweetgum would appear to be involved. The generality of the preference of white-rot fungi for angiosperms, as shown in table 3, suggests that this structural mechanism of resistance may be effective in a wide variety of host wood and white-rot fungal species. Brown-rot fungi appear not to be affected by this mechanism.

Resumé

From the foregoing discussion of differences between the white- and brown-rot types of wood decay, it is apparent that the enzymatic effects of decay organisms of the two types are quite distinct. The present research was undertaken with the expectation that further evaluation of the progressive changes in chemical properties of the decayed wood would provide some insight into the fundamental biochemistry of both types of decay and explain some of the striking differences discussed in this review.

Design of the Experiments and Preparation of Materials for Analysis

Experimental Design

The objective of this study was to determine and compare the progressive changes in certain chemical and physical properties of wood during decay by representative white- and brown-rot fungi. Experimental materials and methods of analysis were employed that would provide indirect evidence of the nature and relative rates of enzymatic effects involved in these two major types of wood deterioration.

The analysis of wood in progressive stages of decay permits the depletion and modification of each wood constituent to be followed in all stages of the decay process. The analytical results refer to the sum total of depolymerization and respiratory reactions carried out on the wood constituents by the decay organisms. Information concerning the nature and relative rates of these enzymatic effects was obtained in this study from particle-size

TABLE 3.—*Host-wood distribution for white- and brown-rot fungi*

| Taxonomic group | Type of decay ¹ | Proportion of total number of fungus species— | | | Total fungus species |
|-----------------------------------|----------------------------|---|---|---|----------------------|
| | | Restricted to angiosperms or found only occasionally on gymnosperms | Found regularly on both angiosperms and gymnosperms | Restricted to gymnosperms or found only occasionally on angiosperms | |
| | | <i>Percent</i> | <i>Percent</i> | <i>Percent</i> | <i>Number</i> |
| Polyporaceae..... | White..... | 75 | 7 | 18 | 184 |
| | Brown..... | 30 | 11 | 59 | 92 |
| Thelephoraceae..... | White..... | 78 | 10 | 12 | 41 |
| | Brown..... | 10 | 50 | 40 | 10 |
| Agaricaceae..... | White..... | 64 | 18 | 18 | 11 |
| | Brown..... | 0 | 0 | 100 | 3 |
| Hydnaceae..... | White..... | 57 | 0 | 43 | 7 |
| | Brown..... | 0 | 0 | 0 | 0 |
| Ascomycetes and Fungi Imperfecti. | White..... | 100 | 0 | 0 | 7 |
| | Brown..... | 0 | 0 | 0 | 0 |
| Total for all groups..... | White..... | 75 | 7 | 18 | 250 |
| | Brown..... | 28 | 14 | 58 | 105 |

¹ Type-of-decay information was available for 100 additional fungi from their oxidase reactions. Adding these organisms to the total for all taxonomic groups raised the total number of white- and brown-rot fungi to 330 and 125, respectively, but in no case altered the proportions associated with each host-wood class by more than 2 percent.

distribution, solubility, composition, degree of polymerization, hygroscopicity, X-ray diffraction, and histological analyses of wood in progressive stages of decay by two representative wood-destroying fungi.

Only one of the standard methods of wood analysis used in this study (74) was originally designed for application to decayed wood. Because of the degraded nature of the material, some modification of the standard procedures was required in almost all determinations. Certain sources of error not normally encountered with sound wood also were introduced. These modifications and potential sources of error are discussed in conjunction with each method.⁷

The presence of mycelium of the test fungi in the wood was a small source of error in all the results. Since the decayed blocks were brushed free of adhering mycelium, and microscopical observations revealed relatively sparse mycelial development within the blocks, it was estimated that mycelium could have accounted for no more than about 2 percent of the moisture-free weight of the samples.

Most of the analytical results in this study were based on single measurements. The differences in extent of decay in the samples submitted to analysis were sufficiently small, and the results of the analyses sufficiently consistent within themselves, that replicate analyses of the same sample were considered unnecessary. In all analyses, however, duplicate determinations were made on the undecayed (control) samples and the average was reported to provide a firm basis for comparison of the decayed-sample results.

The analytical results in this study were calculated as percentages of the moisture-free weight of either (1) the sample submitted to analysis, or (2) the original sound wood from which the sample was obtained. These moisture-free weights were calculated from the moisture content of a separate sample determined by drying it to constant weight under vacuum over magnesium perchlorate.

Selection of Experimental Materials

The sapwood of sweetgum (*Liquidambar styraciflua* L.) was used in this study because it is typical of the important angiospermous timber species of North America (6), is susceptible to decay by both white- and brown-rot fungi, and is a species with which the author and his advisors had had considerable experience both in decay tests and in previous analytical work (67). The representative white-rot fungus, *Polyporus versicolor* L. ex Fries (Madison isolate No. 697), and the brown-rot fungus, *Poria monticola* Murr. (Madison isolate No. 698), were selected as test

⁷ The literature on the chemical changes that occur in wood during decay is conspicuously devoid of information regarding the special problems of technique and interpretation involved in the analysis of decayed wood. Consideration of some of these special problems and a more detailed description of the analytical methods employed in the present study are available in "Methods for Chemical Analysis of Decayed Wood," U.S. Forest Prod. Lab. Rpt. 2177.

organisms. These isolates are obtainable from the American Type Culture Collection in Washington, D.C.

In nature, *Polyporus versicolor* causes a typical, uniform "white rot" of angiospermous slash, stumps, and wood products; *Poria monticola* produces a typical "brown cubical rot" in living coniferous trees and causes a very rapid deterioration of wood products. Both fungi have been used extensively as decay-test organisms (8, 15, 67).

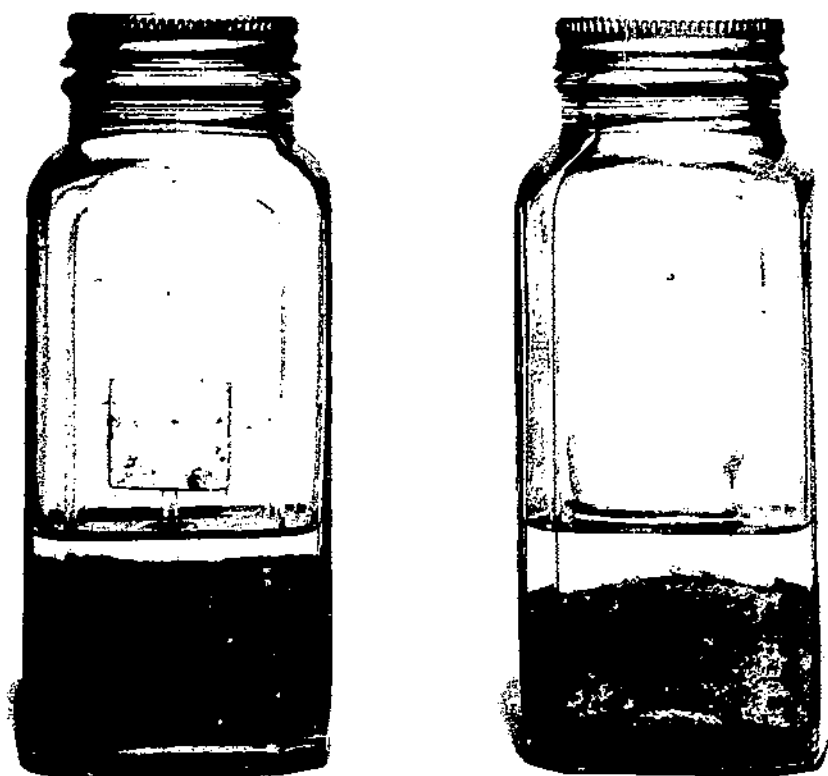
Preparation of Test Blocks

To obtain samples of the test wood in 10 progressive stages of decay by both test fungi, small blocks of wood were inoculated and permitted to decay in the laboratory for various periods of time. The test blocks were obtained from a single board of sweetgum sapwood. It had essentially straight grain, an average specific gravity of 0.51, and an average of eight annual rings per inch. It was apparently free of visible defects, heartwood, or abnormal wood. The board was cut into cubes, 0.75 ± 0.01 inch on a side. A 0.12-inch hole was drilled in the most nearly true tangential face of each block to a depth of 0.45 inch.

After thorough mixing to assure randomization, the blocks were labeled with India ink, equilibrated from the dry side with an atmosphere controlled at $26.5^\circ \pm 0.5^\circ$ C. and 30 ± 2 percent relative humidity, and weighed to the nearest 0.01 gram. They were then impregnated "to refusal" under vacuum with distilled water and dried at laboratory room conditions to an average moisture content of 100 ± 10 percent of their calculated moisture-free weight. The blocks were sterilized in steam at 100° C. for 20 minutes. After they were cooled, the blocks were inoculated by placing them in previously prepared soil-block chambers (1). Each of these chambers contained a 4-week-old culture of one of the test fungi. One block was placed in each chamber, with a transverse surface resting on the mycelium-covered feeder strip. Two days later, the block was inverted. After the fourth day, each block showed mycelial growth at some point on each of its six surfaces. The blocks were then transferred to sponge-rod incubation chambers of the type shown in figure 6 and described in the following section.

Sponge-Rod Incubation Chambers

The sponge-rod incubation chambers used in this study were designed not only to provide favorable circumstances for rapid decay, but also to preclude contamination of the blocks by foreign nutrient materials and leaching of degradation products from the blocks during the incubation periods. Each chamber consisted of an 8-ounce French square bottle with an unlined aluminum screw cap. A 1-inch-high cellulose sponge, cut to fit the bottle, was submerged in approximately 60 milliliters of distilled water. A 1.8-inch length of 2-millimeter glass rod was inserted into the sponge. The chambers were then autoclaved at 120° C. for 30 minutes. After the chambers had cooled, an inoculated test block



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FIGURE 6.—Sponge-rod incubation chambers showing (left) *Polyporus versicolor* and (right) *Poria monticola* growing on sweetgum sapwood test blocks after 6 weeks' incubation. Note the aerial mycelium of *P. monticola* in contact with the sterile distilled water in the chamber at right.

was placed aseptically in each chamber, with the glass rod inserted into the hole in the block. Each block was thus supported 0.25 inch above the free water surface. The caps of all chambers were left loose a quarter turn to permit exchange of gases with the atmosphere in the incubation room. This room was maintained at $26.5 \pm 0.5^\circ \text{C}$. and 70 ± 2 percent relative humidity.

Incubation Periods

The incubation periods ranged from 2 to 40 weeks. It was anticipated that the blocks would not lose appreciable moisture above the free water surface in the incubation chambers. A decrease in rate of decay by *Polyporus versicolor* after 18 weeks' incubation, however, was traced to inadequate moisture in the blocks. Consequently, 50 milliliters of sterile distilled water were added aseptically to each chamber containing this fungus, thereby covering the blocks. The blocks were thus permitted to resorb water for 24

hours. The excess water was then aseptically removed and the chambers reinstalled in the incubation room.

Measurement of the Extent of Decay

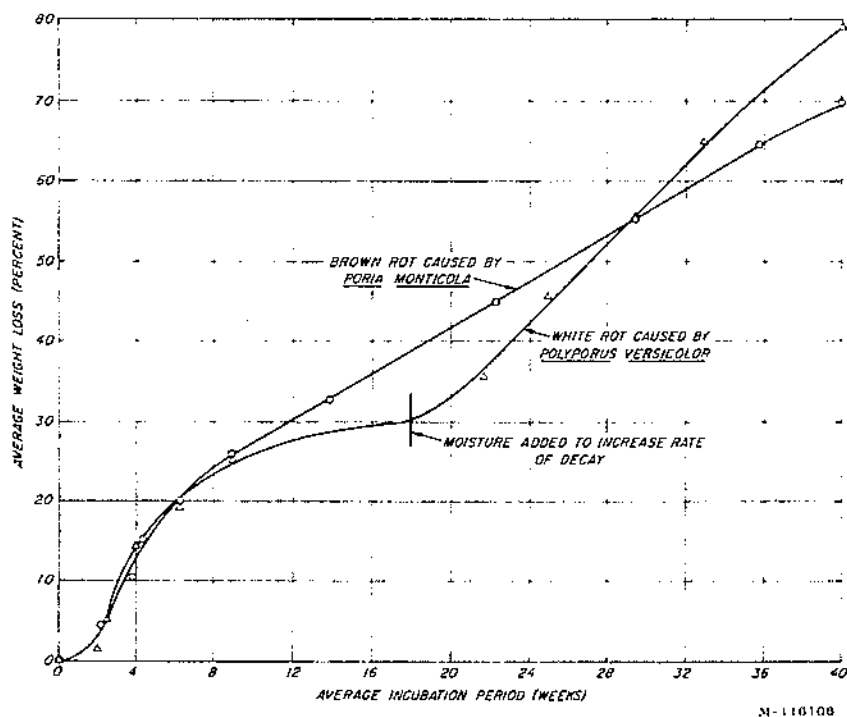
Loss in weight was used as a measure of the extent of decay in the test blocks. After incubation, each block was carefully brushed free of surface mycelium, equilibrated from the dry side with an atmosphere at $26.5^{\circ} \pm 0.5^{\circ}$ C. and 30 ± 2 percent relative humidity, and weighed to the nearest 0.01 gram. This weight of the decayed block was subtracted from the block's original equilibrium weight and the difference expressed as a percent of the latter value. Test blocks with approximately comparable percent weight losses (irrespective of the time required to achieve it) were assembled into groups containing a sufficient number to provide at least 20 grams of material for chemical analysis, and three blocks for microscopical study. The average weight loss, corresponding standard deviation, and the average incubation period for each weight-loss class are given in table 4.

TABLE 4.—Average weight losses and incubation periods for the sweetgum sapwood samples in the various weight-loss classes

| Type of decay and causal organism | Weight loss, based on original equilibrium weight | | Average incubation period |
|---|---|--------------------|---------------------------|
| | Average | Standard deviation | |
| | Percent | Percent | Weeks |
| White rot caused by <i>Polyporus versicolor</i> . | 0.0 | 0.00 | 0.0 |
| | 1.6 | .31 | 2.0 |
| | 5.2 | 1.92 | 2.5 |
| | 14.5 | 1.44 | 4.0 |
| | 19.4 | .82 | 6.3 |
| | 25.3 | 2.84 | 9.0 |
| | 35.5 | 3.51 | 21.7 |
| | 45.5 | 3.09 | 25.0 |
| | 55.2 | 3.19 | 29.5 |
| | 64.7 | 3.36 | 33.0 |
| | 79.0 | 1.09 | 40.0 |
| Brown rot caused by <i>Poria monticola</i> . | .0 | .00 | .0 |
| | 4.5 | .55 | 2.2 |
| | 10.7 | 1.76 | 3.8 |
| | 15.3 | 1.17 | 4.3 |
| | 20.1 | 1.84 | 6.3 |
| | 26.1 | 2.02 | 9.0 |
| | 32.6 | 1.77 | 13.9 |
| | 44.8 | 3.47 | 22.3 |
| | 55.0 | 3.45 | 29.5 |
| | 64.4 | 2.40 | 35.8 |
| | 69.5 | .70 | 40.0 |

In addition to the samples decayed in the sponge-rod incubation chambers and listed in table 4, a group of eight blocks taken from the original lot of test blocks was permitted to decay in soil-block chambers (1) for 38 weeks; an average weight loss of 96.7 (standard deviation 0.2) percent occurred. This sample was ground to pass a 40-mesh screen and submitted to analysis by the sulfuric acid hydrolysis and chromatographic procedure described later in this bulletin together with the samples listed in table 4.

Figure 7 shows the weight loss due to decay by each test fungus during the various incubation periods. Loss in weight may be interpreted as a direct measure of the amount of wood substance converted by the fungus to carbon dioxide and water or other volatile metabolic products during respiration. The initial lag phase of the curves in figure 7 indicates the time required for the fungus to become established in the test blocks. A period of very rapid respiration between 2 and 4 weeks' incubation was followed by a decline in respiration rate. After about 9 weeks, the rate of respiration of the wood substance by *Poria monticola* remained essentially constant. The rate of respiration by *Polyporus versicolor* fell off after 12 weeks, but it increased markedly



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FIGURE 7.—Weight loss due to decay by each test fungus during the various incubation periods. Loss in weight may be interpreted as a measure of the amount of wood substance converted by the fungus to carbon dioxide and water or other volatile products of respiration.

upon addition of water to the blocks after 18 weeks' incubation and remained essentially constant thereafter. The similarity in respiration rate shown by the two test fungi in the early stages of decay is surprising in view of the very different mechanisms of their effects on the test wood, as will be shown later.

Preparation of Wood Meal Samples

The test blocks selected for chemical analysis in each weight-loss class were collectively ground in a Wiley mill to pass a 40-mesh standard sieve. To insure the production of a minimum of fine particles, three grinding periods of 1 minute or less (depending on the extent of decay) were used. The material that passed a 40-mesh standard sieve was collected after each grinding period; only the particles retained by the sieve were reground. A 2-millimeter screen was used in the mill during the first two grinding periods; a 1-millimeter screen was used during the third period. After these three successive fractionation and grinding periods, the entire sample of wood in each weight-loss class had passed the standard sieve.

Progressive Changes in the Distribution of Particle Sizes in the Wood Meal Samples

Two of the TAPPI standard procedures used in this study (74, 77) specify 40- to 60-mesh particle size limits for the samples. The requirement to discard particles smaller than 60 mesh is in contradiction to the conventional rules of sampling and to the TAPPI Standard T 11m-45 (75), which recommends that "... the fine material should not be discarded, because the fractionation of the wood meal may alter the proportion of certain constituents and might lead to erroneous analytical results ... Until agreement is reached, it is recommended that the particle size employed for the determination of [holo-] cellulose be stated as a part of the report." For these reasons, the distribution of particle sizes in the samples in each weight-loss class was determined and an aliquot of the entire sample submitted to each chemical analysis.

Procedure for the Particle-Size Determination

The distribution of particle sizes in the samples in each weight-loss class was determined with a Tyler Testing Sieve Shaker with the tapper engaged. The distribution in each sample was determined as the percent by weight that was retained by the 40-, 50-, 60-, 80-, 100-, and 200-mesh standard sieves (stacked in the order given, above the sieve pan) after 5 minutes on the shaker. A Chi-square test was employed to identify samples whose distributions were significantly different from the distribution observed for the sound wood (control) samples. The particle-size fractions from each weight-loss class were reunited after this fractionation procedure and randomized by shaking the samples thoroughly in their storage bottles.

Results of the Particle-Size Determination

Table 5 shows the distribution of particle sizes in the samples prepared for chemical analysis. Among the weight-loss classes for the white-rot fungus, *Polyporus versicolor*, only the highest class showed a significantly different distribution of particle sizes. This was in accord with a similar observation by Scheffer (67) for this same fungus-host wood combination. However, the brown-rotted wood showed a progressive alteration in distribution of particle sizes with increasing weight loss. In the sound wood, 77 percent of the weight of the sample was composed of particles

TABLE 5.—Distribution of particle sizes in the sweetgum sapwood samples submitted to chemical analysis, based on the total weight of the sample in each weight-loss class

| Type of decay and causal organism | Average weight loss | Mesh size of particles | | | | | | | Probability of a larger value of Chi-square ¹ |
|---|---------------------|------------------------|----------|----------|----------|----------|----------|----------|--|
| | | 0-40 | 40-50 | 50-60 | 60-80 | 80-100 | 100-200 | 200 + | |
| | Per-cent | Per-cent | Per-cent | Per-cent | Per-cent | Per-cent | Per-cent | Per-cent | |
| White rot caused by <i>Polyporus versicolor</i> . | 0.0 | 0 | 29 | 25 | 23 | 11 | 10 | 2 | |
| | 1.6 | 1 | 29 | 24 | 23 | 10 | 11 | 2 | >0.99 |
| | 5.2 | 0 | 22 | 24 | 27 | 12 | 12 | 3 | .70 |
| | 14.5 | 0 | 22 | 24 | 27 | 12 | 12 | 3 | .37 |
| | 19.4 | 0 | 20 | 24 | 28 | 12 | 14 | 2 | .36 |
| | 25.3 | 0 | 25 | 25 | 28 | 10 | 10 | 2 | .78 |
| | 35.5 | 0 | 26 | 24 | 25 | 11 | 11 | 3 | .92 |
| | 45.5 | 1 | 28 | 24 | 24 | 10 | 11 | 2 | .97 |
| | 55.2 | 1 | 24 | 20 | 26 | 11 | 14 | 4 | .33 |
| | 61.7 | 1 | 38 | 17 | 22 | 8 | 11 | 3 | .06 |
| | 79.0 | 2 | 24 | 14 | 27 | 8 | 20 | 5 | <.001** |
| Brown rot caused by <i>Poria monticola</i> . | 0.0 | 0 | 29 | 25 | 23 | 11 | 10 | 2 | |
| | 4.5 | 0 | 22 | 22 | 22 | 13 | 17 | 4 | 0.86 |
| | 10.7 | 0 | 21 | 21 | 24 | 13 | 16 | 5 | .009** |
| | 15.3 | 0 | 17 | 20 | 24 | 13 | 19 | 7 | <.001** |
| | 20.1 | 0 | 22 | 19 | 19 | 13 | 19 | 8 | <.001** |
| | 26.1 | 0 | 27 | 18 | 18 | 11 | 18 | 8 | <.001** |
| | 32.6 | 0 | 23 | 17 | 17 | 11 | 18 | 14 | <.001** |
| | 44.8 | 0 | 23 | 15 | 15 | 11 | 20 | 16 | <.001** |
| | 55.0 | 0 | 22 | 13 | 14 | 12 | 25 | 14 | <.001** |
| | 64.4 | 0 | 17 | 10 | 12 | 10 | 23 | 28 | <.001** |
| | 69.5 | 0 | 13 | 8 | 10 | 10 | 29 | 30 | <.001** |

¹ Double asterisks (**) indicate weight-loss classes whose particle-size distribution was significantly different from that for the sound wood sample as indicated by the Chi-square test.

² Sound wood. The percentages for this sample were the expected values for the calculation of Chi-square.

larger than 80 mesh (177 microns in average diameter); at the highest weight loss 69 percent were smaller than 80 mesh. Only the lowest weight-loss class showed a particle-size distribution similar to the control sample.

These differences in particle-size distribution with increasing weight loss in the two types of decay indicate that the resistance of the wood to fragmentation during grinding was greatly reduced at very low weight losses in brown rot, whereas little change in this property was induced until high weight losses were sustained in white rot. In view of the assumption by wood physicists that the strength of wood may be attributed largely to cellulose of high degree of polymerization, the data of table 5 suggest that *Poria monticola* caused a rapid depolymerization of the cellulose in the wood at low weight losses. Such an effect was apparently not involved in the decay caused by *Polyporus versicolor* until a very advanced stage of decay.

Progressive Changes in Solubility Properties of the Decayed Wood

Determinations of solubility provide one means of measuring the amount and nature of degradation products present in decayed wood. Since the primary degradation products are immediately subject to further metabolism, solubility determinations indicate only the amount of nonvolatile degradation products accumulated at a given stage of decay, rather than the total amount released from the wood during the decay process.

Since the extracting solvents commonly employed in wood analysis are only partially selective for particular degradation products, solubility determinations in themselves have only limited fundamental significance. To enhance the value of the solubility determinations used in this study, an attempt was made to characterize the water and alkali extracts.

Methods of Solubility Analysis

The water solubility determinations were made according to the TAPPI Standard T 1m-51 (78) except that 1- rather than 2-gram samples and correspondingly smaller amounts of distilled water were used. The temperature of the cold water during the 48-hour digestion period was $26.5^{\circ} \pm 0.5^{\circ} \text{C}$. With increasing extent of brown rot above 32 percent weight loss, the addition of 0.5 (w/v) percent sodium dioctyl sulfosuccinate as a wetting agent gave more consistent results. The results for the brown-rotted wood are given for distilled water both with and without the wetting agent.

The solubility of the samples in 1 percent sodium hydroxide was determined according to the TAPPI Standard T 4m-14 (74) except that 1- rather than 2-gram samples and correspondingly smaller amounts of solvent and acetic acid were used. The entire ground sample (particle-size distribution given in table 5) was used in accordance with recommended practice as given in the TAPPI Standard T 11m-45 (75).

The total extractives were determined quantitatively according to the TAPPI Standard T 12m-45 (76). The procedure involved successive extractions with ethanol-benzene, ethanol, and hot water. To assure complete removal of soluble materials, the extraction periods with ethanol-benzene and ethanol were 8 hours for white-rotted wood and 16 hours for the brown-rotted material. A single 3-hour extraction with hot distilled water was used for both types of decayed wood.

The solubility in ethanol-benzene was determined by weighing the extract in a tared flask after it was dried to constant weight at 105° C. The total extractives were determined by transferring the extracted sample into a tared, fritted glass crucible of porosity C. The sample was then washed with hot distilled water and dried to constant weight in a tared weighing bottle under vacuum over magnesium perchlorate. The difference between the original and extracted moisture-free weights was expressed as a percent of the former value. The ethanol-benzene result was expressed on this same basis.

During the ethanol-benzene extraction, the brown-rotted samples showed a progressive tendency, with increasing weight loss above 32 percent, to agglomerate into a coherent elastic mass that resisted fragmentation. These agglomerates did not break up during the ethanol extraction, and some even remained after the water extraction. This adhesive tendency may have reduced the yield of extractives in all three solvents. No tendency to agglomerate was noted for the white-rotted samples. This agglomeration may have resulted from a tendency of the partial degradation products in the brown-rotted wood to polymerize in the presence of the organic solvents used in these analyses. A similar tendency was observed in water solution during the holocellulose analyses as described on page 39. No further explanation can be offered to account for this agglomeration.

Solubility in methyl cellosolve was determined by weighing a 1-gram sample of the air-dry wood meal, whose moisture content was known from a previous determination, in a 30-milliliter shell vial. Twenty-five milliliters of distilled methyl cellosolve were added, and the vial was rotated end over end for 72 hours at room temperature. The contents of the vial were then poured with suction into a tared, fritted glass crucible of porosity M, and washed with cold methyl cellosolve and distilled water. The sample was then air-dried, dried to constant weight at 105° C., and weighed. The difference in moisture-free weight before and after extraction was expressed as a percentage of the former value.

Results of the Solubility Determinations

The results of the solubility determinations are presented in table 6 in the manner conventional for wood analyses; that is, as percentages of the moisture-free weight of the samples submitted to analysis. In table 7, the same results are given as percentages of the moisture-free weight of the original sound wood. The latter basis has the advantage of permitting more ready visualization of the changes in composition of a given sample of wood through

all stages of decay. The data of table 7 are shown graphically in figure 8.

In determining the significance of the curves in figure 8 it should be recognized that two competitive aspects of the decay process were going on simultaneously; that is, at the same time that the insoluble constituents of the wood were being depolymerized by extracellular reactions to soluble partial degradation products, the previously formed partial degradation products were in turn being converted by intracellular respiratory reactions to carbon dioxide and water and other volatile metabolic products.

TABLE 6.—Solubility of sweetgum sapwood in progressive stages of decay, based on moisture-free weight of the decayed samples

| Type of decay and causal organism | Average weight loss | Solubility in— | | | | | |
|---|---------------------|-------------------------|------------------------|----------------------------|-----------------|---|-------------------|
| | | Cold water ¹ | Hot water ² | 1 percent sodium hydroxide | Ethanol benzene | Ethanol, benzene, ethanol, and hot water ³ | Methyl cellosolve |
| | Percent | Percent | Percent | Percent | Percent | Percent | Percent |
| White rot caused by <i>Polyporus versicolor</i> . | 0.0 | 1.7 | 2.6 | 16.9 | 2.5 | 4.3 | 0.8 |
| | 1.6 | | | | 1.9 | 4.0 | |
| | 5.2 | 2.3 | 3.2 | 17.9 | 2.1 | 4.3 | |
| | 14.5 | 2.0 | 2.6 | 18.7 | 2.0 | 4.2 | 1.7 |
| | 19.4 | 2.5 | 3.5 | 20.0 | 2.1 | 4.5 | |
| | 25.3 | 2.3 | 3.2 | 20.5 | 2.2 | 5.5 | |
| | 35.5 | 2.5 | 3.5 | 20.3 | 2.9 | 5.3 | 2.6 |
| | 45.5 | 2.9 | 4.2 | 22.9 | 3.3 | 6.7 | |
| | 55.2 | 4.4 | 5.7 | 25.5 | 3.8 | 8.2 | 3.3 |
| | 64.7 | 5.3 | 7.9 | 29.3 | 4.8 | 10.4 | |
| Brown rot caused by <i>Poria monticola</i> . | 0.0 | 1.7 | 2.6 | 16.9 | 2.5 | 4.3 | .8 |
| | 4.5 | 2.8 | 5.5 | 33.2 | 4.0 | 8.1 | |
| | 10.7 | 4.7 | 7.7 | 47.5 | 6.0 | 13.7 | |
| | 15.3 | 6.4 | 11.6 | 58.6 | 8.0 | 17.4 | 4.9 |
| | 20.1 | 7.4 | 13.5 | 63.1 | 10.4 | 21.5 | |
| | 26.1 | 7.8(8.0) | 15.3(15.4) | 66.0 | 11.9 | 22.7 | |
| | 32.6 | 8.3(8.5) | 16.1(17.0) | 69.3 | 12.7 | 24.6 | 15.0 |
| | 41.8 | 6.8(9.4) | 16.0(20.1) | 74.4 | 14.4 | 26.9 | |
| | 55.0 | 6.9(9.9) | 17.6(21.4) | 81.2 | 18.5 | 32.7 | |
| | 64.4 | 7.8(10.7) | 20.7(23.9) | 87.5 | 25.3 | 40.6 | 38.0 |
| | 69.5 | 7.4(10.4) | 20.0(23.7) | 91.2 | 27.3 | 45.6 | |

¹ Numbers in parentheses indicate solubility in distilled water containing 0.5 (w/v) percent sodium dioctyl sulfosuccinate as a wetting agent.

² Total of successive extractions.

³ Sound wood.

TABLE 7.—*Solubility of sweetgum sapwood in progressive stages of decay, based on moisture-free weight of the original sound wood*

| Type of decay and causal organism | Average weight loss | Solubility in— | | | | | |
|---|---------------------|-------------------------|------------------------|----------------------------|-----------------|--|-------------------|
| | | Cold water ¹ | Hot water ¹ | 1 percent sodium hydroxide | Ethanol benzene | Ethanol benzene, ethanol, and hot water ² | Methyl cellosolve |
| | Percent | Percent | Percent | Percent | Percent | Percent | Percent |
| White rot caused by <i>Polyporus versicolor</i> . | 0.0 | 1.7 | 2.6 | 16.9 | 2.5 | 4.3 | 0.8 |
| | 1.6 | | | | 1.9 | 3.9 | |
| | 5.2 | 2.2 | 3.0 | 17.0 | 2.0 | 4.1 | |
| | 14.5 | 1.7 | 2.2 | 16.0 | 1.7 | 3.6 | 1.5 |
| | 19.4 | 2.0 | 2.8 | 16.1 | 1.7 | 3.6 | |
| | 25.3 | 1.7 | 2.4 | 15.3 | 1.6 | 4.1 | |
| | 35.5 | 1.6 | 2.2 | 13.1 | 1.9 | 3.4 | 1.7 |
| | 45.5 | 1.6 | 2.3 | 12.5 | 1.8 | 3.7 | |
| | 55.2 | 2.0 | 2.6 | 11.4 | 1.7 | 3.7 | 1.5 |
| | 64.7 | 1.9 | 2.8 | 10.3 | 1.7 | 3.7 | |
| Brown rot caused by <i>Poria monticola</i> . | 0.0 | 1.7 | 2.6 | 16.9 | 2.5 | 4.3 | .8 |
| | 4.5 | 2.6 | 5.2 | 31.7 | 3.9 | 7.8 | |
| | 10.7 | 4.2 | 6.8 | 42.4 | 5.4 | 12.2 | |
| | 15.3 | 5.5 | 9.8 | 49.7 | 6.8 | 14.8 | 4.4 |
| | 20.1 | 5.9 | 10.8 | 50.1 | 8.3 | 17.2 | |
| | 26.1 | 5.8(5.9) | 11.3(11.4) | 48.7 | 8.8 | 16.8 | |
| | 32.6 | 5.6(5.7) | 10.8(11.4) | 46.7 | 8.6 | 16.5 | 10.1 |
| | 44.8 | 3.8(5.2) | 8.8(11.1) | 41.1 | 8.0 | 14.9 | |
| | 55.0 | 3.1(4.5) | 7.9(9.6) | 36.6 | 8.3 | 14.7 | |
| | 61.4 | 2.8(3.8) | 7.4 (8.5) | 31.1 | 9.0 | 14.4 | 13.5 |
| | 69.5 | 2.3(3.2) | 6.1 (7.2) | 27.9 | 8.3 | 13.9 | |

¹ Numbers in parentheses indicate solubility in distilled water containing 0.5 (w/v) percent sodium dioctyl sulfosuccinate as a wetting agent.

² Total of successive extractions.

³ Sound wood.

Thus, an ascending line in figure 8 indicates that the extracellular depolymerization reactions were proceeding more rapidly than the products of these reactions were being converted to volatile products by respiration. A descending line, conversely, indicates that the respiratory metabolism of partial degradation products to volatile materials was proceeding more rapidly than new partial degradation products were being formed.⁸ A horizontal line indicates that a stage of equilibrium existed between the two proc-

⁸ Respiration was assumed to account for any decrease, from one stage of decay to the next, in amount of material extracted from the decayed wood.

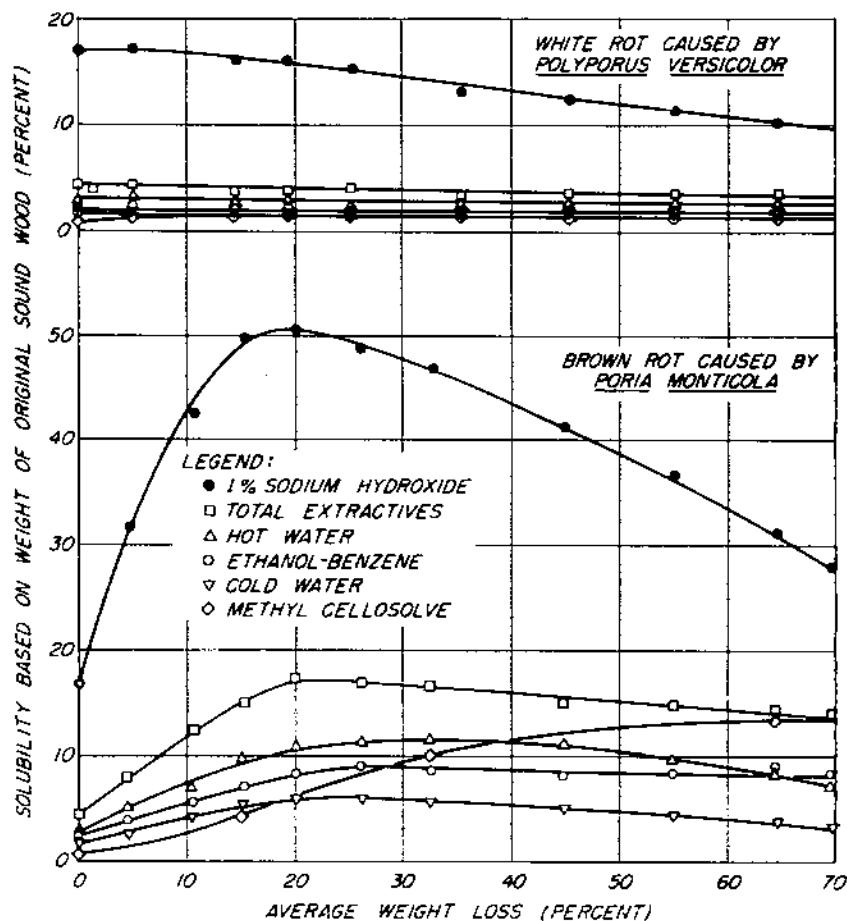


FIGURE 8.—Solubility of sweetgum sapwood in progressive stages of decay. Total extractives were determined after successive extractions with ethanol-benzene, ethanol, and hot water.

esses; that is, the extracellular depolymerization reactions and the intracellular respiratory processes were occurring at similar rates.

In each solvent, the rapid changes in solubility with increasing extent of brown rot present a marked contrast to the essentially constant or slowly decreasing solubility of the white-rotted material. The observed contrast in solubility of the two types of decayed wood in 1 percent sodium hydroxide was noted earlier by Campbell (11) and suggested as a general method for distinguishing the two types of decayed wood. As shown below, these differences indicate that distinctly different mechanisms and relative rates of enzymatic effects were involved in the two types of decay.

At all stages of decay by *Polyporus versicolor*, the rate of extracellular depolymerization of the wood substance to materials

soluble in all the solvents used, except 1 percent sodium hydroxide, approximately equaled the rate of their disappearance due to respiration. In the early stages of decay, this was also true of the alkali-soluble materials; in later stages, however, the rate of respiration of the materials soluble in dilute alkali exceeded the rate of their production.

In the initial stages of the brown rot caused by *Poria monticola*, the extracellular depolymerization of the wood substance to materials soluble in all solvents greatly exceeded the rate of their respiration to volatile materials. This condition was especially pronounced in substances soluble in alkali, and it continued in all stages of decay for the materials soluble in methyl cellosolve. After 25 to 30 percent weight loss in the water-soluble materials, and 20 percent in substances soluble in the other solvents used, however, the rate of respiration of the depolymerization products equaled or exceeded the rate of their production.

These observations suggest that some aspect of the respiration process was the rate-limiting factor in the metabolism of sweetgum sapwood by *Poria monticola*, whereas the depolymerization reactions appeared to be rate-limiting in the case of *Polyporus versicolor*. This distinction is most interesting in view of the very similar rate of respiration of the wood substance shown in figure 7 for the early stages of decay by both test fungi.

Differences in dissolving capacity of the various solvents also are shown in figure 8. One percent sodium hydroxide had by far the greatest solvency for wood in all stages of either type of decay. Hot water was a better solvent than cold water. Methyl cellosolve and ethanol-benzene showed less capacity to dissolve white-rotted wood than did hot water. With brown-rotted material, however, the reverse was true for all samples except those at low weight losses. The last two observations may be explained in part by the affinity of both organic solvents for the decay lignin gradually released by both fungi during decay. This substance accumulated during brown rot due to the inability of *Poria monticola* to metabolize lignin.

The total extractives content of the white-rotted wood was only slightly greater than its content of water-soluble materials. In brown-rotted wood, however, the total extractives were approximately 1.5 times as great as the hot-water solubles in all stages of decay.

Characterization of the Water and Alkali Extracts

To provide information as to the types of materials extracted from the decayed wood, the hot and cold water and 1 percent sodium hydroxide extracts were fractionated and characterized according to the procedures outlined in figure 9 and described below.

The pH of the water extracts was determined with a glass electrode after each extract was diluted to 250 milliliters.

Undecayed sweetgum sapwood contains only very small amounts of soluble tannins and other phenolic materials that show strong

absorption at 280 millimicrons. The absorption at this wave length, therefore, can be used to provide an estimate of the amount of lignin rendered soluble by the decay fungi and thus present in extracts of the decayed wood. Thus, ultraviolet absorption spectra were determined for selected extracts with a recording ultraviolet spectrophotometer and a comparable solvent blank. The amounts of "apparent lignin" in the extracts were calculated from these spectra by means of the following datum obtained by Pew (55): 1 milligram of lignin per liter of solution gives an approximate absorbance of 0.02 at 280 millimicrons.

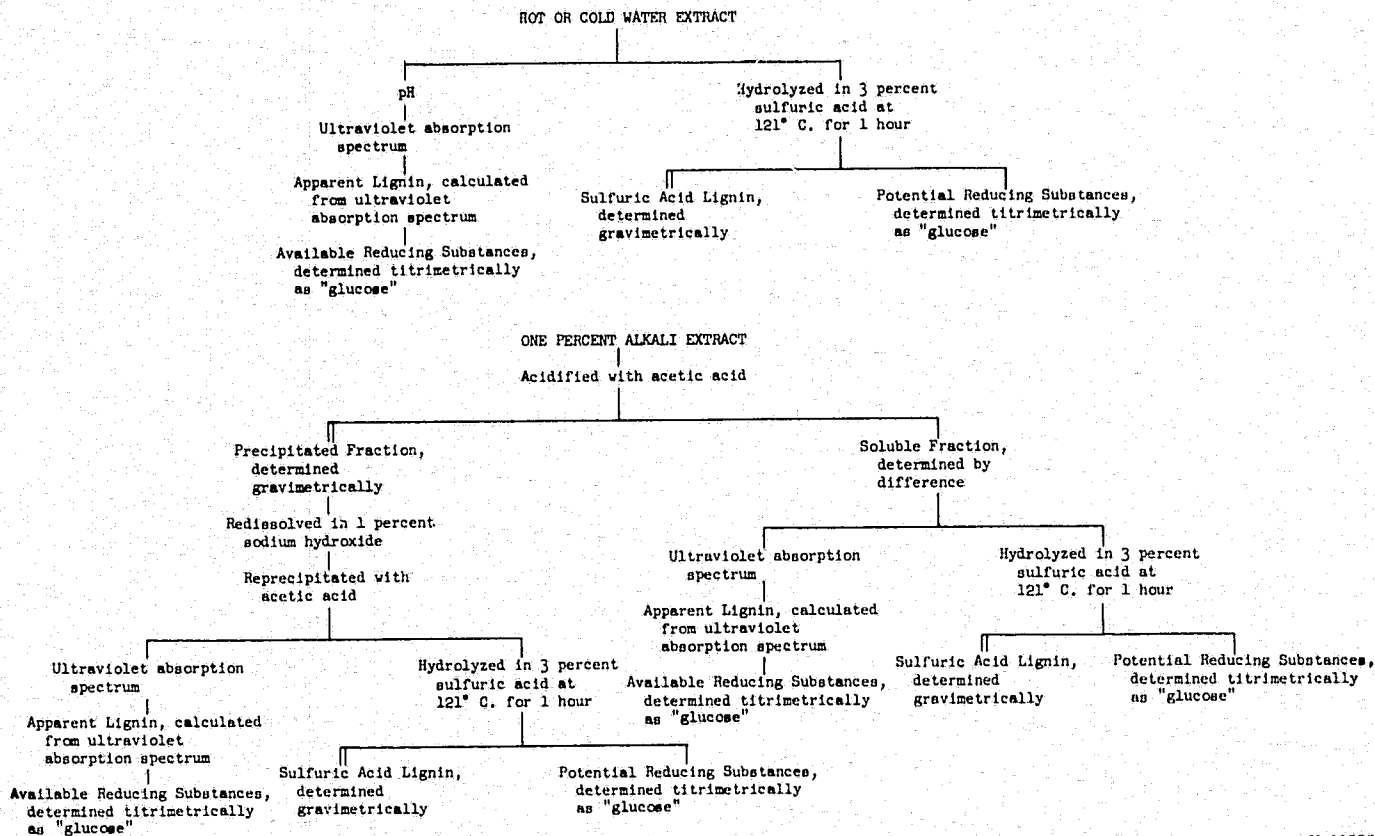
The potential and apparent reducing substances in selected extracts were determined, respectively, with and without hydrolysis in 3 (w/v) percent sulfuric acid at 121° C. for 1 hour. These conditions were equivalent to those of the secondary hydrolysis procedure described by Saeman and coworkers (65). The titrimetric procedure of Shaffer and Somogyi (69) was used to give estimates of the reducing substances present, which were calculated as "glucose."

During the hydrolysis described above, the "sulfuric acid lignin" in each extract was precipitated. It was transferred to a fritted glass crucible of porosity M, washed with hot distilled water, dried to constant weight at 105° C., and weighed.

The fraction of the 1 percent alkali extracts precipitable on acidification was determined by pouring the acidified extract with suction into a tared, fritted glass crucible of porosity M. After the residue was washed on the filter with distilled water, the crucible and its contents were air-dried, dried to constant weight under vacuum over magnesium perchlorate, and weighed. The acid-soluble fraction was determined by difference from the total alkali solubility.

The results of these characterizing determinations are given in figures 10 through 13. All results, except those for the pH determinations, were calculated and plotted as percentages of the moisture-free weight of the sample from which the extract was derived.

The progressive changes in pH of the water extracts shown in figure 10, reveal a more substantial drop in pH in the brown-rot extracts than in those from the white-rotted material. This observation is in accord with an earlier report by Rabanus (57). There was also an initial drop and subsequent increase in pH during the initial stages of white rot, however, that has not been reported. This suggested either (1) rapid production and extracellular release of organic acids that were subsequently metabolized; or (2) removal and subsequent utilization of acidic (probably acetyl) groups attached to the cellulose of the wood. The first alternative would appear more likely since alternative 2 would require (1) that *Polyporus versicolor* possess a cellulose deacetylating enzyme system—a type of enzyme which has not been detected in other cellulolytic organisms despite intensive efforts to do so (59, 60); and (2) that this enzyme system could gain access to a considerable number of acetyl groups attached to the cellulose—a degree of accessibility not observed for the other extracellular enzymes of this organism, as will be shown



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FIGURE 9.—Fractionation and characterization procedures applied to the water and 1 percent alkali extracts of sweetgum sapwood in progressive stages of decay by the white-rot fungus, *Polyporus versicolor*, and the brown-rot fungus, *Poria monticola*.

later. The fact that both the hot and cold water extracts showed this same dip indicates that the responsible acids were relatively nonvolatile.

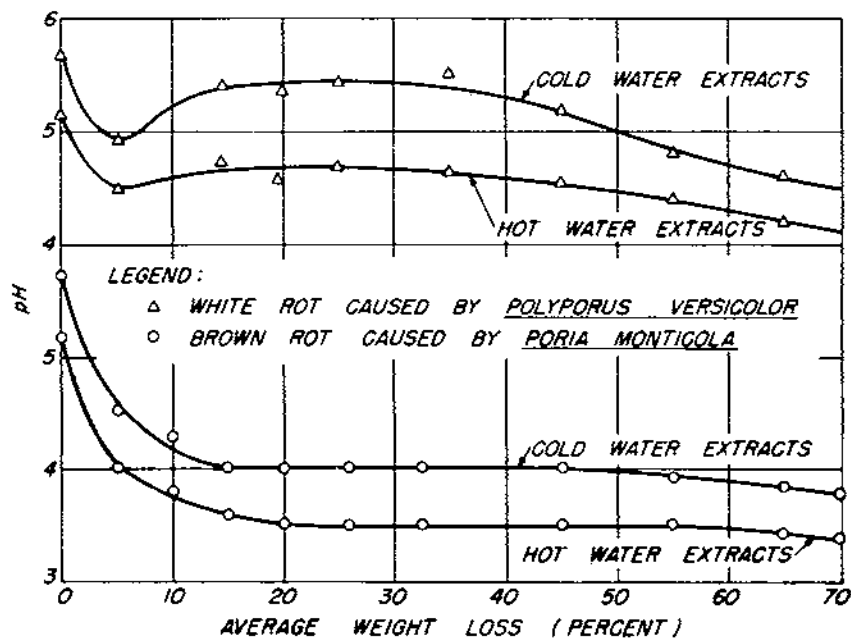


FIGURE 10.—The pH of water extracts of sweetgum sapwood in progressive stages of decay.

As shown in figure 11, the hot water extracts of the white-rotted wood had an essentially constant amount of available and potential reducing substances and apparent lignin, but they contained no sulfuric acid lignin. Potential reducing substances made up the bulk of both the white- and brown-rot extracts.

Figure 12 shows the proportions of the 1 percent sodium hydroxide extracts that precipitated and remained soluble on acidification. Although the solubility properties of these fractions are analogous to those for beta- and gamma-cellulose, respectively, both fractions contained a substantial amount of lignin as well as reducing substances. Almost all of the white-rot extracts remained soluble on acidification. The amount of acid-precipitable material increased with increasing extent of brown rot, whereas the acid-soluble fraction at first increased and then decreased.

As shown in figure 13, the alkali extracts of white-rotted wood contained an essentially constant amount of reducing substances. The comparable brown-rot extracts showed an initial increase followed by a pronounced decrease in proportion of these materials with increasing extent of decay. Sulfuric acid lignin and apparent lignin composed very small fractions of the white-rot

extracts, whereas they made up constantly increasing proportions of the brown-rot extracts.

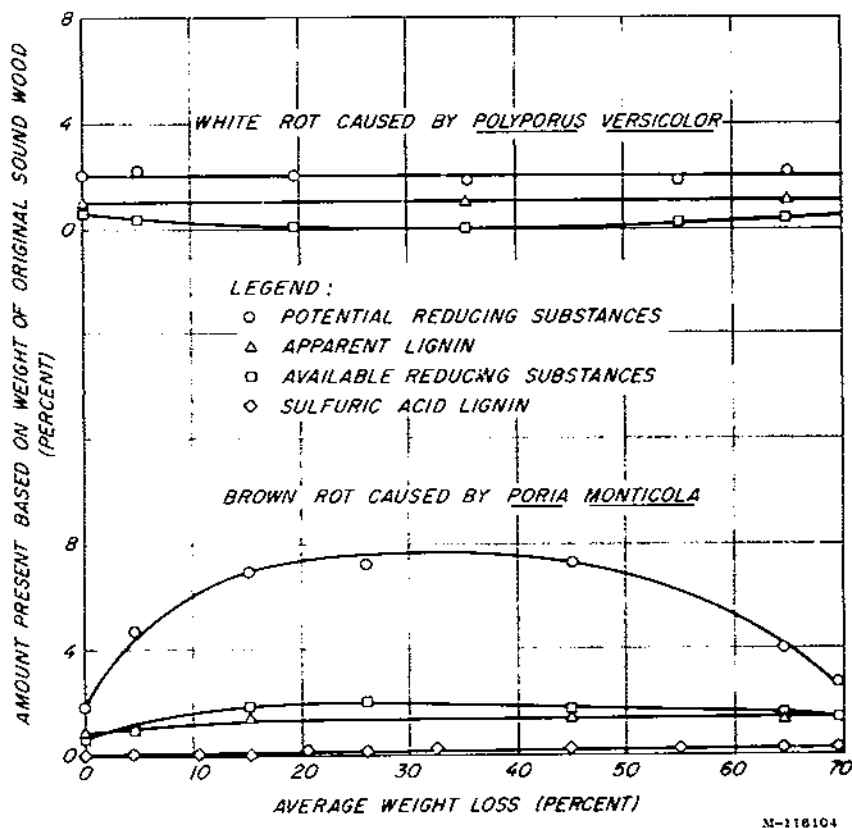


FIGURE 11.—Composition of hot water extracts of sweetgum sapwood in progressive stages of decay.

The accumulation of lignin in both the water and alkali extracts of brown-rotted wood indicates that lignin was so drastically modified during decay by *Poria monticola* that it became soluble in these two solvents. Part of the lignin was unprecipitable in hot dilute sulfuric acid, the reagent commonly used for its quantitative analysis. The very small amount of lignin in the white-rot extracts is indicative of its rapid utilization by *Polyporus versicolor*, as will be shown later.

The observation that lignin made up a large proportion of the alkali extracts of brown-rotted wood, even in the earliest stages of decay, is in contrast to the presumption by Campbell (11), Kennedy (35), and Pechmann and Schaile (53) that the alkali solubility of decayed wood may be attributed to carbohydrate degradation products.

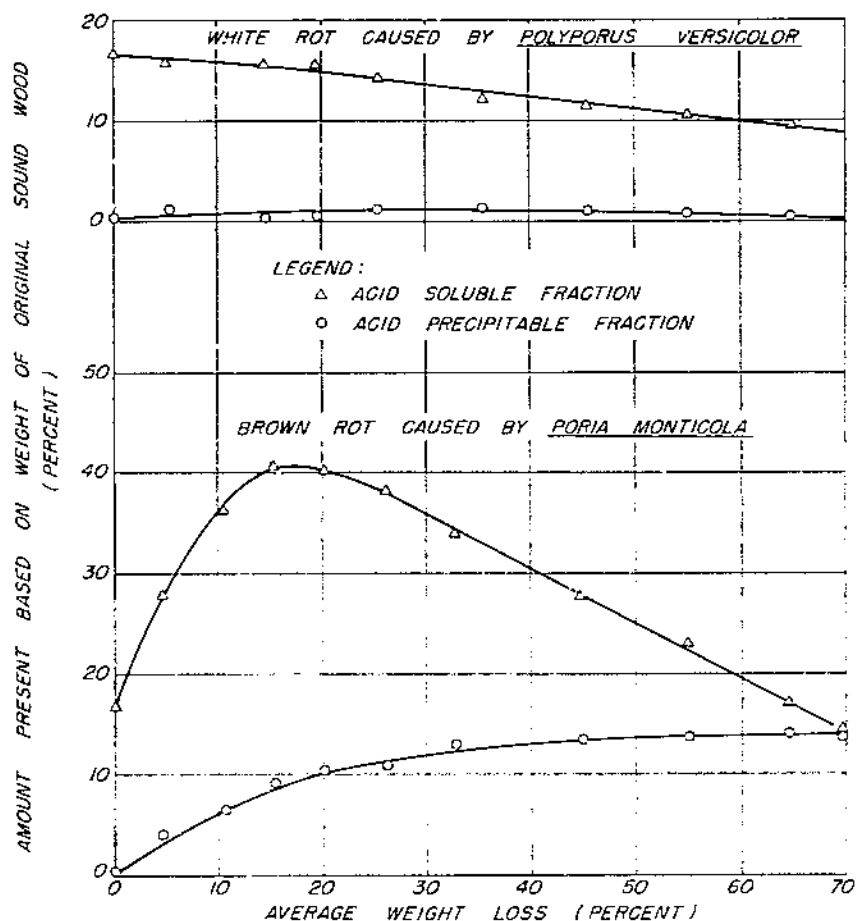


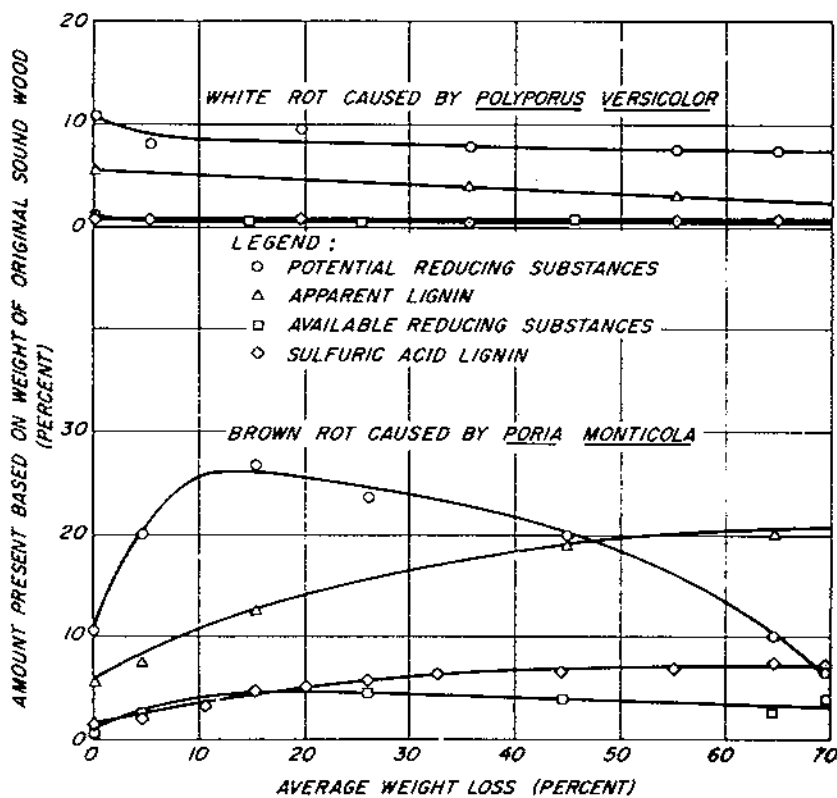
FIGURE 12.—Composition of 1 percent sodium hydroxide extracts of sweetgum sapwood in progressive stages of decay.

Significance of the Solubility Determinations

The results of the solubility determinations indicated the following:

(1) In the brown rot caused by *Poria monticola*, there was a rapid depolymerization of the carbohydrates and significant alteration of the lignin of the wood. The depolymerization of the wood carbohydrates was reflected by their increased solubility in water and dilute alkali. The changes in lignin induced by this fungus were reflected by its increased water and alkali solubility.

(2) In the early stages of brown rot, the rate of depolymerization of the major wood constituents to soluble materials greatly exceeded the rate of respiration of the depolymerization products. In later stages of decay, the reverse was true—the previously



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FIGURE 13.—Composition of 1 percent sodium hydroxide extracts of sweetgum sapwood in progressive stages of decay.

formed degradation products were consumed more rapidly than new ones were formed.

(3) In all stages of decay by the white-rot fungus, *Polyporus versicolor*, the solubility properties and composition of the materials extracted underwent only slight changes. The rate of extracellular depolymerization of the wood constituents was essentially equal to the rate of respiration of the degradation products. The slow depolymerization of the wood carbohydrates was reflected by gradual changes in the ratio of potential to available reducing substances in the alkali extracts.

(4) The more substantial reduction in pH of the water extracts of brown-rotted in comparison with white-rotted wood confirmed earlier observations that the brown-rot fungi release greater quantities of organic acids during the decay process.

Progressive Changes in Chemical Composition of the Decayed Wood

The solubility analyses have shown the amount of various degradation products that remained in the wood at various stages of decay. They have also given some evidence of the relative rates of depolymerization and respiration of the wood constituents. To obtain additional information on the overall rates of utilization of each wood constituent, and on the enzymatic nature of the depolymerization processes, the changes in composition of the wood during progressive stages of decay by the two test fungi were determined.

Methods of Composition Analysis

The composition of the samples in each weight-loss class was determined by two methods. In the first method, the total carbohydrate, individual sugar, and lignin contents of the samples were determined by the sulfuric acid hydrolysis and chromatographic procedure of Saeman and coworkers (65). By this procedure, the polysaccharides of the wood were converted to their constituent monosaccharides, and the lignin was condensed to an insoluble residue. The lignin residue was determined gravimetrically, and the total carbohydrates were determined by difference, as suggested by Van Beckum and Ritter (79).

The monosaccharides in selected hydrolyzates were then resolved by paper chromatography into five identifiable fractions, namely glucose, galactose, mannose, xylose, and arabinose. The relative proportions of these fractions were determined spectrophotometrically by their reducing power after elution from the developed chromatograms. The actual amounts present in the samples were calculated from the relative proportions in the hydrolyzate, using the total carbohydrates content as a basis of calculation. Corrections were applied in the analysis for the relative reducing power of the various monosaccharides and for losses during hydrolysis. No corrections were made for the extraneous and mineral constituents remaining in the samples.

The second method of determining the composition of the decayed samples involved successive preextractions of the samples in each weight-loss class with ethanol-benzene, ethanol, and hot water. Holocellulose was then prepared from the extractive-free samples and subsequently fractionated into alpha-, beta-, and gamma-cellulose. The TAPPI Standard procedure for preparing holocellulose (77) was used, except that the entire ground sample, rather than the specified 40- to 60-mesh particle-size fraction, was used. The distribution of particle sizes in each sample is given in table 5, p. 26, as recommended by the TAPPI Standard T 11m-45 (75).

The holocellulose procedure involved a succession of alternate chlorination and monoethanolamine extraction treatments of the sample. By this procedure, the lignin was removed and the insoluble carbohydrates of the wood were obtained in a presumably unaltered fibrous state. In the brown-rotted wood, a progressive

increase in difficulty of filtration was noted with increasing extent of decay and each successive chlorination of a given sample. This difficulty was apparently due largely to the presence of carbohydrate degradation products in the samples. These materials formed a thick gel that plugged the pores of the fritted glass filter upon addition of the water wash. At weight losses above 44 percent, this plugging made analysis by the Standard method impossible. Thus, at high weight losses, the water wash was omitted from the procedure. This tendency of the partially delignified holocellulose to form a gel in water solution may be related to a similar tendency of the particles of brown-rotted wood to agglomerate during the ethanol-benzene extractions as described on page 28. No further explanation can be offered to account for this gelation. No difficulty was experienced in preparing holocellulose from the white-rotted samples.

The holocellulose was fractionated into alpha-, beta-, and gamma-cellulose according to the following procedure. The sample was placed in a 250-milliliter beaker in a 20° C. water bath, and 25 milliliters of 17.5 (w/v) percent (5.23 N) aqueous sodium hydroxide at 20° C. were added. After 5 minutes, the sample was macerated with a stirring rod while an additional 25 milliliters of alkali were added. The beaker was then covered and left in the water bath, with occasional stirring, for 45 minutes from the time of addition of the first aliquot of alkali. After this mercerizing treatment, 50 milliliters of distilled water at 20° C. were added with thorough stirring, and the contents of the beaker were poured with suction and quantitatively washed with distilled water into a tared, fritted glass crucible of porosity C. The sample was then treated with 25 milliliters of 10 (v/v) percent acetic acid for 5 minutes, and was finally washed with distilled water until acid free. The filtrate was poured into a stoppered flask for determination of beta- and gamma-cellulose. The alpha-cellulose in the crucible was then washed with ethanol and ethyl ether, air-dried, dried to constant weight under vacuum over magnesium perchlorate, and weighed.

Twenty milliliters of glacial acetic acid were added to precipitate the beta-cellulose in the filtrate. After a 24-hour settling period, the contents of the flask were poured with suction into a tared, fritted glass crucible of porosity M, washed with distilled water until acid free, air-dried, dried to constant weight, and weighed. The alpha- and beta-cellulose yields permitted an estimate of gamma-cellulose by difference from the holocellulose content. The results were expressed as percentages of the moisture-free weight of the original unextracted wood from which the holocellulose was prepared.

These two methods of composition analysis are not directly comparable since the wood meal samples were not extracted prior to analysis by the sulfuric acid hydrolysis procedure, but were extracted before the holocellulose procedure.

Composition of the Decayed Wood

As in the case of the solubility analyses, the results of the composition analyses are presented on two bases: in table 8 as per-

centages of the moisture-free weight of the decayed samples, and in table 9 on the basis of the original sound wood. Since progressive changes in composition can be visualized most readily when expressed on the latter basis, the data of table 9 are presented graphically in figures 14 through 17.

The curves of figure 14 indicate that the lignin, total carbohydrates, and each of the individual sugar polymers of the wood were utilized concurrently in all stages of decay by the white-rot fungus, *Polyporus versicolor*. They also show that the rates of utilization of each of these constituents were so nearly constant in all stages of decay, and so nearly proportional to the amounts present in the original undecayed wood, that the relative proportions of each constituent present in any of the decayed samples were only slightly different from those for undecayed wood.

The curves of figure 15 also indicate considerable uniformity in composition of the wood in progressive stages of decay by *Polyporus versicolor*. Holo-, alpha-, and gamma-cellulose as well as lignin were utilized in all stages of the decay process. The rates of utilization of the holo- and alpha-cellulose and the lignin remained essentially constant during the decay process, whereas gamma-cellulose was utilized at a constantly increasing rate. Beta-cellulose accumulated slowly during all stages of decay. The rapid disappearance of alpha-cellulose without corresponding increase in the amount of beta-cellulose present suggests that the comparatively long alpha-cellulose molecules were depolymerized in such a way that only a small amount of intermediate-length degradation products accumulated during the decay process.

As shown in table 9, the holocellulose and lignin contents of the white-rotted samples, as determined by the holocellulose procedure, are in substantial agreement with the total carbohydrate and lignin results of the hydrolysis procedure. The two estimates of lignin content agree within 1.7 percent. The sum of the total extractives and the holocellulose contents agrees with the total carbohydrate yield within 1.6 percent. These facts suggest that most of the materials removed during extraction of the samples prior to the holocellulose determination were present in the hydrolyzate, which was resolved chromatographically in the hydrolysis procedure.

The curves of figure 16 show that lignin was utilized only to a very minor extent by the brown-rot fungus, *Poria monticola*. They also indicate that this organism utilized the total carbohydrates as well as the individual sugar polymers at rates that remained essentially constant in all stages of decay and that were approximately proportional to the amounts present in sound wood.

As shown in table 9, the total carbohydrate and lignin results of the hydrolysis procedure are not in agreement with the holocellulose and lignin contents of the brown-rotted samples, as determined by the holocellulose procedure. This discrepancy was caused by the removal of certain soluble degradation products of the carbohydrates and lignin during the extractions that were necessary prior to the holocellulose analysis. For this reason, the holo-, alpha-, beta-, and gamma-cellulose results should be considered as estimates of the composition of the insoluble carbo-

TABLE 8.—Composition of sweetgum sapwood in progressive stages of decay, based on the moisture-free weight of the decayed samples

| Type of decay and causal organism | Average weight loss | Results of the sulfuric acid hydrolysis and chromatographic procedures | | | | | | | Results of the holocellulose procedure | | | | | |
|---|---------------------|--|---------|----------|---------|---------|---------|----------------------|--|---------------|-----------------|----------------|-----------------|-----------------------|
| | | Total carbohydrate ¹ | Glucan | Galactan | Mannan | Xylan | Araban | Sulfuric acid lignin | Total extractives | Holocellulose | Alpha-cellulose | Beta-cellulose | Gamma-cellulose | "Lignin" ² |
| White rot caused by <i>Polyporus versicolor</i> . | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent |
| | 0.0 | 77.1 | 52.3 | 1.1 | 2.7 | 20.1 | 0.9 | 22.9 | 4.3 | 73.5 | 46.4 | 0.5 | 26.6 | 22.2 |
| | 1.6 | 77.5 | | | | | | 22.5 | 4.0 | 74.1 | 46.1 | .7 | 27.3 | 21.9 |
| | 5.2 | 78.0 | | | | | | 22.0 | 4.3 | 73.3 | 45.5 | 1.0 | 26.8 | 22.4 |
| | 14.5 | 78.4 | | | | | | 21.6 | 4.2 | 75.7 | 44.4 | 1.4 | 30.0 | 20.1 |
| | 19.4 | 78.0 | | | | | | 22.0 | 4.5 | 74.1 | 43.4 | .6 | 30.0 | 21.4 |
| | 25.3 | 77.7 | 52.9 | .7 | 3.4 | 20.0 | .6 | 22.3 | 5.5 | 74.4 | 43.2 | .7 | 30.5 | 20.1 |
| | 35.5 | 78.9 | | | | | | 21.1 | 5.3 | 73.9 | 41.4 | 2.7 | 30.7 | 20.8 |
| | 45.5 | 76.2 | | | | | | 23.8 | 6.8 | 71.1 | 38.5 | 4.7 | 27.9 | 22.1 |
| | 55.2 | 75.7 | 50.8 | .7 | 3.9 | 19.8 | .6 | 24.3 | 8.2 | 68.3 | 36.4 | 7.5 | 24.4 | 23.5 |
| | 64.7 | 74.2 | | | | | | 25.8 | 10.4 | 64.1 | 33.5 | 11.3 | 19.3 | 25.5 |
| | 79.0 | 71.0 | 46.2 | .9 | 4.4 | 18.8 | .8 | 29.0 | | | | | | |
| | 96.7 | 77.0 | 61.8 | .8 | 4.2 | 9.4 | .9 | 23.0 | | | | | | |
| Brown rot caused by <i>Poria monticola</i> . | 0.0 | 77.1 | 52.3 | 1.1 | 2.7 | 20.1 | .9 | 22.9 | 4.3 | 73.5 | 46.4 | .5 | 26.6 | 22.2 |
| | 4.5 | 75.5 | | | | | | 24.5 | 8.1 | 66.5 | 34.3 | 10.6 | 21.6 | 25.4 |
| | 10.7 | 73.5 | | | | | | 26.5 | 13.7 | 56.8 | 21.1 | 19.1 | 16.6 | 29.5 |
| | 15.3 | 72.7 | | | | | | 27.3 | 17.4 | 50.4 | 14.3 | 22.2 | 13.9 | 32.2 |
| | 20.1 | 71.1 | 50.2 | .6 | 1.6 | 18.2 | .5 | 28.9 | 21.5 | 48.1 | 10.3 | 26.0 | 11.8 | 30.4 |
| | 26.1 | 68.0 | | | | | | 32.0 | 22.7 | 44.3 | 7.5 | 26.5 | 10.3 | 33.0 |
| | 32.6 | 66.8 | | | | | | 33.2 | 24.6 | 44.9 | 5.0 | 30.0 | 9.9 | 30.5 |
| | 44.8 | 59.0 | 40.2 | .4 | 1.3 | 16.6 | .4 | 41.0 | 26.9 | | | | | |
| | 55.0 | 52.2 | | | | | | 47.8 | 32.7 | 32.2 | .0 | 22.1 | 10.1 | 35.1 |
| | 64.4 | 40.8 | | | | | | 59.3 | 40.6 | 21.3 | .0 | 12.1 | 9.2 | 38.1 |
| | 69.5 | 30.2 | 18.0 | .4 | .6 | 11.0 | .3 | 69.8 | 45.6 | 12.4 | .0 | 6.1 | 6.3 | 42.0 |

¹ Determined from sulfuric acid lignin by difference.² Determined from holocellulose plus total extractives by difference.³ Sound wood.

TABLE 9.—Composition of sweetgum sapwood in progressive stages of decay, based on the moisture-free weight of the original sound wood

| Type of decay and causal organism | Average weight loss | Results of the sulfuric acid hydrolysis and chromatographic procedures | | | | | | | Results of the holocellulose procedure | | | | | |
|---|---------------------|--|---------|----------|---------|---------|---------|----------------------|--|---------------|-----------------|----------------|-----------------|-----------------------|
| | | Total carbohydrate ¹ | Glucan | Galactan | Mannan | Xylan | Araban | Sulfuric acid lignin | Total extractives | Holocellulose | Alpha-cellulose | Beta-cellulose | Gamma-cellulose | "Lignin" ² |
| White rot caused by <i>Polyporus versicolor</i> . | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent | Percent |
| | 0.0 | 77.1 | 52.3 | 1.1 | 2.7 | 20.1 | 0.9 | 22.9 | 4.3 | 73.5 | 46.4 | 0.5 | 26.6 | 22.2 |
| | 1.6 | 76.3 | | | | | | 22.1 | 3.9 | 72.9 | 45.4 | .7 | 26.9 | 21.5 |
| | 5.2 | 74.0 | | | | | | 20.9 | 4.1 | 69.5 | 43.1 | .9 | 25.4 | 21.2 |
| | 14.5 | 67.0 | | | | | | 18.5 | 3.6 | 64.7 | 37.9 | 1.2 | 25.6 | 17.2 |
| | 19.4 | 62.9 | | | | | | 17.7 | 3.6 | 59.7 | 35.0 | .5 | 24.2 | 17.3 |
| | 25.3 | 58.0 | 39.5 | .6 | 2.6 | 14.9 | .4 | 16.7 | 4.1 | 55.5 | 32.3 | .5 | 22.8 | 15.0 |
| | 35.5 | 50.9 | | | | | | 13.6 | 3.4 | 47.7 | 26.1 | 1.7 | 19.8 | 13.4 |
| | 45.5 | 41.6 | | | | | | 13.0 | 3.7 | 38.8 | 21.0 | 2.6 | 15.2 | 12.1 |
| | 55.2 | 33.9 | 22.8 | .3 | 1.7 | 8.9 | .3 | 10.9 | 3.7 | 30.6 | 16.3 | 3.4 | 10.0 | 10.5 |
| | 64.7 | 26.2 | | | | | | 9.1 | 3.7 | 22.6 | 11.8 | 4.0 | 6.8 | 9.0 |
| | 79.0 | 14.9 | 9.7 | .2 | .9 | 3.9 | .2 | 6.1 | | | | | | |
| | 96.7 | 2.5 | 2.0 | .03 | .1 | .3 | .03 | .8 | | | | | | |
| Brown rot caused by <i>Poria monticola</i> . | 0.0 | 77.1 | 52.3 | 1.1 | 2.7 | 20.1 | .9 | 22.9 | 4.3 | 73.5 | 46.4 | .5 | 26.6 | 22.2 |
| | 4.5 | 72.1 | | | | | | 23.4 | 7.8 | 63.5 | 32.8 | 10.1 | 20.6 | 24.3 |
| | 10.7 | 65.7 | | | | | | 23.7 | 12.2 | 50.8 | 18.9 | 17.1 | 14.8 | 26.4 |
| | 15.3 | 61.6 | | | | | | 23.1 | 14.8 | 42.7 | 12.1 | 18.8 | 11.8 | 27.3 |
| | 20.1 | 56.8 | 40.1 | .5 | 1.3 | 14.5 | .4 | 23.1 | 17.2 | 38.4 | 8.2 | 20.8 | 9.4 | 24.3 |
| | 26.1 | 50.2 | | | | | | 23.7 | 16.8 | 32.7 | 5.5 | 19.6 | 7.6 | 24.4 |
| | 32.6 | 45.0 | | | | | | 22.4 | 16.5 | 30.3 | 3.4 | 20.2 | 6.7 | 20.5 |
| | 44.8 | 32.6 | 22.2 | .2 | .7 | 9.2 | .2 | 22.6 | 14.9 | | | | | |
| | 55.0 | 23.5 | | | | | | 21.5 | 14.7 | 14.5 | .0 | 9.9 | 4.5 | 15.8 |
| | 64.4 | 14.5 | | | | | | 21.1 | 14.4 | 7.6 | .0 | 4.3 | 3.3 | 13.6 |
| | 69.5 | 9.2 | 5.4 | .1 | .2 | 3.4 | .1 | 21.3 | 13.9 | 3.8 | .0 | 1.9 | 1.9 | 12.8 |

¹ Determined from sulfuric acid lignin by difference.² Determined from holocellulose plus total extractives by difference.³ Sound wood.

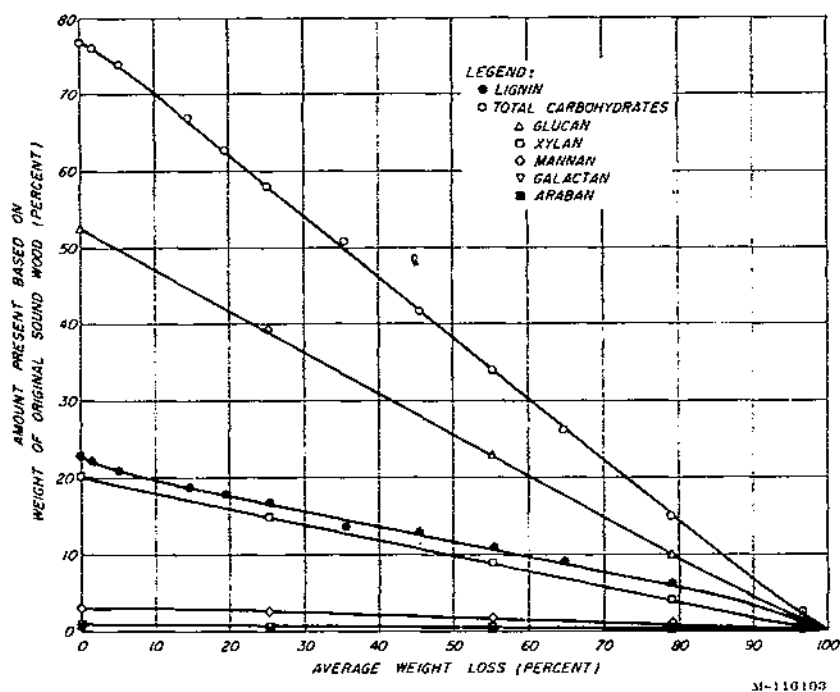


FIGURE 14.—Composition of sweetgum sapwood in progressive stages of decay by the white-rot fungus, *Polyporus versicolor*, as determined by the sulfuric acid hydrolysis and chromatographic procedures.

hydrates rather than of the total carbohydrates in the brown-rotted wood.

With this qualification, the curves of figure 17 indicate very rapid depletion of holo-, alpha-, and gamma-cellulose and accumulation of beta-cellulose in the early stages of decay by *Poria monticola*. After about 20 to 25 percent weight loss, however, the rates of utilization of holo-, alpha-, and gamma-cellulose were reduced and beta-cellulose was utilized at an appreciable rate.

Significance of the Composition Analyses

The changes in composition of sweetgum sapwood induced by the white-rot fungus, *Polyporus versicolor*, were in some respects similar, and in other respects quite different, from the corresponding changes induced by the brown-rot fungus, *Poria monticola*. Both organisms utilized each of the individual sugar polymers of the wood at rates that were essentially constant in all stages of decay and approximately proportional to the amounts present in sound wood. On the other hand, the variable pattern of change in the proportion of alpha-, beta-, and gamma-cellulose in the holocellulose of brown-rotted wood presents a distinct contrast to the uniformity of comparable changes in the wood decayed by *Polyporus versicolor*. Also, *Poria monticola* utilized

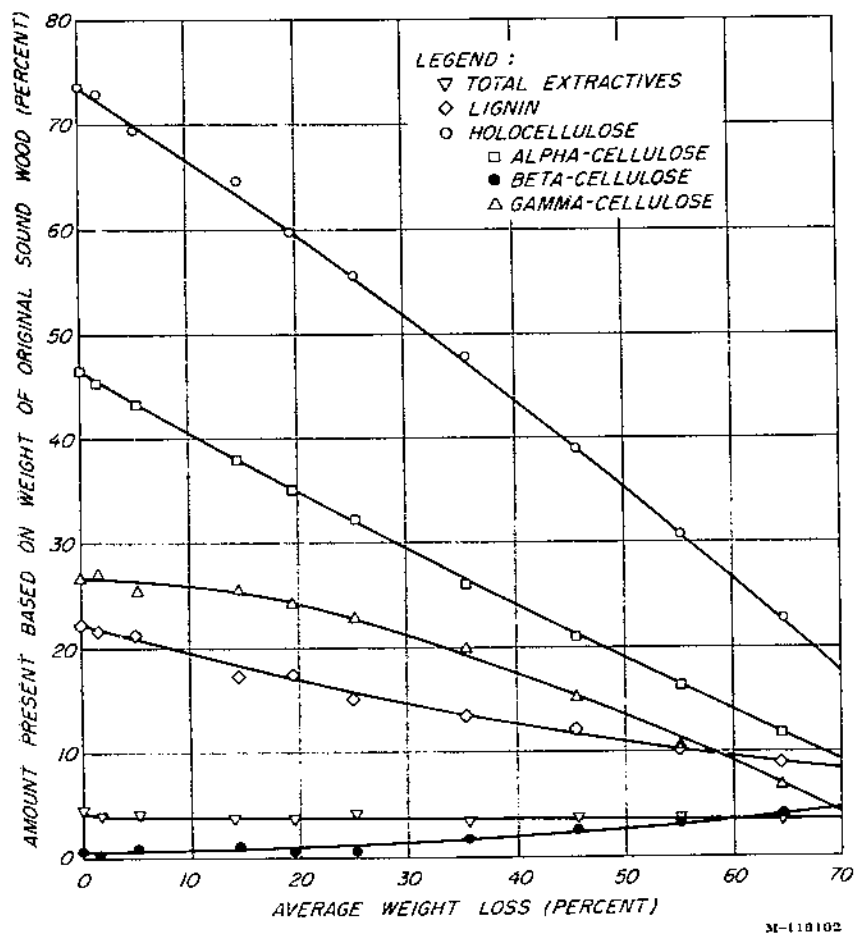


FIGURE 15.—Composition of sweetgum sapwood in progressive stages of decay by the white-rot fungus, *Polyporus versicolor*, as determined by the holocellulose procedure.

lignin only to a minor extent, whereas *Polyporus versicolor* utilized lignin rapidly and at an essentially constant rate in all stages of decay.

The observation of uniformity in the nature of the effects of *Polyporus versicolor* on the major constituents of sweetgum sapwood confirms a similar conclusion drawn from more limited data by Scheffer (67). Utilization of the various sugar polymers at rates that were proportional to the amounts present in sound wood has not been reported previously for brown-rot fungi.

The uniformity in rate of metabolism of the various wood constituents by both test fungi is remarkable in view of the diverse chemical nature of the constituents and their heterogeneous distribution within the wood cell walls. Rapid

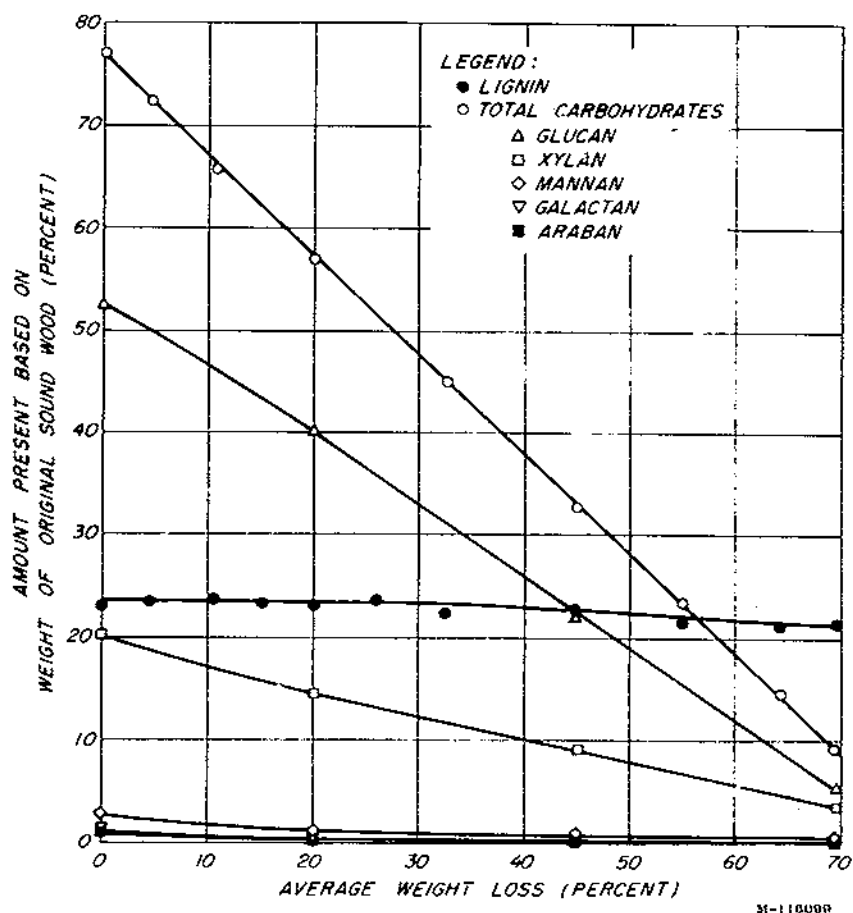


FIGURE 16.—Composition of sweetgum sapwood in progressive stages of decay by the brown-rot fungus, *Poria monticola*, as determined by the sulfuric acid hydrolysis and chromatographic procedures.

utilization of lignin in the early stages of decay by *Polyporus versicolor* is especially difficult to understand, since lignin is concentrated in the compound middle lamella of wood cells.

The very rapid depletion of alpha-cellulose and corresponding accumulation of beta-cellulose in the early stages of decay by *Poria monticola* suggests that the very long alpha-cellulose molecules were depolymerized at random along their length by the carbohydrases of this organism. Such a mechanism has been reported for the isolated cellulolytic enzymes of other fungi (52) and would logically lead to the accumulation of polysaccharide fragments of the intermediate length, beta-cellulose type. On the other hand, the depletion of alpha-cellulose without appreciable increase in the amount of beta- or gamma-cellulose in the wood decayed by *Polyporus versicolor* suggests that this organism

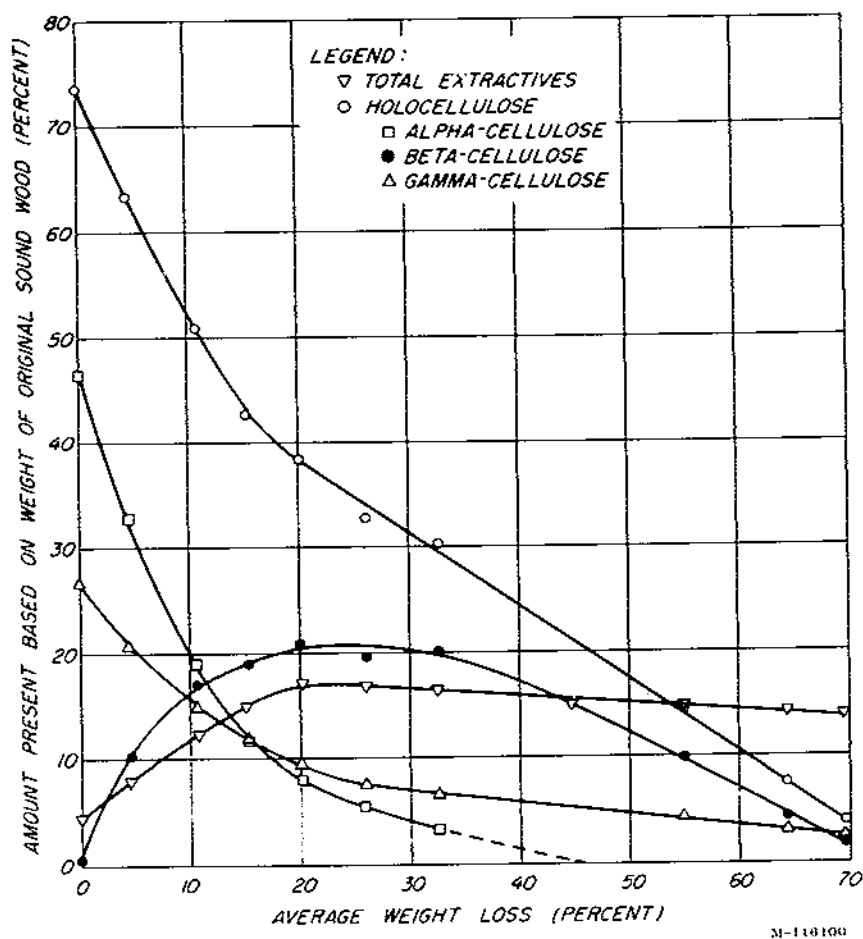


FIGURE 17.—Composition of sweetgum sapwood in progressive stages of decay by the brown-rot fungus, *Poria monticola*, as determined by the holocellulose procedure.

degraded alpha-cellulose to fragments that could be assimilated and respired directly. This could have resulted in two ways: (1) if the carbohydrases of *Polyporus versicolor* very thoroughly depolymerized a small portion of the alpha-cellulose per unit time so that the degradation products could be consumed as rapidly as they were formed; or (2) if the carbohydrases of the organism acted by an endwise mechanism. The first of these alternatives appears most likely in view of the observation by Norkrans and Rånby (52) that the cellulolytic enzymes of other white-rot fungi act by a random mechanism.

The observation that *Poria monticola* did not utilize lignin to an appreciable extent does not mean that the lignin remained unaffected during the decay process. Approximately 2 percent of

the lignin present in sound wood was utilized, as shown by the slope of the lignin curve in figure 16. This slight utilization may have been due to removal of methoxyl groups as suggested by Bray and Andrews (5). Evidence is presented in figures 11 and 13 that the lignin in samples decayed by *Poria monticola* had appreciably greater solubility in water and 1 percent sodium hydroxide than the lignin in sound wood. This latter observation suggests that *Poria monticola* caused at least partial degradation of the lignin. Thus, the failure of this organism to utilize lignin to an appreciable extent may not have been due to its inability to degrade the lignin in sound wood, but rather to its inability to assimilate or to metabolize most of its own lignin degradation products. This suggestion is consistent with the earlier observations of Leopold (40) and Pew (55) regarding the degraded nature of the lignin remaining in brown-rotted wood.

Progressive Changes in Degree of Polymerization of the Holocellulose of the Decayed Wood

The results of the particle-size distribution study, the solubility analyses, and the alpha-, beta-, and gamma-cellulose determinations, suggested that the polysaccharide molecules of the test wood were depolymerized relatively more slowly by the white-rot fungus, *Polyporus versicolor*, than by the brown-rot fungus, *Poria monticola*. This hypothesis was tested directly by a viscosimetric determination of the average degree of polymerization of the holocellulose samples described in the preceding section.

Measurements of intrinsic viscosity provide one means for determining the degree of polymerization (DP) of cellulose. Intrinsic viscosity, $[\eta]$, is defined by the equations:

$$[\eta] = \lim_{C \rightarrow 0} \eta_{sp}/C = \lim_{C \rightarrow 0} \frac{t/t_0 - 1}{C},$$

where η_{sp}/C equals reduced viscosity, t equals the efflux time of a solution of concentration C , and t_0 equals the efflux time of the solvent. DP values calculated from intrinsic viscosity measurements are weight averages. This means that a given number of long molecules will have a greater influence on the average than a similar number of short molecules.

In this study, a modification of the cupri-ethylene diamine disperse viscosity procedure of Straus and Levy (72) and Ostwald-Fenske viscosimeters were used. Intrinsic viscosity was determined by extrapolating to zero concentration a plot of reduced viscosity against concentration of holocellulose. Data for at least four concentrations were plotted. The average DP of the holocellulose was calculated from the intrinsic viscosity by means of the equation $DP = K[\eta]$, with the value 190 for K , as proposed by Conrad and coworkers (17). No corrections were made for rate of shear. The results are given in table 10 and are shown graphically in figure 18.

TABLE 10.—*Intrinsic viscosity and degree of polymerization of holocellulose prepared from sweetgum sapwood in progressive stages of decay*

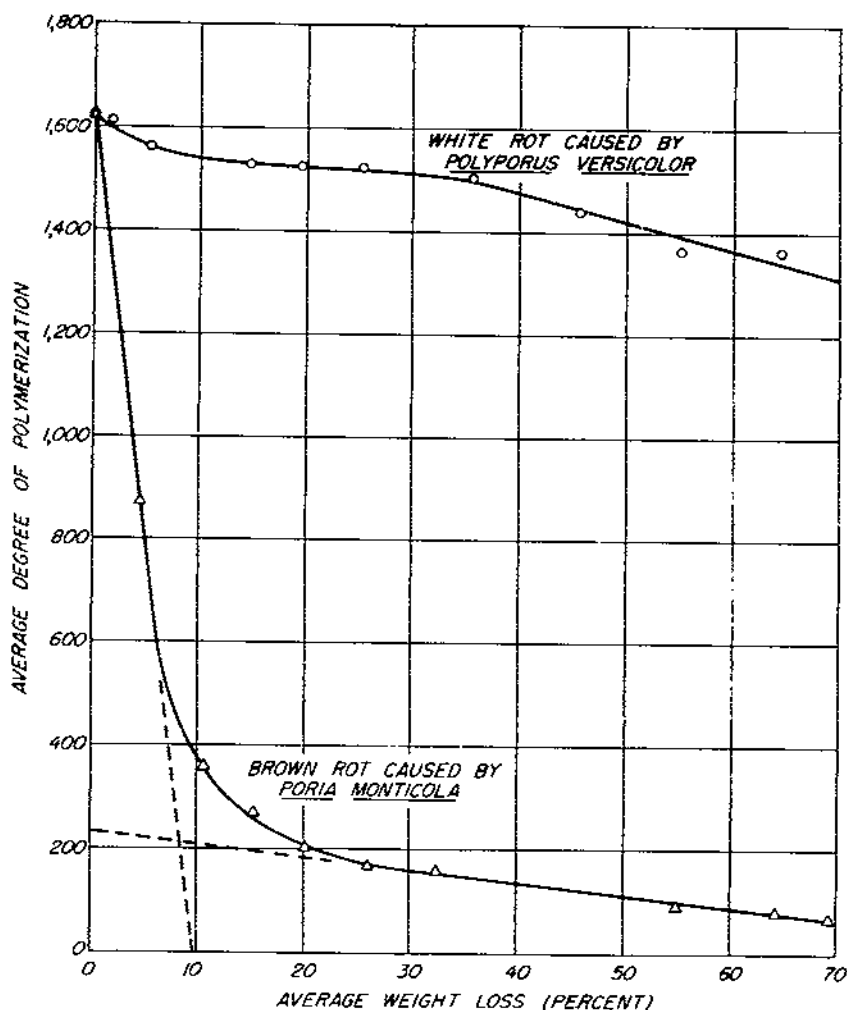
| Type of decay and causal organism | Average weight loss | Intrinsic viscosity $[\eta]$ | Average degree of polymerization ¹ (DP) |
|---|---------------------|------------------------------|--|
| | Percent | | |
| White rot caused by <i>Polyporus versicolor</i> . | ±0.0 | 8.6 | 1,635 |
| | 1.6 | 8.5 | 1,615 |
| | 5.2 | 8.2 | 1,560 |
| | 14.5 | 8.05 | 1,530 |
| | 19.4 | 8.0 | 1,520 |
| | 25.3 | 8.0 | 1,520 |
| | 35.5 | 7.9 | 1,500 |
| | 45.5 | 7.55 | 1,435 |
| | 55.2 | 7.15 | 1,360 |
| | 64.7 | 7.15 | 1,360 |
| Brown rot caused by <i>Poria monticola</i> . | ± 0 | 8.6 | 1,635 |
| | 4.5 | 4.6 | 875 |
| | 10.7 | 1.9 | 360 |
| | 15.3 | 1.4 | 265 |
| | 20.1 | 1.1 | 210 |
| | 26.1 | .90 | 170 |
| | 32.6 | .85 | 160 |
| | 44.8 | | |
| | 55.0 | .50 | 95 |
| | 64.4 | .42 | 80 |
| | 69.5 | .36 | 70 |

¹ Calculated from intrinsic viscosity, $[\eta]$, by means of the equation $DP = K[\eta]$, with the value 190 for K , proposed by Conrad and coworkers (17).

² Sound wood.

Figure 18 shows the strikingly different patterns of progressive change in average degree of polymerization (DP) of the holocellulose induced by the two test fungi. *Polyporus versicolor* caused a very slow, gradual change in average DP, whereas the effect of *Poria monticola* was at first very rapid, and then similarly gradual, and linear. Even in the most advanced stage of decay, the average DP of the white-rot holocellulose was 1,360 DP units, or 83 percent of the sound wood value of 1,635. This same stage of depolymerization occurred in brown-rot at an estimated weight loss of only 1.5 percent. The average DP of the brown-rot holocellulose reached 70 DP units, or 4 percent of the sound wood value, at 70 percent weight loss.

The average DP values shown in figure 18 would have been somewhat lower, especially in the brown-rotted material, if the carbohydrates removed during the extractions prior to the



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FIGURE 18.—Average degree of polymerization of the holocellulose of sweetgum sapwood in progressive stages of decay. Extrapolation of the rapidly descending portion of the curve to zero DP indicates the proportion of the wood easily hydrolyzable by the carbohydrases of *Poria monticola*. Extrapolation of the gradually descending portion of the curve to zero weight loss indicates the average DP of the resistant (presumably crystalline) fraction of the holocellulose.

holocellulose determinations had been present in the samples submitted to the viscosity determinations.

The differences between the curves in figure 18 reflect pronounced differences in the rate of depolymerization of the wood carbohydrates during decay by the two test fungi. These differences in rate are in agreement with the hypothesis suggested by the solubility and composition analyses, and earlier by Pechmann and Schaile (59).

The gradual decrease in average DP during all stages of decay by *Polyporus versicolor* suggests that the carbohydrases of this organism either (1) selectively attacked only a small part of the holocellulose at a time, so that the average degree of polymerization of the molecules remaining at a given stage of decay was not greatly affected; or (2) depolymerized the holocellulose molecules by an endwise mechanism. Since the cellulolytic enzymes of other white-rot fungi cleave cellulose molecules by a random mechanism (52), the first alternative appears most reasonable.

The gradual decrease in average DP shown in figure 18 also suggests that the carbohydrases of *Polyporus versicolor* did not act preferentially in the amorphous regions of the wood cell walls as has been reported for isolated cellulolytic enzymes of other fungi by Norkrans (51) and Walseth (83). Cellulose molecules are in general much longer than individual crystallites so that they traverse several crystalline and amorphous regions. Thus, preferential hydrolysis of the amorphous polysaccharides would necessarily have led to a rapid reduction of the average DP of the holocellulose, such as that shown by the lower curve in figure 18.

The curve in figure 18 for the brown-rotted wood is very similar to those reported for the hydrolysis of cellulose by isolated cellulolytic enzymes (51, 83) and by strong mineral acids (44), except that in the latter two cases the tendency for a true "leveling off DP" is even more pronounced. The similarity in these curves confirms the early suggestion of Campbell and Booth (12) that decay of the brown-rot type may be considered analogous to acid hydrolysis. It also suggests that the factors that have been shown to account for the shape of curves of this type for enzymatic and acid hydrolysis may be used as a precedent for the interpretation of the curve in figure 18 as well.

The rapid initial decrease in average DP of the brown-rot holocellulose suggests that the more accessible, amorphous carbohydrates were hydrolyzed preferentially in the initial stages of decay by *Poria monticola*. Extrapolation of the initial linear portion of the curve to zero DP indicates that the fraction of sweetgum sapwood that was readily hydrolyzed by this organism constituted about 10 percent of the original wood substance. This estimate is in substantial agreement with estimates of the proportion of amorphous cellulose in several native cellulosic materials as determined by Millett and coworkers (44). Extrapolation from only two data points in figure 18, however, makes the significance of this coincidence somewhat uncertain.

The more gradual decrease in average DP after 20 percent weight loss suggests that the less accessible crystalline cellulose was degraded primarily in later stages of decay by *Poria monticola*. Extrapolation of the second linear phase of the DP curve to zero weight loss indicates that the more resistant fraction of the holocellulose consisted of entities with an average length of about 230 DP units. This value is in substantial agreement with estimates of the length of crystallites in other cellulosic materials as determined by acid hydrolysis (44). The small difference in the two estimates may have resulted from differences in the size of the catalytic agents involved, as

suggested by Norkrans and Rånby (52). The very small hydronium ions may be able to penetrate and act upon parts of the amorphous structure of the wood that are inaccessible to the much larger cellulolytic enzyme molecules.

The rapid initial decrease in average DP during decay by *Poria monticola* indicates that the carbohydrases of this organism split the polysaccharide molecules of the wood by a random mechanism. The more gradual decrease in average DP after 20 percent weight loss, however, indicates that the more resistant, presumably crystalline, fraction of the holocellulose was hydrolyzed, at least partially, by a mechanism that proceeded parallel to the major axis of the crystallites. The continual decrease in average DP during decay by *P. monticola* is in distinct contrast to the true "leveling off DP" observed in acid hydrolysis (44), and with isolated cellulolytic enzymes by Reese and coworkers (60, 62).

The extreme rapidity of DP change before 10 percent weight loss due to decay by *Poria monticola* also suggests that the depolymerization proceeded rapidly throughout the wood cell walls. It is almost inconceivable that so rapid a decrease in average DP could have been achieved without affecting the bulk of the total carbohydrate fraction of the wood. This suggestion is reinforced by the fact that estimates of average DP from viscosity measurements are weight averages, where long molecules have a greater influence on the average than a corresponding number of short ones.

Progressive Changes in Hygroscopicity of the Decayed Wood

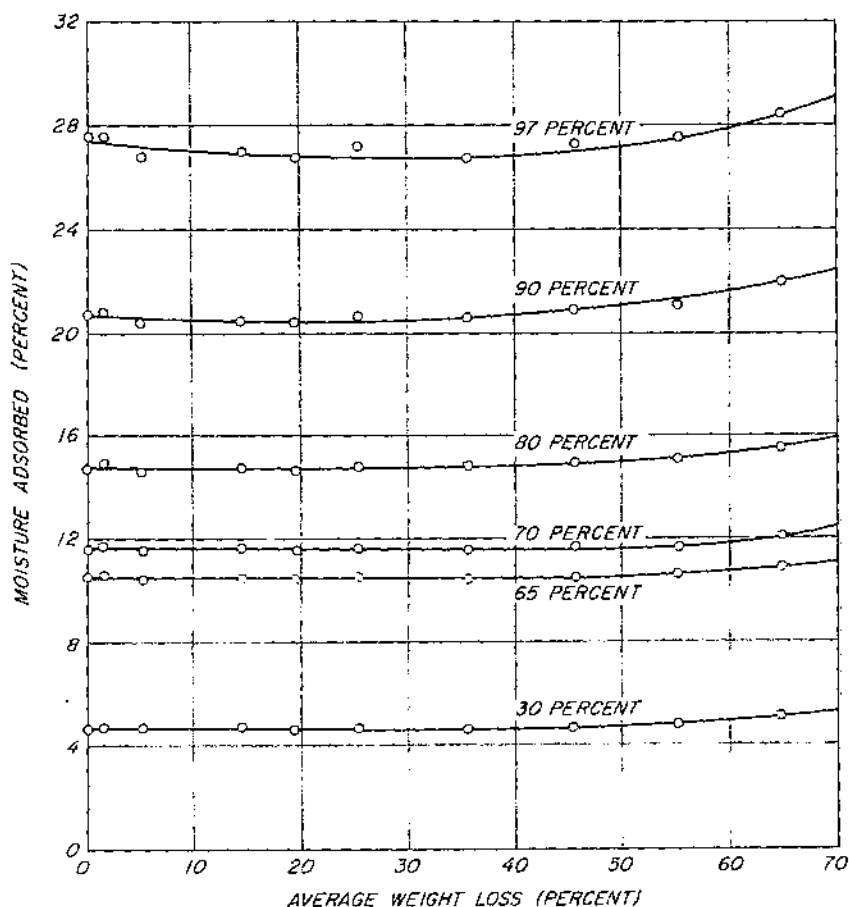
The progressive changes in hygroscopicity of the decayed wood were determined in order to ascertain the effects of decay on the degree of crystallinity of the wood cellulose, and the fundamental moisture adsorptivity of the decayed-wood constituents.

Since moisture is adsorbed largely in the amorphous regions of the cell walls, the amount of moisture adsorbed by wood is inversely related to the degree of crystallinity of the cellulose. The hygroscopicity of wood also is influenced by its composition. The major constituents of wood may be listed in the following order of decreasing moisture adsorptivity: hemicellulose, cellulose, and lignin (16). These differences reflect, in part, the relatively lower concentration of hydroxyl groups in lignin as compared to the carbohydrates, and the partial crystallinity of the cellulose as opposed to the amorphous nature of the hemicelluloses.

The progressive changes in hygroscopicity of the decayed wood in this study were determined on 1-gram samples of the wood meal prepared for the chemical analyses. Differences in external sorbing surface of the samples due to differences in their particle-size distribution (see table 5, p. 26) were considered negligible in comparison with the magnitude of their internal sorbing surfaces (71). Each sample was placed in a tared weighing bottle, and its moisture-free weight was determined after it was dried to constant weight under vacuum over magnesium perchlorate.

The samples were then equilibrated, successively, with atmospheres in humidity rooms controlled at $26.5^{\circ} \pm 0.5^{\circ} \text{C}$. and 30, 65, 70, 80, 90, and 97 (each ± 2) percent relative humidity. Equilibrium weights were determined at each relative humidity after the samples had reached constant weight. After each weighing the samples were thoroughly shaken in their weighing bottles to avoid packing during handling and to break up possible moisture gradients. The equilibrium moisture contents measured for the samples at each relative humidity were expressed as percentages of their respective moisture-free weights. The results are presented in figures 19, 20, and 21.

As shown in figure 19, no change in hygroscopicity of the wood

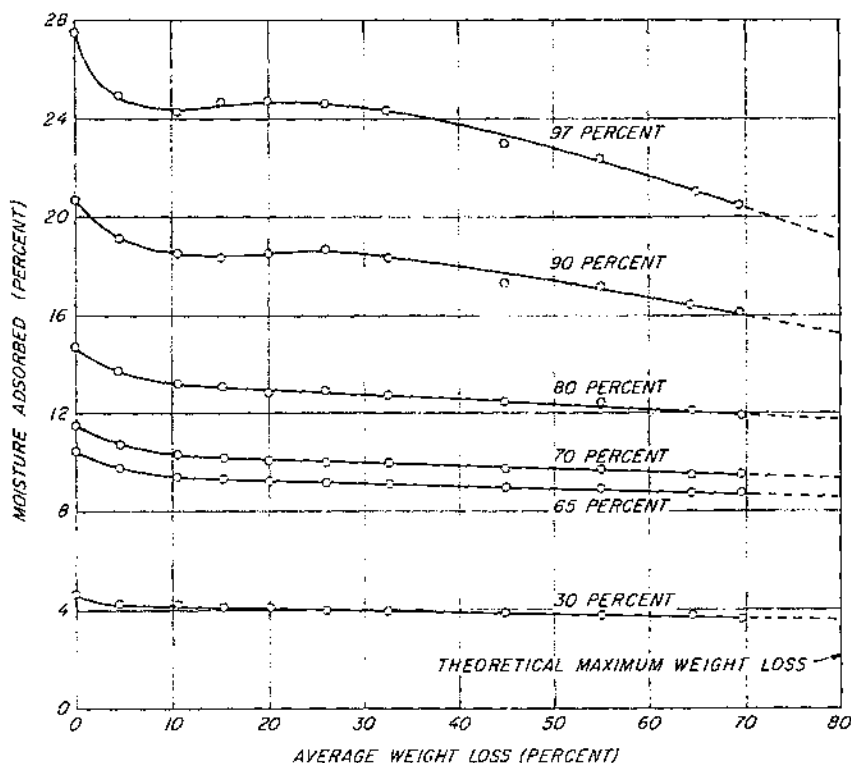


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FIGURE 19.—Moisture adsorbed at 26.5°C . and various relative humidities by sweetgum sapwood in progressive stages of decay by the white-rot fungus, *Polyporus versicolor*. Moisture adsorbed by each sample is expressed as a percentage of the moisture-free weight of the decayed sample.

due to decay by *Polyporus versicolor* was observed before 40 percent weight loss. This observation is in contrast to the consistently lower hygroscopicity reported for white-rotted wood by Scheffer (67) and Komarov (36). At higher weight losses, there was a significant increase in moisture adsorptivity. In view of the essentially uniform composition of the white-rotted wood (see table 8, p. 42), this increase may have been due to diminution in the amount of crystalline material in the wood. The X-ray diffraction analyses described in the following section confirm this suggestion.

The hygroscopicity changes shown in figure 20 for the wood decayed by *Poria monticola* present a marked contrast to those induced by *Polyporus versicolor*. In the brown-rotted wood, moisture adsorptivity at relative humidities below 80 percent diminished at first rapidly and then in a more gradual and linear manner. This reduction in hygroscopicity is in agreement with the



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FIGURE 20.—Moisture adsorbed at 26.5° C. and various relative humidities by sweetgum sapwood in progressive stages of decay by the brown-rot fungus, *Poria monticola*. Moisture adsorbed by each sample is expressed as a percentage of the moisture-free weight of the decayed sample. Extrapolation of the various curves to the theoretical maximum weight loss of 80 percent indicates the probable amount of moisture adsorbed by the lignin residue in brown-rotted wood.

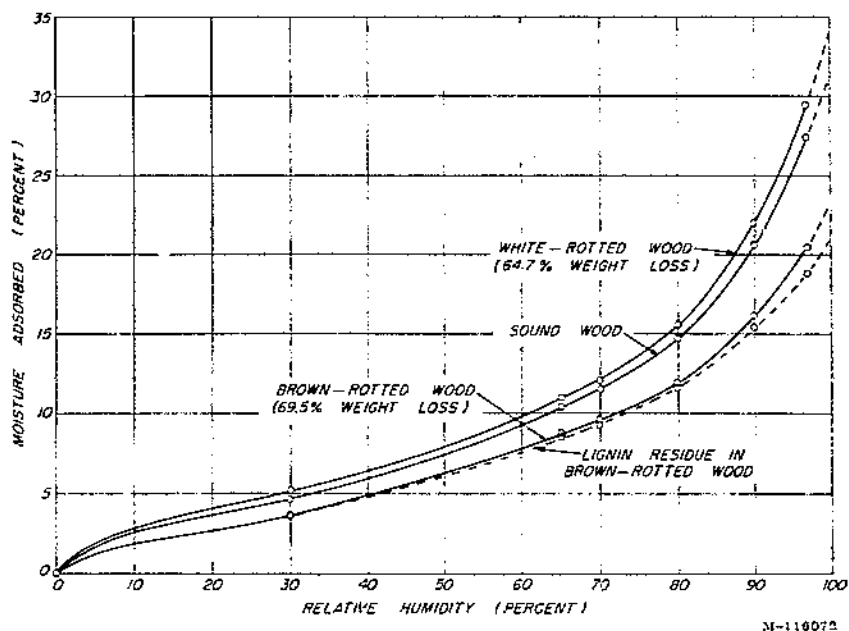


FIGURE 21.—Moisture-adsorption curves for sound and decayed sweetgum sapwood at 26.5° C. Moisture adsorbed by each sample is expressed as a percentage of the moisture-free weight of the decayed sample. Extrapolation of the curves to 100 percent relative humidity indicates the approximate fiber saturation point of the respective samples.

observations of Buro (7) and Komarov (36). The rapid initial drop may have been due to preferential attack on amorphous cellulose, which lead to the accumulation of less hygroscopic crystalline cellulose. This inference also is supported by the degree of polymerization data shown in figure 18.

The more gradual and linear diminution in hygroscopicity after about 15 percent weight loss may have been due to gradual accumulation of lignin as a result of preferential attack on the wood carbohydrates by this organism (see table 8). The materials that analysis indicated were beta-cellulose apparently had little influence on the hygroscopicity of the decayed wood. This is shown by the linearity of hygroscopicity changes in those stages of decay after 15 percent weight loss, during which the amount of beta-cellulose reached a maximum and then diminished.

At 90 and 97 percent relative humidity, a slight increase in hygroscopicity followed the initial rapid decrease. This increase is similar, although much smaller in magnitude, to that observed by Millett and coworkers (48) for cellulosic materials in progressive stages of hydrolysis by constant-boiling hydrochloric acid. It is not known why this inflection was not observed at lower relative humidities.

On the assumption that the slope of the linear part of the curves of figure 20 was due primarily to accumulation of less hygroscopic

lignin, the curves were extrapolated to 80 percent weight loss (100 minus the percent lignin content of highly decayed sweetgum sapwood). These extrapolated values indicate the probable moisture adsorptivity of the carbohydrate-free lignin residue that remains after decay by *Poria monticola*. The product of these extrapolated values and the proportion of lignin in sound wood, divided by the moisture adsorptivity of the sound wood at the same relative humidity, gave values in substantial agreement with the theoretical hygroscopicity calculated for lignin in sound wood by Christensen and Kelsey (16). The observed values ranged from 16.2 to 18.0 percent, depending on the relative humidity at which the comparison was made; the theoretical values were equal to or greater than 16 percent. The observed values are in much better agreement with the theoretical values than are those for isolated sulfuric acid and "native" lignins reported by Christensen and Kelsey (16) and Runkel and Lüthgens (64).

The moisture adsorptivity shown in figures 19 and 20 for the samples in the most advanced stages of both types of decay, for the sound wood, and the lignin residue in brown-rotted wood, were plotted in figure 21 against relative humidity. Differences between these moisture adsorption curves indicate the direction and magnitude of the deviation in hygroscopicity of the severely decayed wood from that of sound wood. If it is assumed that the difference in moisture adsorbed at 30 percent relative humidity was proportioned to the adsorbing area in each sample (A. J. Stamm, personal communication), the changes in area were about 10, 20, and 22 percent of the sound wood value in the white-rot, brown-rot, and decay-lignin samples, respectively.

Extrapolation of the curves of figure 21 also indicates that the fiber saturation point (moisture content in equilibrium with 100 percent relative humidity) for sweetgum sapwood was changed from a sound-wood value of about 31 percent to 23 in the advanced brown rot and 34 percent in the advanced white rot. The data in figures 19 and 21 for the white-rotted material confirm the generalization that the residues remaining after various stages of white rot are not greatly different from sound wood.

The moisture-adsorption data of figures 20 and 21 support the suggestion made earlier that the amorphous regions of the cellulose were attacked preferentially in the early stages of brown rot, and that a gradual and much slower utilization of the relatively less hygroscopic crystalline materials took place thereafter.

Progressive Changes in the X-Ray Diffraction Patterns of the Decayed Wood

The results of the hygroscopicity and degrees of polymerization determinations suggested that the brown-rot fungus, *Poria monticola*, preferentially hydrolyzed the amorphous cellulose of sweetgum sapwood. They also suggested that the white-rot fungus, *Polyporus versicolor*, attacked the amorphous and crystalline cellulose simultaneously, but in such a way that the

hygroscopicity of the decayed wood was increased in advanced stages of decay. Both fungi would thus be expected to alter either the relative amount or degree of parallelism of the crystalline material present. This hypothesis was tested directly by X-ray diffraction analyses applied to the following selected samples: undecayed wood, two stages of white rot—35.5 and 55.2 percent weight loss—and three stages of brown rot—10.7, 32.6, and 69.5 percent weight loss. Part of the material in each of these weight-loss classes was further ground to pass an 80-mesh screen before it was analyzed.

The Bragg theory of X-ray diffraction proposes that X-radiation incident upon a crystalline material is diffracted by the planes of the crystal, much in the manner that light is diffracted by a diffraction grating. In polyphasic systems of minute crystallites separated by amorphous materials, such as occur in wood, X-ray diffraction patterns can be obtained most conveniently on a finely divided sample in which a large number of small crystallites are randomly oriented with respect to one another. Each of these randomly oriented crystallites diffracts an incident beam of X-rays in a characteristic manner that varies with the angle of incidence of the beam. If the finely divided sample is rotated in an X-ray beam, and the sum of the diffractions caused by its component crystallites is recorded at a series of angles from the incident beam, a diffractometer pattern is obtained. The form of these patterns, obtained for a series of comparable samples, may then be used to give a relative measure of the amount and degree of parallelism of the crystalline material in each sample in the series.

The various peaks in an X-ray diffraction pattern are characteristic of certain interatomic distances. If such distances are relatively common, as in highly crystalline materials, the signal intensity is high. Thus the height of the peak is an index of the relative amount of crystalline material present. If the crystalline material is of a low order of perfection (parallelism), there is some variation in the interatomic distances, and the peak is correspondingly broad. The breadth of the peak is thus an inverse index of the degree of parallelism of the crystalline phase.

The diffractometer patterns obtained in this study are shown in figure 22. Pattern A is for the sound wood and is the basis for comparison of patterns B and C for the white-rotted material, and patterns D, E, and F for the brown-rotted material. The peak in each pattern at 22° indicates the relative proportion and degree of parallelism of the crystalline fraction of the wood.

Comparison of the amplitude and width of the peak at 22° in patterns A, B, and C indicates a progressive decrease in amount of crystalline material, with little change in degree of parallelism of the crystallites present, as the extent of white rot increased. The change in amount of crystalline material was most pronounced between 35 and 55 percent weight loss (patterns B and C). This observation is in agreement with expectations from the hygroscopicity measurements and an earlier report by Cartwright and Findlay (15).

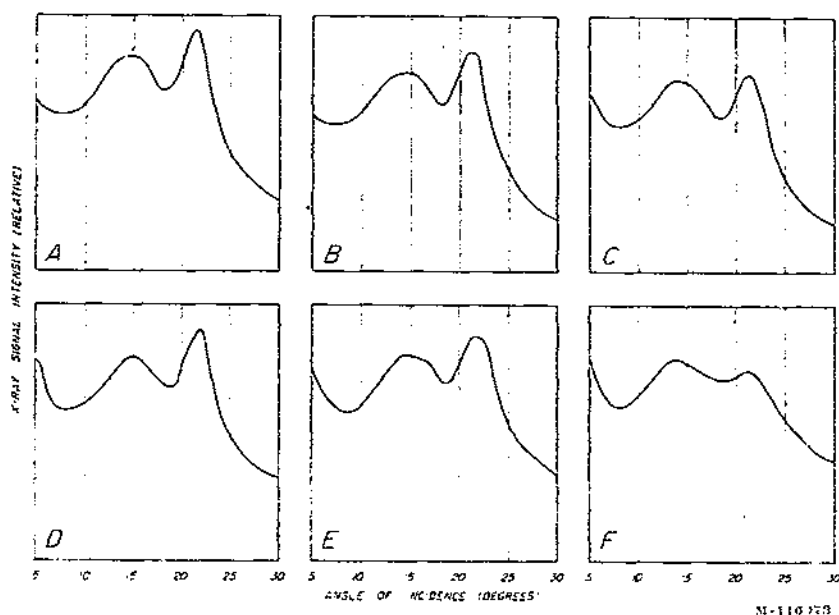


FIGURE 22.—X-ray diffractometer patterns for selected samples of sweetgum sapwood in progressive stages of decay by the white-rot fungus, *Polyporus versicolor*, and the brown-rot fungus, *Poria monticola*. Pattern A is for the undecayed wood; patterns B and C are for the white-rotted material at 35.5 and 55.2 percent weight loss, respectively; patterns D, E, and F are for the brown-rotted material at 10.7, 32.6, and 69.5 percent weight loss, respectively.

Comparison of patterns A, D, E, and F for the brown-rotted material indicates a progressive decrease in amount of crystalline material with little change in degree of parallelism of the crystallites, except possibly at the most advanced stage of decay. The observation of a diffuse pattern at the most advanced stage of brown rot confirms the earlier observation of Schulze and coworkers (68).

Interpretation of these changes, like those of the hygroscopicity determinations, is complicated by the gross changes in composition of the decayed wood, as shown in table 8. The observed increase in ratio of presumably amorphous beta- and gamma-cellulose and lignin to the potentially crystalline alpha-cellulose would be expected to render the diffraction patterns more diffuse. At the earliest stages of brown rot, however, differences in composition were less pronounced.

Since the sample at 10 percent weight loss showed distinctly lower hygroscopicity than the control, it was anticipated that it would also show a higher degree of crystallinity. However, pattern D for this sample is essentially identical with pattern A. This similarity indicates either that no net increase in ratio of crystalline to amorphous material occurred, or that the opposing effects of enrichment in lignin and beta-cellulose and decrease in amorphous cellulose compensated each other.

Microscopical Observations of the Decayed Wood

An attempt was made to correlate the progressive changes in chemical composition noted in the foregoing sections of this paper with changes in the microscopically visible structure of the wood blocks in the same weight-loss classes.

Tangential, radial, and transverse sections of test blocks in each weight-loss class were prepared for light microscopy. Blocks in the initial stages of decay were sectioned without being embedded; those with weight losses above 40 percent in the case of the white-rotted wood, and above 20 percent in the case of the brown-rotted material, were sectioned after they were embedded in a commercial, water-soluble, waxlike material. The sections were cut 6 to 30 microns in thickness, and were examined unstained or after being stained with Pianezze IIIb (80). Phase-contrast, polarized, and bright-field illumination by transmitted light were used at magnifications up to 1,250 diameters.

Sections of sound wood and the material decayed by *Polyporus versicolor* to 25 percent weight loss were examined in the electron microscope. The specimens were stained in osmium tetroxide, dehydrated in ethanol, impregnated with monomeric n-butyl methacrylate, and polymerized at 45° to 50° C. according to the method of Newman and coworkers (45, 46). The embedded material was then sectioned with glass knives, at 0.025 micron thickness, according to the method of Latta and Hartman (39).

The following generalizations were drawn from these studies: In the wood decayed by *Polyporus versicolor*, the hyphae were abundant only in the wood rays until 25 percent weight loss. Up to this stage of decay, the cell walls were penetrated almost exclusively through bordered and simple pit pairs so that bore holes were very sparse. Bore holes became progressively more numerous and large (up to 8 or 10 microns in diameter) in samples above 40 percent weight loss. At no stage of decay, however, were either bore holes or hyphae in longitudinal cells as numerous as in the wood decayed by *Poria monticola*.

Erosion of the pit borders and gradual thinning of the walls of the fiber tracheid cells from the lumen toward the middle lamella were evident in specimens with weight losses from about 10 to 65 percent. This gradual thinning was equally evident in cells that contained hyphae and those in which none could be found. This general thinning pattern of dissolution of the cell-wall substance would account for the utilization of the carbohydrates of the wood. It does not adequately explain the utilization of lignin, however, especially in the early stages of decay, since this constituent is concentrated in the compound middle lamella. Despite diligent examination of several hundred sections of white-rotted wood at the highest magnifications available with the light microscope, the only evidence of attack on the compound middle lamella observed at any stage of decay was hyphal penetration of pit membranes.

Several sections of the wood decayed to 25 percent weight loss by the white-rot fungus, *Polyporus versicolor*, were examined in the electron microscope. This examination revealed that the

general thinning of cell walls observed in the light microscope was not uniform, either in all cells or in all regions of a given cell wall. Figure 23 shows the appearance of typical undecayed fiber tracheid cells. Figure 24 shows the general thinning pattern of dissolution of the cell wall material (at left). The light area evident in the outer region of the cell wall (at right) indicates



FIGURE 23.—Electron micrograph of a cross section of sweetgum sapwood showing the normal structure of contiguous fiber tracheid cells and a bordered pit pair. The micrograph was taken at a magnification of 1,700 times and enlarged to 8,140 times.



FIGURE 24.—Electron micrograph of a cross section of sweetgum sapwood decayed by *Polyporus versicolor* to 25 percent weight loss. Note general thinning of the fiber tracheid wall at left, evidence of internal dissolution of the cell wall at right, and hyphae in both cell lumens. The micrograph was taken at a magnification of 3,200 times and enlarged to 9,340 times.

removal of material from the interior regions of the cell wall. Two hyphae are also shown in cross section in this figure.

Figure 25 shows the general thinning pattern of dissolution of the cell-wall material (at right), as well as a distinct increase in porosity of the secondary walls and compound middle lamella of two contiguous fiber tracheid cells. This porous pattern of removal of the wood substance during decay by *Polyporus versicolor* was evident in many of the cells of the decayed wood. The pores usually developed first near the lumens, and in many cases appeared to precede the general thinning of the cell wall from the lumen toward the middle lamella. The dimensions of these cell-wall pores were highly variable, as shown in figure 25, but they were in general 0.1 to 2 microns in diameter.



FIGURE 25.—Electron micrograph of a cross section of sweetgum sapwood decayed by *Polyporus versicolor* to 25 percent weight loss. Note porous pattern of dissolution of the secondary walls and compound middle lamella and general thinning of the fiber tracheid wall at right. The micrograph was taken at a magnification of 3,200 times and enlarged to 12,000 times.

The pores shown in the compound middle lamella of the cells depicted in figure 25 were in greater abundance than was typical in the sections examined at 25 percent weight loss. Pores in this region were evident with some regularity, however, and together

with the destruction of pit membranes suggest definite removal of material from this lignin-rich layer. Too few sections were examined to ascertain the extent to which lignin utilization could be accounted for by digestion of the compound middle lamella substance.

In the wood decayed by *Poria monticola*, the hyphae ramified through the wood elements, usually as individual filaments. The hyphae penetrated the entire block before 5 percent weight loss, largely by way of bordered and simple pit pairs. Bore holes were formed with increasing frequency after this stage of decay. The few hyphal tips that were found in the process of bore-hole formation measured about 0.5 micron in diameter. The bore holes were subsequently enlarged, but rarely exceeded 4 to 5 microns in diameter. The amount of mycelium increased in both the longitudinal and ray cells until about 25 percent weight loss. After this stage of decay, the amount of mycelium remained about the same or decreased somewhat. Bore holes were the only evidence of cell-wall deterioration visible in the light microscope at any stage of decay.

The volume of wood substance removed during bore-hole formation appeared wholly inadequate to account for the weight losses sustained by the blocks at any stage of decay. An estimate of bore-hole volume for the samples at 25 percent weight loss was obtained by measuring the number and average dimensions of bore holes in 50 randomly selected areas in tangential and radial sections. This determination showed that bore holes could account for only about 1.5 percent weight loss, or 6 percent of the total loss observed. This observation suggested that sub (light) microscopical voids must be created during decay by *Poria monticola*, as has been shown by Meier (42) for two other brown-rot fungi. The existence of these voids was confirmed in the electron microscope, but too few sections were examined in this way to indicate average dimensions for the voids.

General Conclusions and Discussion of the Results

The general conclusions that may be drawn from the diverse experimental results obtained in this study are enumerated in the following sections, together with the evidence upon which each conclusion is based. The points of evidence are presented in the order of the strength of support they provide for the conclusion. Reference is made to the figures and tables of the text that show each point of evidence. Although this method of presentation requires some repetition, the diverse nature of the experimental phases of this study make it necessary in the interest of clarity.

Also included in the following sections are certain suggestions that are consistent with the results of this study but not proved to be true by them. These suggestions are submitted as working hypotheses for possible future investigations into the fundamental biochemistry of the white- and brown-rot types of wood decay.

A final section of this discussion of experimental results offers an explanation for several of the differences between white- and brown-rotted wood that were enumerated earlier in this bulletin.

Utilization of Major Cell-Wall Constituents

The white-rot fungus, *Polyporus versicolor*, utilized each of the major constituents of sweetgum sapwood in all stages of decay. The rate of utilization of each constituent was essentially constant in all stages of decay, and approximately proportional to the amount present in sound wood. These conclusions are demonstrated by direct measurement of the amount of each constituent present in samples in progressive stages of decay by *P. versicolor* (figs. 14 and 15).

In all stages of decay by *Polyporus versicolor*, the wood constituents were depolymerized only about as rapidly as the depolymerization products were converted to volatile products of respiration. This conclusion is supported by the following evidence: (1) the very gradual decrease in average degree of polymerization of the holocellulose (fig. 18); (2) the uniform rate of loss of holc- and alpha-cellulose without corresponding accumulation of beta- or gamma-cellulose (fig. 15); (3) the uniformly low solubility of the decayed wood in all of the solvents used in the solubility analyses (fig. 8); and (4) the uniform rate of loss of lignin from the wood (figs. 14 and 15) and lack of corresponding increase in the amounts of apparent or sulfuric acid lignin in the water and 1 percent alkali extracts (figs. 11 and 13).

The residue that remained after various stages of decay by *Polyporus versicolor* had substantially the same composition and many physical properties in common with sound wood. This conclusion is supported by the same points of evidence that support the preceding two conclusions and also by (1) the uniform resistance of the wood to fragmentation during grinding (table 5); (2) the relatively slight changes in hygroscopicity of the decayed wood (figs. 19 and 21); and (3) the slight changes in X-ray diffraction patterns of the decayed wood (fig. 22).

The brown-rot fungus, *Poria monticola*, primarily utilized the carbohydrates of sweetgum sapwood; lignin was metabolized only to a minor extent. The rate of utilization of each sugar polymer present in the wood remained essentially constant in all stages of decay, and it was approximately proportional to the amount present in sound wood. The alpha-, beta-, and gamma-cellulose fractions of the holocellulose were utilized, each at a different rate in all stages of decay. Each of these conclusions is supported by direct measurement of the amount of each constituent present in the wood in progressive stages of decay by *P. monticola* (figs. 16 and 17).

In the initial stages of decay by *Poria monticola*, the wood constituents were depolymerized much more rapidly than the degradation products were converted to volatile products of respiration. This conclusion, as applied to the wood carbohydrates, is supported by the following evidence: (1) the extreme rapidity of decrease in average degree of polymerization of the holocellulose (78-percent decrease in average DP after only 10 percent weight loss, fig. 18); (2) the rapid decrease in alpha-cellulose and accompanying increase in beta-cellulose content of the wood

(fig. 17); and (3) the rapid accumulation in the wood of materials extractable in all the solvents used in the solubility analyses (fig. 8). The validity of this conclusion as applied to lignin is demonstrated by the very slight loss of lignin from the wood (fig. 16) and the accompanying increase in amount of apparent and sulfuric acid lignin in the water and 1 percent alkali extracts (figs. 11 and 13).

After about 20 to 30 percent weight loss due to decay by *Poria monticola*, the carbohydrates of the wood were depolymerized much less rapidly than the previously formed degradation products were converted to volatile products of respiration. This conclusion is supported by the following evidence: (1) the gradual decrease in average degree of polymerization of the holo-cellulose (8-percent decrease in average DP between 20 and 70 percent weight loss, fig. 18); (2) the decreasing amount of materials extractable from the decayed wood by the various solvents used in the solubility analyses (fig. 8); and (3) the rapid decrease in beta-cellulose content of the wood without corresponding increase in gamma-cellulose content (fig. 17).

Uniformity in the rates at which *Polyporus versicolor* utilized all major cell-wall constituents and *Poria monticola* metabolized the various sugar polymers is remarkable for several reasons: (1) the lignin and various sugar polymers of the wood are very different chemically and are probably metabolized by very different biochemical pathways; (2) the enzymes that catalyze the various steps in the metabolism of each wood constituent probably differ appreciably as to amounts produced and relative rates of activity, so that differences in overall rates of metabolism would logically be anticipated; and (3) the distribution of lignin and each sugar polymer within the wood cell walls is quite distinct, so that differences in accessibility to extracellular enzymes released by the decay fungi in the lumens of the wood cells could be expected.

For these reasons, it appears unreasonable that the uniformity in rate of metabolism of the various wood constituents by either test fungus could have resulted by chance. It appears much more plausible that this uniformity was the result of some physiological dependence of the metabolism of one or more of the constituents on the metabolism of the others. For example, in the event that one or more of the steps in the metabolism of lignin by *Polyporus versicolor* was an energy-requiring reaction, the necessary energy may have been supplied by the oxidative metabolism of one or several of the wood carbohydrates. In this way, the rate of utilization of lignin may have been determined by the rate at which energy was made available from carbohydrate metabolism.

An alternative to the hypothesis of physiological dependence is that the nature of the physical or chemical association between the lignin and the various carbohydrates of the wood was such that the rate of metabolism of one or more of the constituents was determined by the rate at which the others were removed from the association. Further research is needed to test both of these hypotheses.

Distribution of Enzymatic Effects Within the Wood Cell Walls

Both test fungi possessed readily diffusible enzyme systems that catalyzed the dissolution of the wood cell-wall substance at considerable distances from the hyphae. This conclusion is supported by the following observations: (1) the volume of wood substance removed in the formation of bore holes was wholly inadequate to account for the weight loss induced by *Poria monticola*; (2) the general thinning and porous patterns of dissolution of the wood substance that were apparent in microscopical sections of the wood decayed by *Polyporus versicolor* (figs. 24 and 25); and (3) the relatively sparse mycelial development observed in microsections of the test blocks decayed by both test fungi.

The lignin-depolymerizing enzymes of *Polyporus versicolor* apparently were able to penetrate the secondary walls of the wood cells and to act on the compound middle lamella substance at a relatively early stage of decay. This conclusion is based on the following observations: (1) lignin was metabolized at an essentially uniform rate in all stages of decay (figs. 14 and 15), although the bulk of the lignin of angiospermous woods, of which sweetgum is typical, exists in the compound middle lamella of the wood cells (fig. 2); and (2) electron micrographs of sections taken from the samples at 25 percent weight loss showed areas of low electron density in the compound middle lamella region (fig. 25). These areas were not apparent in sections of undecayed samples (fig. 23).

Except in the most advanced stages of decay, *Polyporus versicolor* utilized the constituents of the crystalline and amorphous regions of the wood cell walls at rates that were approximately proportional to the amounts present in sound wood. This conclusion is demonstrated by (1) the uniformity in hygroscopicity of the decayed wood (fig. 19), and (2) the very slight changes in X-ray diffraction patterns of the decayed wood samples (fig. 22).

The rapid initial depolymerization of the wood constituents during decay by the brown-rot fungus, *Poria monticola*, apparently occurred throughout the wood cell walls rather than just within regions close to the lumens or other restricted regions within the wood cell walls. This suggestion is supported by: (1) the extreme rapidity of decrease in average degree of polymerization of the holocellulose (fig. 18), and (2) the rapidity of increase in alkali solubility and total extractives content of the decayed wood (fig. 8).

The extracellular enzymes of *Poria monticola* penetrated the amorphous regions of the wood cell walls and concentrated their depolymerizing effects primarily in these regions in the early stages of decay. This conclusion is supported by the following evidence: (1) the proportion of the test wood easily hydrolyzable by the carbohydrases of this organism corresponded closely with the proportion of amorphous carbohydrate in cellulosic materials in general, as shown by Millett and coworkers (44) (fig. 18); and (2) the rapid initial drop in hygroscopicity that would be expected in the event of preferential utilization of the more hygro-

scopic, amorphous materials was observed (fig. 20). The rapid initial increase in proportion of crystalline material in the wood that would also have been expected was not observed, however (fig. 22).

As discussed in the "Orientation" section of this bulletin, the isolated enzymes of several fungi have been shown to attack the more accessible, amorphous cellulose much more rapidly than they do crystalline cellulose (51, 83). The observation in the present study that the carbohydrases of *Poria monticola* acted in this same manner in the presence of the organism lends support to these earlier findings. It is difficult, however, to explain why the carbohydrases of *Polyporus versicolor* apparently attacked the crystalline and amorphous cellulose at essentially similar rates.

It is reasonable to assume that the carbohydrases of *Polyporus versicolor* would have attacked the polysaccharide molecules with which they were able to come into contact. Their failure to act preferentially on the amorphous cellulose therefore suggests that they were either physically excluded from the transient cell wall capillaries (for example because they were larger in molecular size than the corresponding enzymes of *Poria monticola*) or for some other reason were unable to enter or to act within the amorphous regions of the wood cell walls. Thus, their depolymerizing effects were very likely confined to the more gross capillaries of the wood—the lumen surfaces and the surfaces between the microfibrils of the cell wall.

Evidence in support of this hypothesis is provided by the following observations: (1) the general cell-wall thinning and porous patterns of dissolution of the cell-wall substance observed in the electron microscope (figs. 24 and 25); (2) the degree of polymerization of the holocellulose in the decayed wood decreased very slowly in all stages of decay (fig. 18). Very few cellulose molecules, if any, cross over from one microfibril to another (58). Thus, dissolution of the wood polysaccharides could very well proceed from the surface of microfibrils in such a way that the molecules in the centers of the microfibrils remained more or less unaffected. In this way, the average degree of polymerization of the holocellulose that remained in the wood after various stages of decay could have been maintained at a high level; and (3) lignin was utilized in the earliest stages of decay (figs. 14 and 15). Since this constituent is insoluble in sound wood and, in the secondary walls is concentrated in the spaces between microfibrils (84),⁹ it appears likely that lignin was removed from the interfibrillar spaces in the earliest stages of decay. This would have rendered the polysaccharide molecules on the surfaces of microfibrils accessible to the carbohydrases of *Polyporus versicolor*.

The general thinning of the wood cell walls shown in figures 24 and 25 could very well have been the end result of the dissolution of the microfibrils closest to the cell lumens.

Further research will be required to explain the differences in distribution of enzymatic effects of the two test fungi. Research to determine and compare the molecular size and shape as well as

⁹ See footnote 2, p. 4

other physical properties of the carbohydrases of these fungi would be especially helpful in this connection.

Mechanism of Polysaccharide Depolymerization

The polysaccharides of sweetgum sapwood were depolymerized by a random mechanism in the early stages of decay by the brown-rot fungus, *Poria monticola*. This conclusion is supported by the following evidence: (1) the rapid initial decrease in average degree of polymerization of the holocellulose (fig. 18); (2) the rapid depletion of alpha-cellulose and accompanying accumulation of beta-cellulose (fig. 17); (3) the rapid initial increase in solubility of the decayed wood in each solvent used in the solubility analyses (fig. 8); and (4) the rapid diminution in resistance of the wood to fragmentation during grinding (table 5).

This random mechanism of polysaccharide hydrolysis reflects the probable inherent mechanism of action of the carbohydrases of *Poria monticola*. The first three of the four observations listed above in support of a random mechanism would have been impossible, and the last highly unlikely, if an endwise mechanism had been involved. This random mechanism has also been demonstrated for the isolated cellulolytic enzymes of other fungi (52).

The mechanism of action of the carbohydrases of the white-rot fungus, *Polyporus versicolor*, cannot be established with certainty from the experimental results of this study. The following observations, however, are pertinent to consideration of the probable mechanism involved: (1) the very gradual decrease in average degree of polymerization of the holocellulose (fig. 18); (2) the depletion of alpha-cellulose without corresponding increase in the amount of beta- or gamma-cellulose in the decayed wood (fig. 15); (3) the very slight solubility of the wood at all stages of decay in each of the solvents used in the solubility analyses (fig. 8); and (4) the uniform resistance of the wood to fragmentation during grinding (table 5).

These observations could have resulted from a random mechanism of carbohydrase action if the depolymerizing effects of these enzymes were concentrated on a small part of the holocellulose at a time so that most of the cellulose molecules that remained at a given stage of decay were not greatly affected. These observations could also have resulted from an endwise mechanism of carbohydrase action. The former alternative is consistent with the hypothesis discussed in the previous section on "Distribution of Enzymatic Effects Within the Wood Cell Walls." It also appears most likely in view of the observation by Norkrans and Rånby (52) that the isolated cellulolytic enzymes of other white-rot fungi cleave cellulose molecules by a random mechanism.

Hygroscopicity of the Constituents of the Decayed Wood

The hygroscopicity of the lignin residue in brown-rotted wood was very similar to that calculated for lignin in sound wood by Christensen and Kelsey (16). The beta-cellulose that was produced during the initial stages of decay was apparently not as much more hygroscopic than the other wood constituents as were the

isolated hemicelluloses studied by Christensen and Kelsey (16) and Runkel and Lüthgens (64). This conclusion is shown by the linearly diminishing hygroscopicity of the brown-rotted wood (fig. 20) over stages of decay in which both a manyfold increase and similar decrease in beta-cellulose content were observed (fig. 17).

Explanation of Differences in the Properties of White-Rotted and Brown-Rotted Wood

The test organisms used in this study were selected because they are representative of the two major types of wood destroying fungi. They are typical with respect to each of the distinguishing features outlined in the "Review of Differences . . .," p. 10. Although they are probably not representative in all aspects of their metabolism, their progressive effects on the strength, composition, dimensional stability, and solubility properties of wood are in substantial agreement with changes in these properties reported for other white- and brown-rot fungi. Each of these four effects may be explained largely by the strikingly different patterns of progressive change in the average degree of polymerization of holocellulose shown in figure 18. Thus, the following explanations of these effects are proposed as generalizations for the white- and brown-rot types of decay.

Strength Properties: Although experimental proof is lacking specifically for wood, physicists have demonstrated that the strength of other cellulosic materials may be attributed largely to cellulose with a high degree of polymerization (86). Any treatment that drastically decreases the length of cellulose molecules may be expected to decrease the strength properties of the material correspondingly. The rapid and very gradual changes, respectively, in average degree of polymerization of the cellulose in brown- and white-rotted wood correspond with and provide an explanation for the generalization that, at comparable losses in weight due to decay, the strength properties of brown-rotted wood are much lower than those for white-rotted material (14, 28).

Pulping Properties: The higher yields and quality of wood pulp obtained from white-rotted as compared with brown-rotted wood are explained by the higher cellulose content and average degree of polymerization of the cellulose in wood decayed by white-rot fungi. The amount of cellulose in white-rotted wood is higher than in brown-rotted material at comparable weight losses. Thus, the pulp yields are correspondingly higher.

During the chipping or grinding operations preliminary to chemical or chemi-groundwood pulp manufacture, the approximately uniform resistance to fragmentation shown by white-rotted wood in all stages of decay permits a higher yield of pulpable material. The friable nature of brown-rotted wood, however, causes severe losses during these preliminary mechanical treatments. The differences in resistance of decayed wood to fragmentation during grinding have been shown in this study to be correlated directly with (and probably to be caused by) the

changes in degree of polymerization of the holocellulose fraction of the wood.

The strength of pulp and pulp-derived products such as paper and rayon or cellophane is a function of the average degree of polymerization of their cellulose molecules (86). Thus, products obtained from brown-rotted wood may be expected to be low in strength because of their drastically reduced DP. White-rotted wood, on the other hand, would be expected to give pulp products of quality not greatly different from that obtained from sound wood. These expectations have been borne out by studies of pulp yield and quality where decayed wood of the two types were involved (42, 70).

Solubility Properties: The slowly increasing solubility of white-rotted wood and the rapidly increasing solubility of brown-rotted wood with increasing extent of decay may also be explained in large measure by the rapidity of changes in average degree of polymerization of the wood constituents. The solubility of polymer fragments is an inverse function of their average molecular size. Thus, with increasing extent of brown rot, where a rapid decrease in average DP of the holocellulose was observed, solubility increased markedly. In the white-rotted wood, a comparatively minor decrease in average DP was observed, and the solubility of the wood increased only moderately. (This correlation is best shown by comparison of figure 18 with the solubility data expressed as percentages of the sample weight, as in table 6.)

A very close correlation was observed by Kennedy (35) between solubility in 1 percent alkali and strength loss of wood decayed to low weight losses by *Poria monticola*. A fundamental explanation for this correlation is afforded by the dependence of both strength and solubility properties on the average DP of the wood constituents.

Dimensional Stability: The abnormal longitudinal shrinkage and resultant tendency of brown-rotted wood to check perpendicular to the grain, and the absence of these abnormalities in white-rotted wood, may also be traced to differences in the average degree of polymerization of the carbohydrates in the two types of decayed wood.

The swelling of wood is a result of moisture sorption between the linear cellulose molecules. When this fact is considered in relation to the orientation of cellulose molecules in the various layers of the secondary walls of wood cells (see fig. 2), the outer and inner (S1 and S3) layers can be seen to introduce a component of swelling and shrinkage largely parallel to the grain. This tendency, however, would be restrained by the high tenacity of the cellulose strands oriented nearly parallel to the cell axis in the middle (S2) layer of the secondary wall. The resistance of the middle layer to longitudinal dimensional changes has been suggested as the reason for the dimensional stability of wood parallel to the grain (86).

Abnormal longitudinal swelling and shrinkage would result in decayed wood from one or more of three possible causes: (1) if a great many cellulose strands within the secondary walls were broken, the middle layer would be correspondingly less able to

restrain the natural tendency of the outer and inner layers to cause longitudinal dimensional changes; (2) if the amorphous cellulose between crystallites were removed preferentially, the crystallites would become free entities and would be subject to mutual forces of attraction because of the high concentration of hydroxyl groups on their surfaces; and (3) if the glucosidic links in the cellulose molecules were hydrolyzed, the resulting hydroxyl groups facing each other parallel to the fiber axis would become sites for moisture sorption that could introduce a longitudinal component of swelling and shrinkage.

As indicated by the extent of degree of polymerization changes shown in figure 18, each of these factors would increase the tendency of brown-rotted wood to swell and shrink parallel to the grain more than it would white-rotted material. Thus, these factors may be responsible for differences in shrinkage properties noted previously for white- and brown-rotted wood (28).

As a consequence of its greater tendency for longitudinal shrinkage, and its lower resistance to the tensile stresses imposed by moisture gradients during drying, checking perpendicular to the grain would also be expected in brown-rotted wood much more than in white-rotted material. As shown in figure 5, the cubical pattern of checking typical of brown-rotted wood is also typical of wood subjected to pyrolysis, combustion, hydrolysis, ionizing radiation, and soft rot. It is known that the average degree of polymerization of the cellulose is drastically reduced in the case of hydrolyzed and irradiated wood (34, 44), and is very likely reduced similarly by pyrolysis and combustion (F. L. Brown, personal communication). Thus it appears likely that this cubical pattern of checking would result whenever the average DP is drastically reduced. This hypothesis, if proved, would provide a macroscopic indicator for drastic DP changes in wood.

SUMMARY

The purpose of this research was to determine and compare the progressive changes in certain chemical and physical properties of sweetgum sapwood (*Liquidambar styraciflua* L.) as it was being decayed by the representative white-rot fungus, *Polyporus versicolor* L. ex Fries, and the brown-rot fungus, *Poria monticola* Murr. Samples of sweetgum sapwood were obtained in progressive stages of decay by permitting artificially inoculated blocks to decay in the laboratory for various periods of time. Information concerning the nature and relative rates of the enzymatic effects of the two fungi were obtained from particle-size distribution, solubility, composition, degree of polymerization, hygroscopicity, X-ray diffraction, and histological analyses of the decayed wood samples.

The results of the particle-size distribution study showed that the resistance of the decayed wood to fragmentation during grinding was significantly reduced only in the most advanced stages of decay by *Polyporus versicolor*. The wood decayed by *Poria monticola*, on the other hand, showed progressive reduction in resistance to fragmentation from the earliest stages of decay.

The results of the solubility analyses, when expressed on the basis of the original sound wood, revealed little change in solubility of the wood with increased extent of white rot. Brown-rotted wood showed progressively increasing solubility in the initial stages of decay, and decreasing solubility in later stages. Analyses of the water and alkali extracts of brown-rotted wood showed that part of the lignin was rendered soluble in these solvents during decay by *Poria monticola*.

The composition analyses revealed that both *Polyporus versicolor* and *Poria monticola* utilized the various sugar polymers of sweetgum sapwood at rates that were essentially constant in all stages of decay and approximately proportional to the amounts present in sound wood. The relative proportions of alpha-, beta-, and gamma-cellulose in the holocellulose underwent only slight changes during decay by *Polyporus versicolor*. During all stages of decay by *Poria monticola*, however, alpha-, and gamma-cellulose decreased in concentration while beta-cellulose accumulated rapidly in the initial stages and diminished in later stages. Whereas *Polyporus versicolor* utilized lignin rapidly in all stages of decay, *Poria monticola* utilized lignin only to a minor extent.

The average degree of polymerization of the holocellulose that remained after various stages of white rot was only slightly lower than that of sound wood. The corresponding changes in brown-rotted wood were very rapid in the initial stages of decay and much more gradual thereafter.

The hygroscopicity analyses of white-rotted wood revealed a small increase in moisture adsorptivity in advanced stages of decay. The brown-rotted wood showed a rapid initial decrease followed by a more gradual and linear reduction in hygroscopicity in later stages of decay.

A small decrease in the proportion of crystalline material in the wood decayed by *Polyporus versicolor* was revealed by the X-ray diffraction analyses. A much more pronounced decrease in amount of crystalline material was shown in the brown-rotted wood after 10 percent weight loss. Little or no change in degree of parallelism of the crystalline material present took place during either type of decay.

The histological analyses of white-rotted wood, by means of both the light and electron microscopes, revealed general thinning and increase in the porosity of the cell walls, dissolution of pit pairs, and bore-hole formation during decay by *Polyporus versicolor*. In the wood decayed by *Poria monticola*, bore holes were the primary evidence of cell-wall deterioration visible through the light microscope.

The following major conclusions are supported by the analytical results enumerated above.

The samples in progressive stages of decay by the white-rot fungus, *Polyporus versicolor*, differed only slightly from sound wood in resistance to fragmentation during grinding, solubility properties, ratio of carbohydrate to lignin content, relative proportions of the various carbohydrate fractions present, degree of polymerization of the holocellulose, hygroscopicity, degree of crystallinity, and dimensional stability. This uniformity in properties of white-rotted wood resulted from the fact that *P. versicolor* utilized all major constituents of both the crystalline and amorphous regions of the wood cell walls at rates approximately proportional to the amounts present in sound wood. Each constituent was depolymerized only about as rapidly as the partial degradation products were converted to volatile products of respiration.

The lignin-depolymerizing enzymes of *Polyporus versicolor* apparently gained access to the compound middle lamella substance at a very early stage of decay by penetrating the secondary walls of the wood cells. The carbohydrases of this organism depolymerized the wood polysaccharides in such a way that the holocellulose molecules that remained in the wood after various stages of decay were only slightly reduced in average degree of polymerization.

The brown-rot fungus, *Poria monticola*, primarily utilized the wood carbohydrates; lignin utilization was slight, although profound changes were induced in its solubility properties. The various sugar polymers in the wood were utilized at rates that were approximately proportional to the amounts present in sound wood. In the initial stages of decay, the wood constituents were depolymerized much more rapidly than the partial degradation products were converted to volatile products of respiration; the reverse was true in later stages. The carbohydrases of *P. monticola* acted preferentially in the amorphous regions of the wood cell walls in the early stages of decay, and there catalyzed a rapid depolymerization of the wood polysaccharides by a random mechanism. Degradation of the crystalline cellulose was delayed and proceeded much more slowly.

The representative white- and brown-rot fungi used in this

study showed appreciable differences in the rate at which they depolymerized the wood polysaccharides. This difference provides an explanation for differences in the strength, pulping properties, solubility, and dimensional stability that may be applicable to white- and brown-rotted wood in general.

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