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CHANGES IN WOOD MICROSTRUCTURE THROUGH PROGRESSIVE STAGES OF DECAY

ABSTRACT

Successive stages of decay in the sapwood of sweetgum (Liquidambar styraciflua L.), a hardwood, and of southern pine (Pinus sp.), a softwood, were observed microscopically. The white-rot fungus. Polyporus versicolor L., and the brown-rot fungus, Poria monticola Murr., were the fungi used For the observations, light microscopy, plus the techniques of polarization and ultraviolet-absorption microscopy, was used on sections 4 microns thick cut from celloidin-embedded specimens. For both fungi, hyphae were widespread in the early stages of decay in both types of wood. The hyphae of the white rotter were much more numerous than were those of the brown rotter. The white-rot fungus in penetrating the cell walls went through both pits and numerous bore holes: the brown-rot fungus penetrated pits almost exclusively. Pit canals were enlarged by both fungi to the extent that they could not be distinguished from true bore holes in any but the early stages of decay. Results indicated that the action of the cellulolytic enzymes of the white-rot fungus was restricted to the cell wall surfaces, whereas the cellulolytic enzymes of the brown-rot fungus and the lignin-destroying enzymes of the white-rot fungus were able to penetrate and act within the cell wall. The resistance of the cell walls to brown rot was positively correlated with the lignin content. Essentially all loss in weight of wood could be accounted for by microscopically visible loss of cell wall substance.

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CHANGES IN WOOD MICROSTRUCTURE THROUGH PROGRESSIVE STAGES OF DECAY [1]

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Introduction

Although numerous observations of the microscopical and the submicroscopical characteristics of wood decay have been made, few have involved many discrete stages of decay. Usually they have been concerned with only the most advanced stages, A study reported in 1961 (11)[3] of the chemical changes in wood through successive stages of decay posed questions best answered by microscopical examinations. Therefore, this work was undertaken to provide information on the changes occurring during the progression of decay as observed by microscopy.

To compare results of different studies, microscopical observations must he related to a standard stage of decay, regardless of variations in experimental conditions. In many microscopical studies the stage of decay has been measured Solely by the length of period of incubation with the decay fungus. Results of these studies are difficult to compare because they do not measure the degree of wood destruction, which may differ greatly under various experimental conditions.

Loss in weight of wood substance has been widely used as a measure of decay, although certain chemical and mechanical tests may be more sensitive to the destructive effects of the attacking fungus. \\eight loss was chosen here to compare changes observed microscopically in wood during progressive stages of decay because it reflects directly the quantity of wood substance removed by the decay fungus and because it is a nondestructive test that can be applied to each specimen.

The light microscope was used throughout so that large numbers of cells could be observed and ultraviolet absorption and polarized light microscopy could be applied to matched specimens to aid in detecting certain chemical changes.

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[1] Portions of this report are from work for a thesis in partial fulfillment of the requirements for the Ph.D. degree in Plant Pathology at the University of Wisconsin. The research was supervised by Dr. T. C. Scheffer, U.S. Forest Products Laboratory, and Dr. J. E. Kuntz, University of Wisconsin. Dr. Wilcox is now at the Forest Products Laboratory, University of California, Richmond, Calif.

[2] Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

[3] Underlined numbers in parentheses refer to Literature Cited at the end of this paper.

For principal variables, a hardwood and a softwood, representative of each of the two major classes of wood, and a white-rot fungus and a brown-rot fungus, causal agents of each of the major classes of decay, were used.

Background

Wood Cell Wall Structure and Composition

The terminology applied here (fig. 1) to the layers of the wood cell wall was proposed by Kerr and Bailey (2, 25). It was used because it was developed from observations made with polarized light (also utilized in this study) and because alternative terminologies have caused considerable controversy. Layers of the secondary wall are assigned numbers, a simplification used extensively since its adoption by Meeuse (34).

The term "compound middle lamella" denotes the unit composed of the true middle lamella and the two adjacent primary walls. References to direction within the cell wall also correspond to the concept of Kerr and Bailey (25) of "inner" and "outer" layers. Therefore, "outward" denotes the direction from lumen toward middle lamella and "inward" the middle lamella to lumen.

Microscopically Observed Effects of Decay on Wood

In this work deterioration of wood by fungi of the class Basidiomycetes is considered. Two types of decay are recognized: brown rot, in which essentially only the carbohydrate fraction of the wood is removed; and white rot, in which both the lignin and the carbohydrate fractions of the wood are removed. The characteristics of these two types of rot have been described as follows (15, 16):

1) Brown rot--The cells are attacked by solitary hyphae; the structure of the cells appears to be maintained throughout decay: decomposition is rapid and uniform: there is a shrinkage of the completely attacked wood substance resulting in the formation of shrinkage splits, which finally leads to a cubical appearance of the rot: the residual material is dark colored.

2) White rot--The cells are attacked by a network of hyphae and finally are completely decomposed; decomposition is slow and nonuniform; there is no appearance of a friable condition or of shrinkage of residual wood substance: cavities are formed within the cell walls: there is no dark-colored residual material, merely the skeleton-like remains of the wood substance.

Much information on the microscopical characteristics of wood decay has been reported since this early description. Furthermore, within the same type of rot, various fungus-wood species combinations may produce different results.

Hyphal distribution.--Certain differences fairly characteristic of each decay type have been reported in the concentration and distribution of the hyphae of decay fungi in wood.

In white-rotted hardwoods the hyphae first occupied the vessels or rays and entered the fibers only as decay progressed (3, 11, 18, 44). The hyphae of brown-rot fungi generally were more uniformly distributed throughout the wood even in early stages of decay (11, 15, 35, 40, 52), although distribution similar to that of white-rot fungi also has been reported (18).

Channels of hyphal advance from cell to cell.--It is generally accepted that both white-rot and brown rot fungi produce bore holes as one means of crossing cell walls. Bore holes are commonly used as an indicator of incipient decay, and it has been suggested that the number of holes may furnish some evidence of the stage of decay (53, 55). Bore holes have been observed to be more numerous in advanced stages of brown rot than in the advanced stages of white rot (11, 53), but in either it seems that in the early stages of decay, movement of the fungus from cell to cell may in large measure be accomplished by penetration through the pits (3, 11, 18, 40, 44, 46).

Effects on wood microstructure.--A major difference noted in the literature between white rot and brown rot is in the microscopical appearance of the cell walls following removal of wall substance. White rot often involved a progressive thinning of the secondary wall, beginning at the lumen and progressingoutwardtoward the middle lamella (8, 11, 19, 31, 44, 46,53), whereas brown rot reportedly involved no such thinning of the secondary wall, or if thinning occurred it was only in very late stages of decay (8, 31, 40). Also, it has been observed (40) that strength loss in residual wood far exceeded the

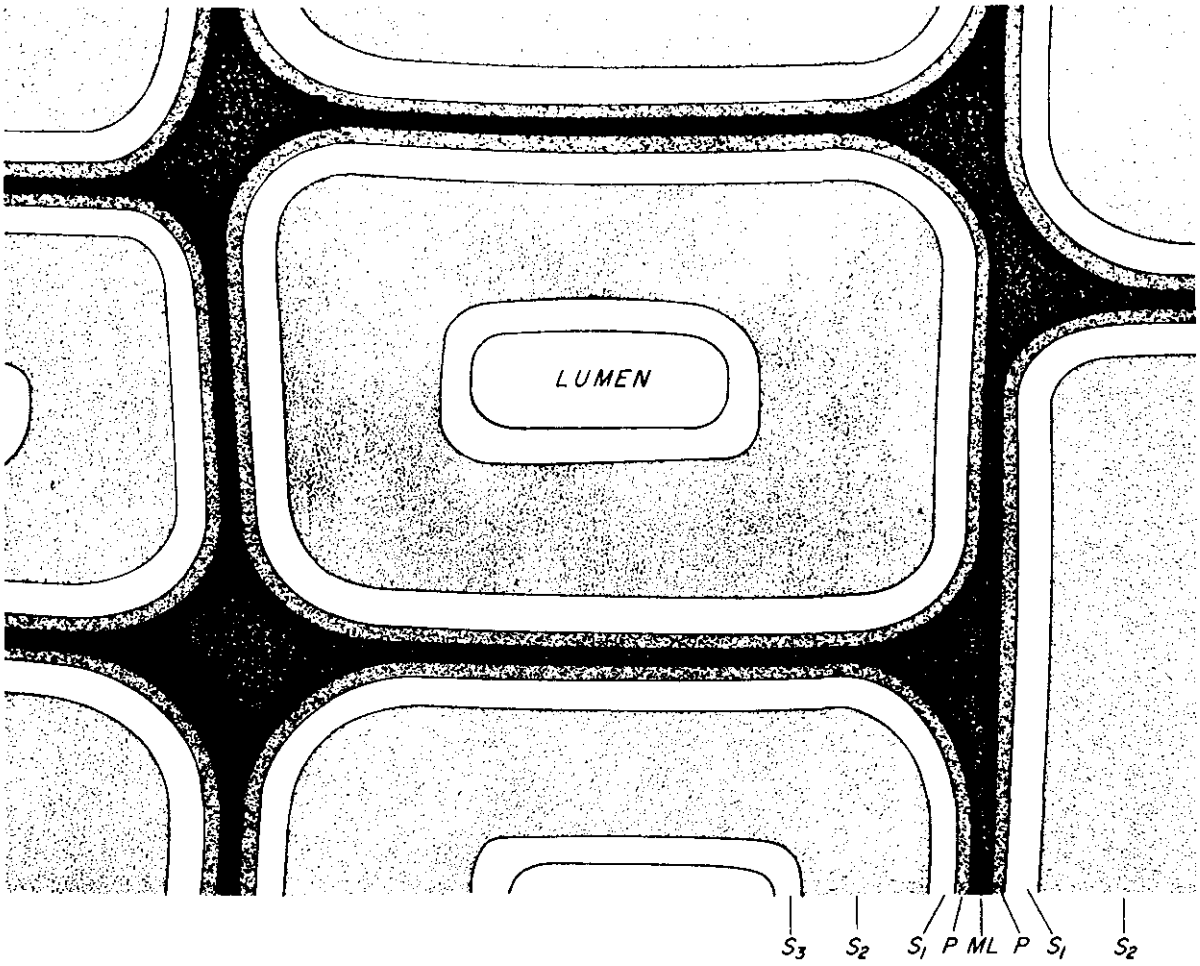

Figure 1.--Diagram of typical wood cell showing middle lamella ML, primary wall P, and secondary wall consisting of an outer layer S1, central layer S2, and inner layer S3. (After Kerr and Bailey (25) and Meeuse (34).)

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visible effects on the walls in brown-rotted wood but closely paralleled these effects in white-rotted wood.

Some of the more significant characteristics reported for white rot are:

a) The fungi appeared to follow one of two different modes of action in their attack on wood cell walls depending on whether lignin is preferentially decomposed early in decay or lignin and cellulose are decomposed simultaneously throughout decay (4, 35).

b) The fungi progressively decomposed the ray cells (3, 5, 18, 53) and greatly enlarged the pit canals (11, 44). It has been reported that even in advanced stages of white rot little or no shrinkage or cell collapse occurred and the original shape and the outward appearance of the wood were maintained (8, 44). Greaves and Levy (18), however, found a loss of structure in advanced stages of white rot in beech.

c) Cell wall thinning that with the light microscope seemed uniform within each cell and from cell to cell (11, 44) appeared less uniform on observation with the electron microscope (11, 35). Minute pockets. indicating the removal of cell wall material, were found in both the secondary wall and the compound middle lamella prior to overall thinning of the walls (11).

Ability of the enzymes of a white-rot fungus to diffuse through cell walls was postulated by Cowling (11) based on the observation that the thinning of cell walls occurred at approximately the same rate in cells lacking hyphae as in cells containing hyphae.

A number of detailed microscopical characteristics have been reported that distinguish the effects of certain brown-rot fungi from those of white-rot fungi:

a) The extensive cell wall thinning typical of white rot has not been reported in brown-rotted wood or has been observed in a totally different form (31, 40). Thinning may occur, however, in late stages of brown rot (8, 35). The form of the cells apparently was maintained by the residual lignin framework (8, 35, 40).

b) Cell wall decomposition may be very irregular from cell to cell, being well advanced in some and seemingly slight in adjacent cells.

c) Extensive cracking within the cell wall has been associated with moderate degrees of brown rot; in later stages of decay the summerwood tracheids were penetrated throughout with spiral cracks (40).

d) Ray parenchyma cells were attacked in early stages of brown rot, and the walls were completely destroyed by advanced stages of decay (40).

e) An increase in cell wall porosity at the submicroscopical level, similar to that observed in white-rotted wood, was detected in cells attacked by a brown-rot fungus (11).

In a detailed electron-microscopical study, Meier (35) observed that the effects of two different brown-rot fungi were similar whether the attack was on a softwood (spruce) or a hardwood (birch). In both types of wood the S2 layer of the secondary wall was the first to be attacked by the fungi. It was progressively decomposed toward the middle lamella, whereas the S3 layer remained essentially intact. The removal of the S2 layer from the birch, however, was not uniform, for in some places it appeared essentially intact and in others cavities existed. These cavities were not observed in the spruce, because the form of the deteriorated S2 layer was maintained by a loose lignin framework that still appeared homogeneous even in the electron microscope. Meier assumed this to be an indication that the S2 layer of birch was much less lignified than that of spruce. Following complete removal of the cellulose from the S2 layer, and in birch the removal of the entire layer, the S3 layer was decomposed; however, even in this advanced stage the S1 layer, the primary wall, and the middle lamella were still intact.

Relative Decay Resistance of Various Wood Elements and Cell Wall Layers

Variations have been detected in the ability of the different layers of the cell wall to resist degradation. It was reported that the S3 layer of the secondary wall was resistant to the action of acids and alkalis (29, 30, 52). This layer was also highly resistant to degradation by brown-rot fungi (23, 30, 35, 39). Its resistance may have been due to a greater degree of order in the cellulose microfibrils (28, 35), to a greater degree of lignification (22, 23, 30), or to actual differences in the chemical composition of the microfibrils (35). Meier (35) observed that apparently the S3 layer retained its resistance to the action of brown-rot fungi, even when it had first been delignified chemically. He reported that the S3 layer showed resistance even to the action of certain white-rot fungi and that the S1 layer was resistant to both white rot and brown rot. This resistance of the S1 layer was attributed to a greater density in it than in the S2 layer or to differences in chemical composition (35, 37).

Meier (35) found that some of the differences between the effects upon wood of white-rot and brown-rot fungi disappeared if the wood to be decayed was first macerated. He subjected macerated spruce wood, considered to be essentially pure cellulose, to attack by several brown-rot and white-rot fungi and observed that both types of fungi decomposed this material at approximately the same rate and produced similar morphological effects.

The compound middle lamella appeared to be the region most resistant to attack of both white-rot and brown-rot fungi (11, 35). Nevertheless, this region was eventually attacked in advanced stages of white rot; the thickened areas of the middle lamella at the cell corners resisted degradation the longest (35). Attack on the pit membranes was the only decomposition of the middle lamella by a white-rot fungus observed by Cowling (11) in sweetgum with weight losses ranging up to 79 percent. In birch, Meier (35) found that the vessels also were resistant to the attack of brown-rot fungi.

In general, it has been suggested that a high degree of parallelism in the cellulose micro-

fibrils imparts resistance to chemical and enzymatic attack (12, 28, 29). Similarly, Mann and Sharples (33) reported that water and aqueous reagents penetrated and swelled only the amorphous regions of the cellulose, leaving the crystallites untouched.

Differences in decay resistance Of various regions within annual rings also have been reported, Schulze and Theden (47) observed that earlywood of pine and spruce was more resistant to brown rot than was latewood. Conversely Meier (35) reported that latewood of spruce was more resistant to the action of brown-rot fungi than was earlywood, but that earlywood was more resistant to white-rot fungi than was latewood.

Materials and Methods

Materials

Sapwood of sweetgum (Liquidambar styraciflua L.), a hardwood, and of southern pine (Pinus sp.), a softwood, were chosen as being representative of each type of wood. They are particularly appropriate for microscopical study of the effects of decay because the fibers of the sweetgum and the latewood tracheids of the pine possess extremely thick secondary walls. The thickness of the S1 and the S3 layers of the secondary walls of plant cells is relatively constant (2); therefore, the considerable thickness of the secondary walls in these woods must consist mainly of the S2 layer. Most mechanical properties of wood have been attributed to this layer (17). This is also the region of the cell wall in which most effects of decay are observed.

The fungi used were Polyporus versicolor L. ex Fr. (Madison 697), a white-rot fungus, and Poria monticola Murr. (Madison 698), a brown-rot fungus. Both are common decay fungi, and both are highly destructive in wood and wood products. Throughout the report the two fungi will be termed the brown-rot fungus. or brown rotter, and the white-rot fungus, or white rotter, and the type of decay produced, white rot or brown rot.

Culture Methods

Decayed wood specimens were prepared by the soil-block culture method (1) except for several modifications. The culture bottles with soil substrate and feeder block were sterilized in an autoclave for 1 hour at 15 pounds per square inch on each of 3 successive days for a total sterilization time of 3 hours to assure soil sterility.

To avoid any changes that might be produced by heat in the structure, composition, or decay resistance of the wood. the wood sample blocks were sterilized by exposure to approximately 0.5 gram ethylene oxide per liter of space in the sterilizing vessel for 12 hours at room temperature. The ethylene oxide was applied as a nonflammable mixture of 12 percent ethylene oxide with several hydrocarbon gases. For sterilization, the blocks—with a moisture content of approximately 12 percent (based on ovendry weight)--were held in petri plates wrapped with heavy paper and placed in a vacuum desiccator, The air was withdrawn from the desiccator, and the ethylene oxide mixture was introduced into it until equilibrium with room pressure was reached. Expeximentation showed that this procedure introduced approximately the correct amount of ethylene oxide into the container.

The size of the wood sample blocks was reduced from that specified by the soil-block method to approximately 3/4 by 1 by 1/8 inch (along the grain). The thinness in the grain direction made it possible to prepare specimens suitable for embedding and sectioning with a minimum of damage or distortion simply by splitting the blocks along the grain with a sharp knife.

To determine weight losses (the basic measure of decay) during the course of decay, the blocks were first air-dried at constant temperature (80 ±2° F.) and relative humidity (70 ±4 percent), and weighed to the nearest 0.001 gram. They then were sterilized, inoculated. and incubated as described. Finally, each sample block again was dried under the same controlled conditions and weighed. Noninoculated blocks similarly treated served as controls. Blocks were removed from culture at weekly intervals for a total of 12 weeks to provide a series of specimens in progressive stages of decay. In addition. a small number of sample blocks were incubated for 16 and 20 weeks to provide highly advanced stages of decay.

Preparation of Specimens for Microscopical Examination

Most specimens were embedded in celloidin prior to sectioning. Unless otherwise stated, the data in this report were obtained from celloidin-embedded specimens sectioned at a thickness of 4 microns. A small number were embedded in paraffin (43) or were observed without having been either embedded or sectioned.

Standard procedures for embedding, sectioning, and staining (21, 43) were employed with slight modification. The sample blocks of air-dried wood were gently split along the grain with a sharp knife to yield cubes approximately 1/8 inch on a side. The height along the grain was equal to the thickness of the sample block. The solvent for celloidin was 2-methoxyethanol, and embedding was according to methods described (54) in which specially designed hardening chambers were utilized in the final stages of thickening the celloidin solution. The celloidin-embedded specimens were sectioned after storage for 6 months or more in glycerin-alcohol. A sliding microtome was used for sectioning. The sections were stored in glycerin-alcohol in small vials for later use or mounted on glass slides with Haupt's adhesive (21, 54) and dried at 50°C. The celloidin was removed from the sections mounted on glass slides by soaking overnight in 2-methoxyethanol at 50" C.

For most observations, sections of each weight loss sampled were prepared by the following methods: (1) stained in 1 percent safranin in 50 percent ethanol and 1 percent fast green in ethanol-clove oil, (2) stained in azure B, (3) stained in 1 percent aqueous chlorazol black E, and (4) unstained. Details of the first two procedures were outlined by Wilcox (54). The sections to be stained with chlorazol black E were covered with a 1 percent aqueous solution of the dye (color index 30235 (9), and the slide was placed on a hot plate maintained at a temperature sufficient to cause the solution to steam without actually bubbling. The solution was allowed to evaporate until its margin approached but was not quite in contact with the sections. The sections then were washed with water, and 50 percent, 95 percent, and absolute ethanol; cleared in clove oil; washed in xylene; and mounted in Permount. The unstained sections were prepared by transferring them from 2-methoxyethanol to absolute ethanol, clearing in clove oil, washing with xylene, and mounting in Permount.

Most observations were of sections stained with safranin and fast green. This procedure was used primarily to emphasize cell wall Structure: however, it has been reported that red staining of wood cell walls by safranin may be closely correlated with lignin content under some conditions (13). This method also aided detecting certain effects of advanced stages of brown rot on the wood. Staining with chlorazol black E was useful with the area measuring device of Ladell (26) because it colored the entire section black, emphasizing the distinction between cell wall and cell lumen. Unstained sections were prepared to give the required clarity and flatness for observation under polarized light.

A small number of sections were stained by the picro aniline blue-safranin method toconfirm the presence of hyphae, revealed only faintly by staining with safranin and fast green. The method was essentially that of Cartwright (7) with minor modifications as outlined by Wilcox (54). The modifications entailed the use of more dilute staining solutions than originally specified. By this procedure wood cell walls appeared Pink, and hyphae appeared blue.

The iodine-potassium iodide method for the detection of starch was employedon some sections (20, 54). The sections were mounted and observed beneath a cover glass in this stock solution. The wood substance became yellow, and the starch was colored dark blue to black.

For observation under ultraviolet radiation, sections stored in glycerin-alcohol were placed in glycerin on a quartz slide and covered with a quartz cover slip.

Not all microscopical features of the decayed wood were clearly visible in sections. Bore holes were best observed by examining the entire cell walls of isolated wood elements. For these observations, thevarious woodelements were separated by Jeffrey's macerationprocedure (21, 54). The macerated specimens were stored in 50 to 70 percent ethanol and observed directly in this storage fluid as wet mounts. The surfaces of some specimen blocks were observed microscopically without microtechnical treatment by incident-illumination optics.

Microscopy

Most microscopical examinations were made with a binocular research microscope in which the various optical systems were incorporated. Most observations were carried out under bright-field illumination. The observations and the photography under polarized light were made on the same equipment by introducing two polarizing filters, one as polarizer and one as analyzer, With the two polarizing filters crossed, the microscopic field appeared dark when no subject was present. However, areas of the cell wall containing crystalline cellulose, in which the microfibrillar orientation was approximately at right angles to the light beam, appeared bright, and noncrystalline areas were dark

This polarizing system used with a first-order red compensator, facilitated observations of changes in quantity and location of crystalline cellulose in macerated, decayed wood. The occurrence and distribution of bore holes and similar features were also more easily detected. By this method crystalline areas in the macerated material not stained or colored by chemical means appeared either yellow or blue against a magenta background; cavities or noncrystalline areas in the cell wall also were magenta. A few unstained sections were observed under phase-contrast illumination. By this method the boundary between cell wall and cell lumen was accentuated, and cavities in the cell wall were easily observed. Several specimens were viewed without sectioning under .incident illumination.

The ultraviolet absorption microscopy was performed on a microscope equipped with quartz optics and a first-surfaced, aluminized mirror. A grating monochromator with focal length of 500 millimeters, grating containing 1,200 grooves per millimeter, and high-intensity mercury-arc light source was used as the illuminator. Sections mounted in glycerin were observed under illumination at a wavelength of 2780 angstroms--a wavelength strongly absorbed by lignin. By this method, dark, highly absorbent areas in the cell wall were presumed to indicate regions of high lignin content.

Throughout this paper certain assumptions have been made on wood composition based on results of various chemical and optical methods applied in this and other studies. Under the conditions employed, many detection procedures could not be considered specific for a given wood component. However, the acquisition of the same results by several different methods was considered adequate evidence of the presence or absence of the component.

Therefore, any substance that stained red with safranin and fast green, blue-green with azure B, strongly absorbed ultraviolet radiation at 2780 angstroms—and occurred in a region of the cell wall known to contain lignin--was termed "lignin."

Any substance that stained green with safranin and fast green, appeared birefringent when viewed between crossed polarizing filters, and occurred in a region of the cell wall known to contain cellulose was termed "cellulose" or, more correctly, "crystalline cellulose." "Birefringence" was used here simply to denote the production of a bright image between crossed polarizing filters: no measurement of the angle or sign of the alteration in light path was implied by use of this term

Mensuration

Two methods of measurement were employed here. Individual objects such as hyphae or bore holes were measured with an ocular micrometer placed in the eyepiece tube of the microscope. Measurements of cross-sectional area were made by a rapid procedure developed by Ladell (26). Slight modifications, however, of the original equipment were made to adapt the research microscope as part of this apparatus (54).

The method for measuring cross-sectional area consisted of projecting the image of a section from the microscope onto a white card containing 100 pinholes on grid coordinates determined from a table of random numbers. The card containing the randomly spaced pinholes was illuminated from below, so that the holes appeared as spots of light on the projected image of the specimen. In the area occupied by the 100 spots, the number of spots falling upon a given structure provided a measure of the percentage of the area occupied by the structure. Sections measured by this means were stained with chlorazol black E to increase the contrast between cell wall substance and cell lumen.

Random placement of the sampling points avoided any bias that might result if regularly spaced points were to fall in sequence with regularly spaced wood elements (26), as were

present in the pine tracheids viewed in cross section, Subsequently, evidence has appeared, however, that randomly and regularly spaced sampling points can produce comparable results even when applied to pine (49).

Experimental Design

The effects of a representative white rotter and a representative brown rotter were observed in two woods—arepresentative hardwood and a representative softwood. Wood sample blocks with various degrees of decay were selected for sectioning and microscopical study. In microscopical examinations the substance remaining is observed, rather than the substance removed, so the results reported are based on residual weight rather than on weight loss. The percent residual weight was calculated by subtracting the percent weight loss from 100. Residual weights of blocks from which specimens were selected for study were as follows:

Species	Residual weight	
	White rot	Brown rot
	Percent	Percent
Sweetgum	97	96
	85	81
	76	67
	40	40
	28	32
Southern pine	97	91
	85	85
	15	70
	65	55
	50	50
	--	35

The periods of incubation in which these residual weights were progressively developed are shown in figure 2.

In addition to selecting the sample blocks to represent various stages of decay, it was necessary to select small regions of each block for sectioning and observation. The selection procedure was as follows:

1) A block of each of the two sound woods

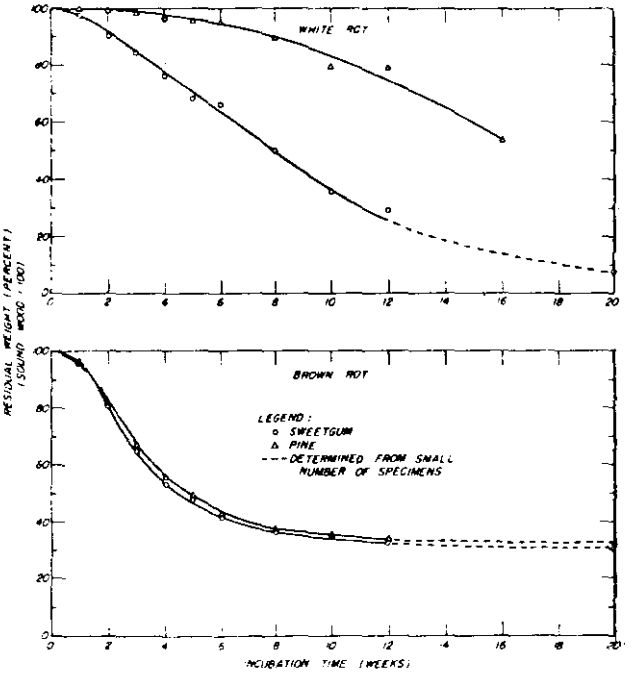

Figure 2.--Relation of average residual weights of decayed wood sample blocks to incubation time with the decay fungus. M 127 336

from the same sticks that provided the decayed sample blocks was split into three or four specimens for embedding and sectioning.

2) From the decayed wood, where possible, three blocks in each residual weight category (within 1 percent) were selected, and each was split into several specimens for embedding and sectioning.

3) Two cross sections from each of three to four embedded specimens representative of each residual weight category were stained with safranin and fast green and critically examined.

4) From those two cross sections a single area judged most representative of the predominant microscopical appearance of the cell wall structure in the two sections was photographed under high magnification.

5) The three to four photomicrographs representing a particular residual weight category were visually compared with one another and with those of the other residual weight categories to select the one from each category that most accurately depicted the trend of microscopically visible changes in the cell walls. The principal feature appraised for this purpose was the relative area of cell wall substance in cross

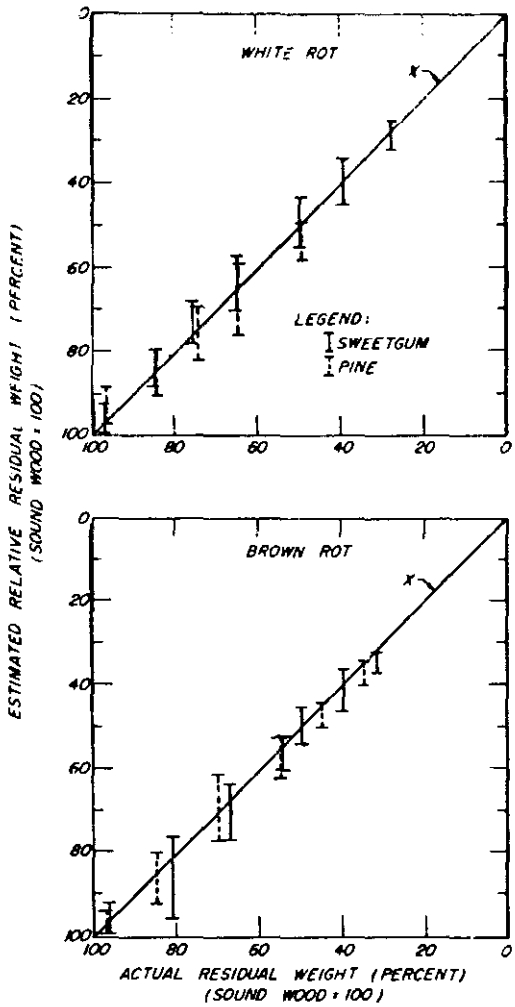

Figure 3.--Relationship of estimated relative residual weights in cross sections prepared from embedded specimens to actual residual weights. Each bar represents a single residual weight category, and length of bar indicates range of estimated residual weights in sections from that category. Line X represents points where values on the two coordinates are equal. M 133 888

section, although features such as cell separation and checking of the secondary wall also were considered.

6) From this trend and knowing the actual residual weight of the block from which each photomicrograph was prepared plus the range of variation in microscopically visible characters present in the photomicrographs representing a given residual weight, it was possible to estimate the residual weight of a specific region. These estimated residual weights (fig. 3) provided a measure of the variability in microscopically visible changes in the cell walls encountered in sample blocks of the same residual weight.

7) Finally, the estimated residual weights were plotted against the actual residual weights, and a line was drawn to represent all points where the values on the two coordinates were equal (fig. 3, line x). The single photomicrograph in each residual weight category represented by the plotted position nearest line x was selected as the representative of that residual weight category; usually the associated estimated residual weight fell near the midpoint of the range as shown in figure 3. Further observations and measurements of a particular residual weight were made on sections from the same embedded specimen as that represented by the selected photomicrograph.

The results reported were based on critical examination of more than 800 sections of sound and decayed wood. Fifteen to 20 sections of each sound wood and 25 to 35 sections representative of each residual weight category of the decayed woods were examined microscopically.

Proliferation of Hyphae in Decaying Wood

The photomicrographs selected as representative of the various stages of decay are shown in figures 4 through 8. Because the major criterion for selection was the microscopical appearance of the cell wall structure. many do notadequately illustrate the hyphae that were present. Nevertheless, because some photomicrographs are referred to in this as well as subsequent sections and because the progression of decay effects is most readily visible by examining the photomicrographs as a group, the entire series representing the various residual weights is presented in the order of increasing decay.

Hyphal Distribution

One of the striking differences between effects of the white-rot fungus and those of the brown-rot fungus was the distribution of hyphae throughout the wood. In sweetgum. the hyphae of the white-rot fungus initially were small (1 to 2μ in diameter) but numerous (fig. 5A through F).

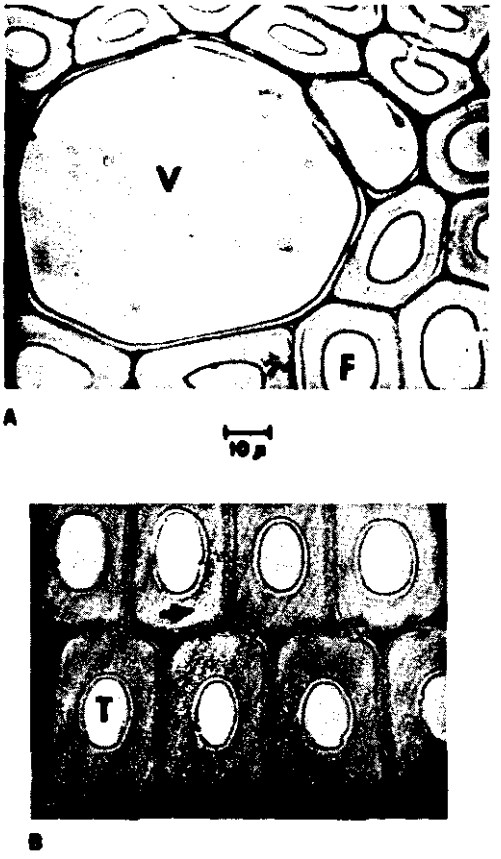

Figure 4.--Bright-field photomicrographs of cross sections of sound wood:

A, Sound sweetgum showing small, thick-walled fibers F and large, thin-walled vessels V.

B, Sound pine slowing thick secondary wall (⇒) and small lumens of tracheids T.

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When 85 percent residual weight had been reached, at least several hyphae could be found in the lumen of almost every fiber, whereas even larger numbers occurred in the vessels and rays (fig. 5B). However, the concentration of observed hyphae decreased in later stages of decay, and when 50 percent residual weight was attained hyphae were sparse in the fibers and the rays, although significant numbers were still present in the vessels (fig. 5D). Hyphae still present in advanced stages in decay were larger (approximately 2 to 2.5μ in diameter) than those in the early stages.

In pine (fig. 6A through D) the hyphae of the white-rot fungus were more numerous in the ray cells than in the tracheids in early stages of decay, but when 85 percent residual weight had been reached hyphae had become numerous in both (fig. 6B). At least several were found in the lumina of most tracheids. The longitudinal resin canals of pine, likethevessels ofsweetgum, became packed with hyphae in early stages of decay (97 percent residual weight). In later stages the concentration of hyphae decreased considerably.

The hyphae of the brown-rot fungus in sweetgum were as uniformly distributed throughout the wood as those of the white rotter, but they occurred in smaller numbers (fig. 7A through F). With 96 percent residual weight, hyphae could be found in almost all cells (fig. 7A). Commonly only a single hypha or pairs of hyphae were present in the lumina of many fibers. Hyphae were seen in the rays but not in great numbers. Also, they were not as concentrated in the vessels as were those of the white-rot fungus. Below 60 to 70 percent residual weight the concentration of hyphae decreased, until in advanced stages of decay they were sparse. The hyphae of the brown-rot fungus were similar in size to those of the white-rot fungus and likewise were larger in advanced than in early stages of decay.

In contrast to their appearance in sweetgum, the hyphae of the brown-rot fungus in pine were sparse in early stages of decay and remained so in the tracheids into advanced stages (fig. 8A through F). However, by 85 percent residual weight the concentration of hyphae of the brown rotter had increased in the ray cells to a high level, which was maintained into advanced stages of decay.

Channels of Hyphal Advance from Cell to Cell

The method of hyphal advance from cell to cell differed between the white-rot fungus and the brown-rot fungus, but with each there was little difference in this advance between sweetgum and pine. The hyphae of the white-rot fungus penetrated directly through the cell wall in the earliest observed stages of decay, producing numerous bore holes (figs. 5A and 6A). Conversely, no clear-cut example of bore hole formation was observed with the brown rotter.

In early stages of bore hole formation by the white-rot fungus, the diameter of the hypha inside the cell wall appeared to be only slightly

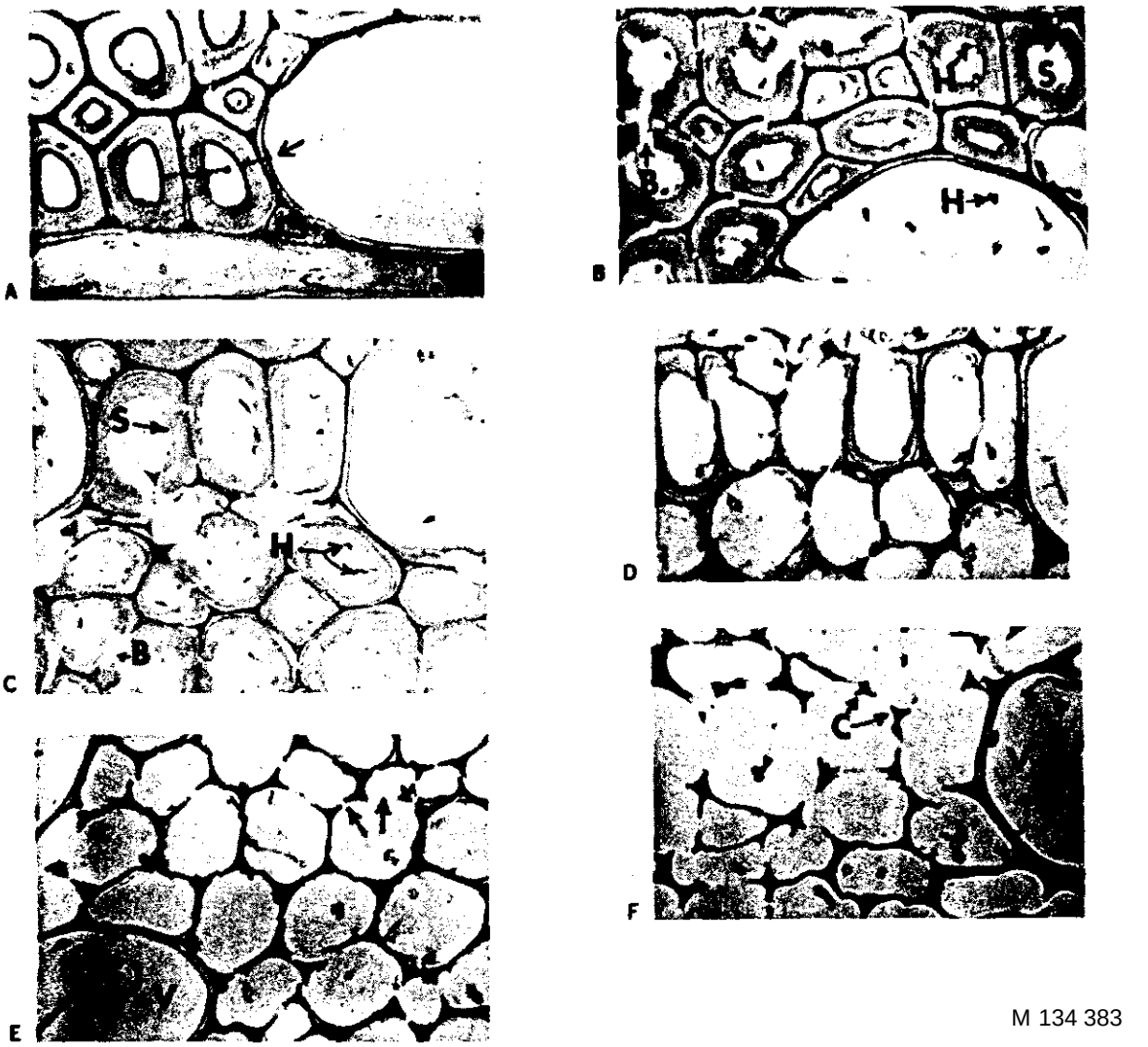

Figure 5.--Bright-fieldphotomicrographs showing increasing amounts of white rot in cross sections of sweetgum with residual weights of:

A, 97 percent. Note fine hypha penetrating transversely through cell walls of a vessel and several fibers (⇒).

B, 85 percent. Note dark hyphae usually present within the cell lumina H, bore holes or pit canals enlarged by penetrating hyphae B, and cell separation S.

C, 76 percent. Shows large number of hyphae in cell lumina H, enlarged pit canals and bore hales B, and serrated appearance of boundary between secondary wall and lumen in several fibers S.

D, 50 percent. Note extreme thinness of secondary wall of fibers.

E, 40 percent. Shows almost total removal of secondary wall of fibers, extensive removal of areas of compound middle lamella (⇒). and essentially intact condition of vessel walls V.

F. 28 percent. Note absence of secondary wall in fibers and extensive removal of regions of compound middle lamella. Note also residual thickened areas of compound middle lamella at corners of fibers C and essentially intact appearance of vessel walls V.

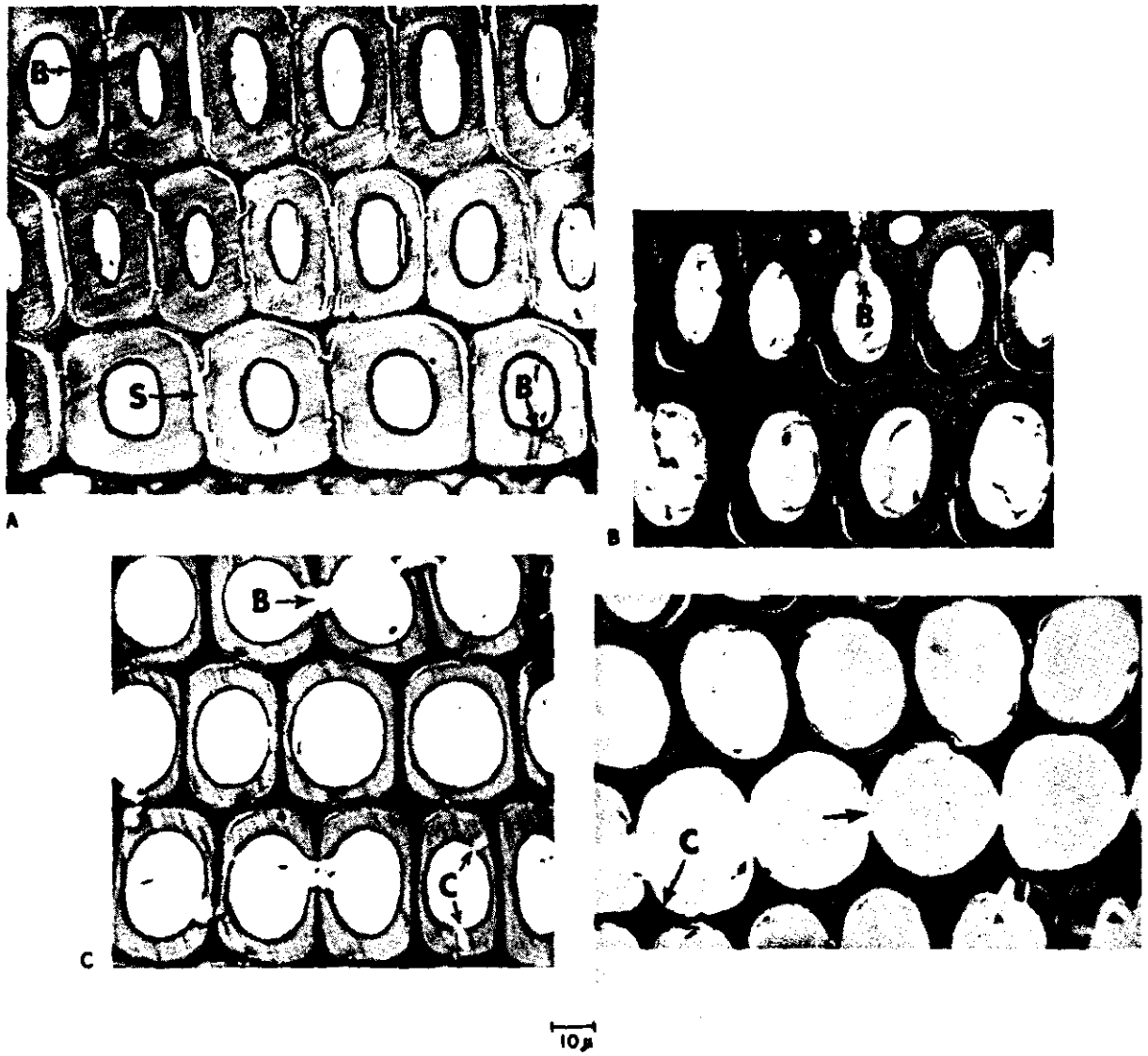

Figure 6.--Bright-field photomicrographs showing increasing amounts of white rot in cross sections of pine with residual weights of:

A, 97 percent. Note early formation of bore holes in secondary walls of tracheids B and cell wall separation S.

B, 85 percent. Shows thinning of secondary wall of tracheids and enlargement of bore holes by penetrating hyphae B.

C, 65 percent. Note increased thinning of secondary wall of tracheids, increased diameter of bore holes B, and occurrence of checks within secondary wall C.

D, 50 percent. Shows almost total removal of secondary wall in some tracheids and removal of some regions of compound middle lamella (⇨). Note also thickened region of compound middle lamella at cell corners remained longer than other regions C.

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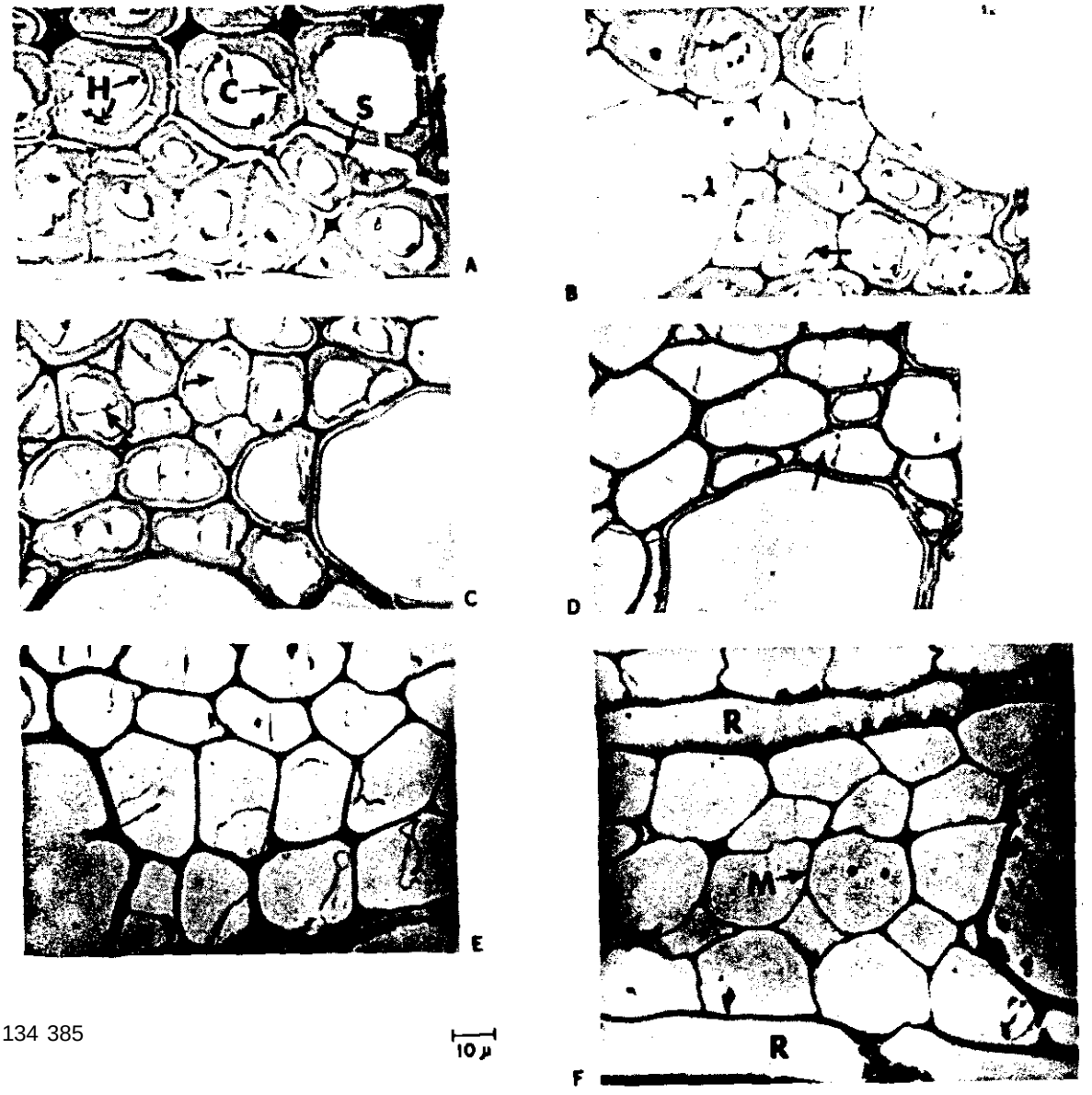


Figure 7.--Bright-field photomicrographs showing increasing amounts of brown rot in cross sections of sweetgum with residual weights of:

A, 96 percent. Shows hyphae in lumina of most fibers H, cell separation S, and checking C of the secondary wall.

B, 81 percent. Shows removal of S2 layer behind an apparently intact S3 layer in fibers (⇒).

C, 67 percent. Note continued removal of S2 layer behind apparently intact S3 layer in fibers (⇒).

D, 55 percent. Note almost complete removal of S2 layer, whereas both the S1 and S3 layers remain intact in many fibers (⇒).

E, 40 percent. SI layer is not apparent in most fibers whereas thin remnants of S3 layer are still visible.

F, 32 percent. The fiber compound middle lamella M and walls of vessels V and rays R appear intact.

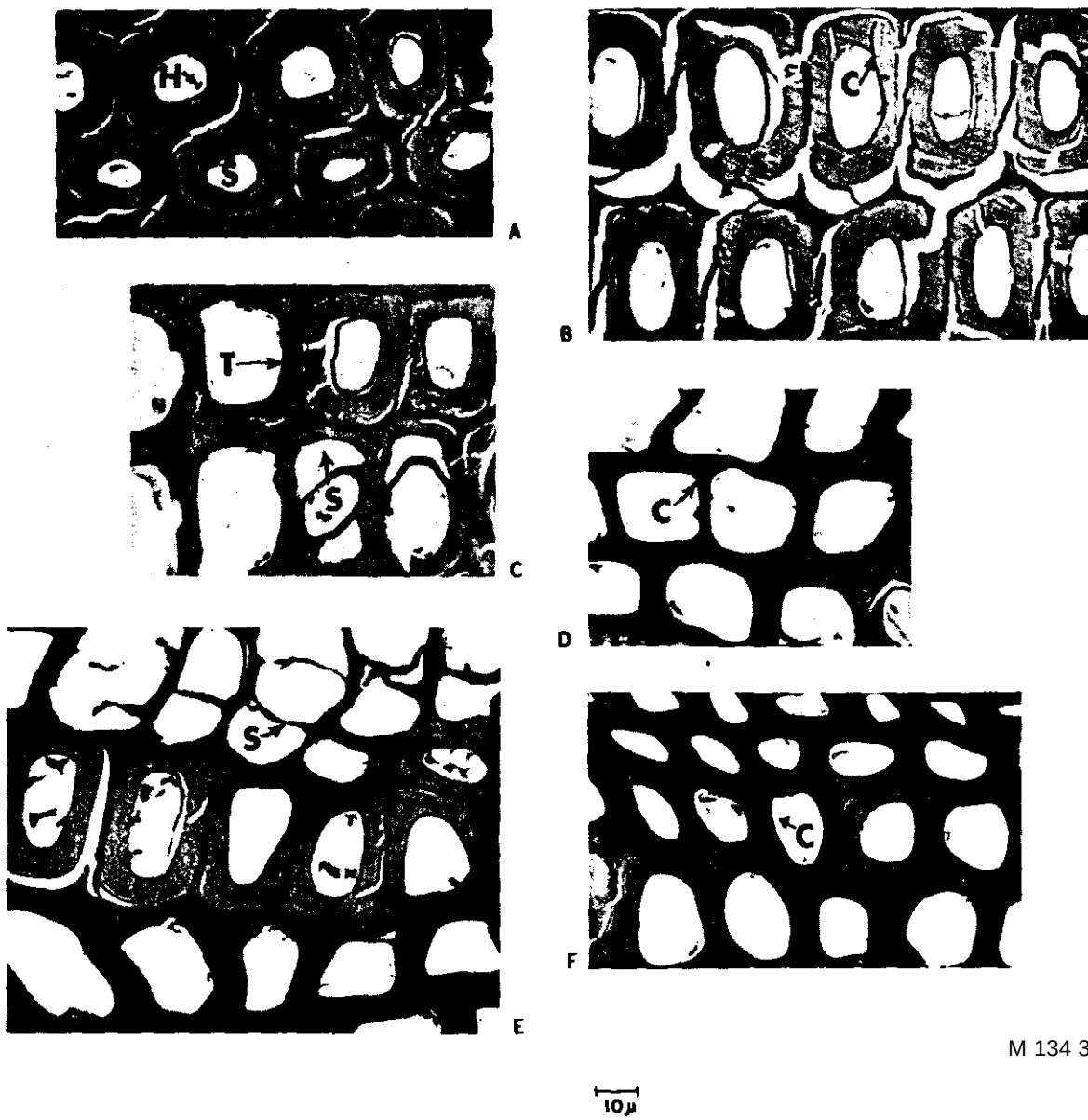


Figure 8.--Bright-field photomicrographs showing increasing amounts of brown rot in cross sections of pine with residual weights of:

A, 97 percent. Shows a few hyphae in lumina of tracheids H and cell separation S.

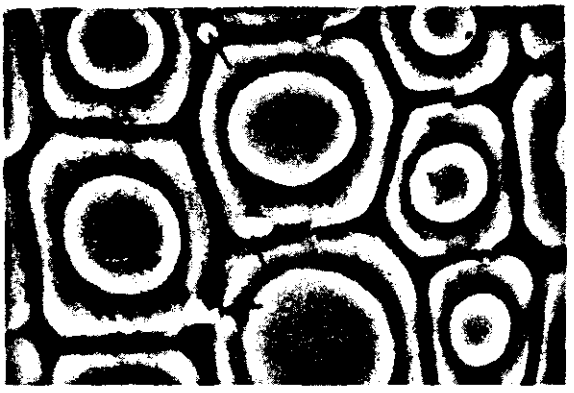
B, 85 percent. Note extensive cell separation S and checking C within secondary wall of tracheids.

C, 70 percent. Note removal of S2 layer behind an intact 53 layer in some tracheids S, and an apparent narrowing from lumen outward in others T.

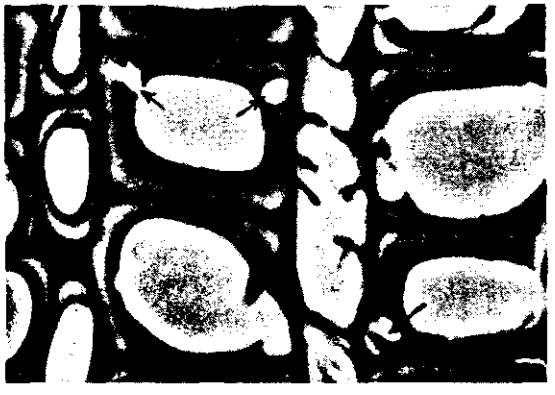
D, 55 percent. Shows collapse of cell walls of some tracheids C.

E, 45 percent. Shows intact appearance of secondary walls of tracheids in center of figure surrounded by cells in which wall collapse occurred. Note residual fragments of S3 layer in some tracheids S.

F, 35 percent. Shows extensive wall collapse C in many tracheids with resulting decrease in cell size.



A



B

10 μ

Figure 9.--Phase-contrast photomicrographs of cross sections of decayed pine showing cavities produced in the cell wall.

A, White rot; residual weight, 65 percent. Shows cavities within cell wall (⇒).

B, Brown rot; residual weight, 70 percent. Shows cavities within cell wall (⇒). M 134 387

less than the diameter of the hypha in the lumen. Neither district constriction nor subsequent enlargement of a hypha on passage through a bore hole was observed. The bore holes initially were the same diameter as the penetrating hypha or only slightly larger. However, in both woods the diameter of the bore holes and the diameter of the canals of penetrated pits increased greatly as decay progressed. By 65 to 75 percent residual weight in both woods, the bore holes and the pit canals had been enlarged four to eight times the diameter of the penetrating hypha At this point the origin of the hole in the cell wall--as a true bore hole or an enlarged pit canal--was obscure.

Differences also occurred between the two fungi in the location of the hyphae within the wood. The hyphae of the white rotter usually extended longitudinally in the cell lumina and penetrated the cell walls perpendicularly through bore holes or in pit canals in both woods. The hyphae of the brown rotter likewise usually extended longitudinally in the cell lumina but they seemed to penetrate the walls only through pit canals. Occasionally, cavities were observed within the cell wall of pine, which indicated that in this wood both fungi might grow within the cell wall parallel to the cell longitudinal axis. It could not be determined from cross sections whether or not the internal cavities were truly longitudinal. These cavities were observed within the secondary wall andcompound middle lamella in white-rotted pine (fig. 9A) but only within the secondary wall in brown-rotted pine (fig. 9B).

Effects of Decay on Walls of Sweetgum Fibers and Pine Tracheids

Changes in Microstructure

Because the thick-walled fibers of sweetgum and latewood tracheids of pine provide the bulk of the cell wall material in these woods, the effects of decay on the quantity of wall substance removed from each cell was most obvious in these elements. Moreover, these thick-walled fibers and latewood tracheids were of special interest because they are the main strength-giving components of these woods. Therefore, the principal information in this paper was obtained from study of these cells (fig. 4A and B).

Sequence of visible cell wall deterioration.--With white rot, a significant effect was the progressive removal of the secondary wall, called cell wall thinning (figs. 5 and 6). The thinning proceeded from the S3 layer outward, eventually including the compound middle lamella. In sweetgum by 85 percent residual weight (fig. 5B), only fragments of the S3 layer remained, and by 76 percent residual weight, the S3 layer had totally disappeared (fig. 5C). The extent of wall thinning was fairly uniform from cell to

cell. In sweetgum, the deteriorated cell wall surface adjacent to the lumen was irregularly eroded, and in many cells it appeared deeply serrate (fig. 5C). In pine, the surface adjacent to the lumen appeared smooth, and the thinning was fairly uniform in all portions of each cell wall (fig. 6B through 6D). There was no evidence of attack on the compound middle lamella until removal of the secondary wall was complete--at approximately 50 percent residual weight (figs. 5D and E, and 6D). In both woods, when the decomposition had progressed into the compound middle lamella, the thickened areas of the middle lamella at the cell corners were the last to he destroyed (figs. 5F and 6D).

With brown rot, the progressive thinning of the cell walls, which characterized the action of the white-rot fungus, was not observed. In the brown rot of sweetgum, decomposition began in the S2 layer of the secondary wall causing the S3 layer to become separated so that it lay free in the lumen (fig. 7B). The S2 layer disappeared before appreciable attackonthe remainder of the cell wall was detected (fig. 7D). The S1 layer of the secondary wall was visibly attacked next, whereas in many cells the S3 layer still remained intact although distorted and somewhat thinner (fig. 7D). Examination under ultraviolet illumination indicated that a thin portion of the S2 layer remained attached to the isolated S3 layer, thereby increasing its apparent thickness. This S2 layer residue disappeared prior to complete destruction of the S3 layer in advanced stages of decay. The compound middle lamella remained intact even in advanced stages of decay, although it appeared somewhat thinner in some regions than it did in sound wood (fig. 7F).

In early stages of brown rot in pine, a few cells exhibited preferential early removal of the S2 layer as in the sweetgum, but most showed little removal of cell wall substance other than as indicated by a slight narrowing of the walls (fig. 8B and C). Throughout these stages the secondary wall took on a green color when stained with safranin and fast green as it did in sound wood. However, when decay had neared a residual weight between 60 and 70 percent, the secondary wall of some cells became much narrower, and these cells also became smaller in cross section (fig. 8D); in this condition the residual secondary wall stained red rather than green. This change in staining properties suggested a pronounced reduction in cellulose in the collapsed cell walls. In these cells the secondary wall structure appeared simply to have collapsed or shrunk. The effect was irregular, and it was not uncommon to find small groups of cells or even single cells possessing a thick secondary wall that was stained green by safranin and fast green, surrounded by cells with thin and collapsed secondary walls that stained red (fig. 8E and F).

The decomposition due to brown rot in both sweetgum and pine was highly irregular, both from cell to cell and within each cell; this appearance was in striking contrast totheuniform attack observed in white-rotted wood In the brown-rotted wood it was not uncommon to find a small group of apparently intact cells adjacent to cells in which almost the entire secondary wall had been removed. In sweetgum the attack on the S2 layer did not progress at the same rate on all sides of a given cell. In many cells, decomposition of the S2 layer was greatest in regions adjacent to areas where hyphae in the lumen were appressed to the S3 layer (fig. 7A and B). However, this did not always occur: occasionally extensive decomposition occurred even on the side of the cell opposite that contacted by a hypha.

Differences in the action of the two types of decay fungi on the wood microstructure are shown in figure 10. The greater heterogeneity of attack by the brown-rot fungus than that by the white-rot fungus is illustrated by the smaller percentages of cells affected in early stages of brown rot. This dissimilarity was most evident in pine.

Cell shrinkage and wall collapse resulting from brown rot.--It was of interest to know whether the cell wall shrinkage or collapse observed in the brown-rotted pine tracheids could occur while the decaying wood remained moist or if it would require drying to produce it. Also of interest was what effect leaching with 2-methoxyethanol might have on these phenomena because both leaching and drying occurred during specimen preparation. This information would indicate the amount of the visible dimensional changes actually induced by preparation of the decayed wood for microscopical examination, It was observed that the size of the brown-rotted sample blocks was similar to

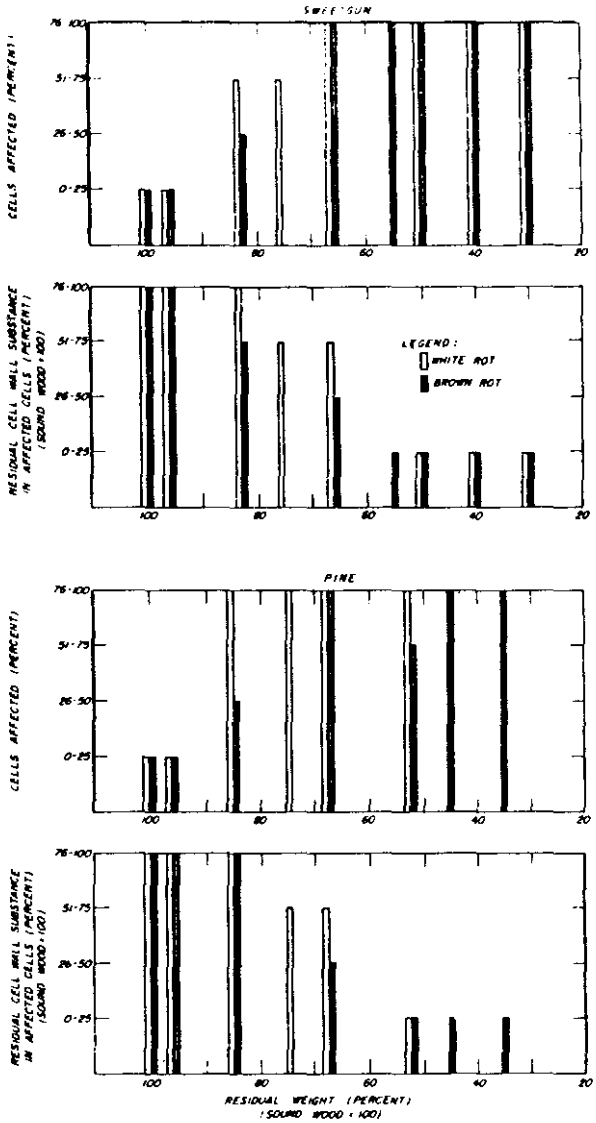


Figure 10.--Relation of residual weight of sweetgum fibers and pine latewood tracheids to percent of cells affected and to residual cell wall substance in affected cells. Because of width of bars, the position of each bar on horizontal axis indicates only approximate residual weight of sample. M 127 334

that of the sound blocks immediately after removal from the culture bottles. After drying, however, considerably more shrinkage occurred in the brown-rotted blocks than in the sound blocks. This great decrease in external dimensions after drying of the brown-rotted blocks suggested that internal dimensional changes, such as the observed cell shrinkage and wall collapse, might also occur during drying. Therefore a small experiment was undertaken. This experiment was performed under conditions comparable to those previously employed in preparing the wood for microscopical examination.

A block of brown-rotted pine with a residual weight of approximately 50 percent and a block of sound pine were treated as follows: (1) They were saturated with water under vacuum (The brown-rotted block was taken directly from the moist conditions of a soil-block culture bottle.); (2) They were air-dried; (3) They were saturated with 2-methoxyethnol under vacuum and again air-dried (4) The length and the width of a cross-sectional face on each block were recorded at each of the two swollen and the two air-dry conditions. From these dimensions, the cross-sectional area and the percent change in cross-sectional area were computed (table 1). A cross-sectional surface of each block at each of these conditions also was observed and photographed microscopically by means of incident illumination (fig. 11A through D).

The surface-microscopical study of sound and brown-rotted wood in both the wet and air-dry conditions (table 1) provided information on the nature of the extensive cell wall shrinkage and collapse that was observed in the sections of brown-rotted pine.

The comparative data for the two blocks indicated that the brown-rotted pine shrank considerably more than the sound pine during drying and that not all of this shrinkage was recovered by resaturation of the brown-rotted wood, as it was by the sound wood. In addition, the microscopical observations of the block surfaces--water saturated and air-dry--suggested that changes in cell size andwallthickness were closely correlated with the measured changes in dimensions (fig, 11A through D). Apparently much of the cell and wall shrinkage occurred during drying of the decayed wood. Contact with 2-methoxyethanol before drying appeared to accentuate the shrinkage, presumably through leaching of degradation products of the decay.

Cell separation and checking of secondary wall.--Other features of decay examined in detail were a tendency for the cells to separate and for checks to appear in the secondary walls (fig. 12). Cell separation was seldom observed in sound wood, but in the decayed wood it was apparent

Table 1.--Comparativedimensional changes in sound and brown-rotted pine blocks during drying

Condition of block	Treatment of block	Linear dimensions of cross-sectional surface[1]		Cross-sectional area	Relative cross-sectional area
		x	y		
		Millimeter	Millimeter	Millimeter	
Sound	Water-saturated	20.5	19.8	405.9	100
	Air-dried (from water)	19.3	18.9	364.8	90
	As above, then saturated with 2-methoxyethanol	20.4	19.8	403.9	100
	Air-dried (from 2-methoxyethanol)	19.4	18.9	366.7	90
Brown-rotted (approximately 50 percent residual weight)	Water-saturated	19.3	19.0	366.7	100
	Air-dried (from water)	15.6	15.2	237.1	65
	As above, then saturated with 2-methoxyethanol	18.6	18.0	334.8	91
	Air-dried (from 2-methoxyethanol)	[2]13.7	[2]11.5	[2]157.6	43

[1] x and y were not truly radial or tangential because the annual rings ran diagonally across the cross-sectional faces of both blocks. However, the cross-sectional surfaces were rectangular throughout treatment except as noted in footnote 2, and they could be so considered in computing area.

[2] Measurements and calculations were based on a parallelogram rather than a rectangle because the cross-sectional surface had assumed a rhomboidal shape due to differential shrinkage.

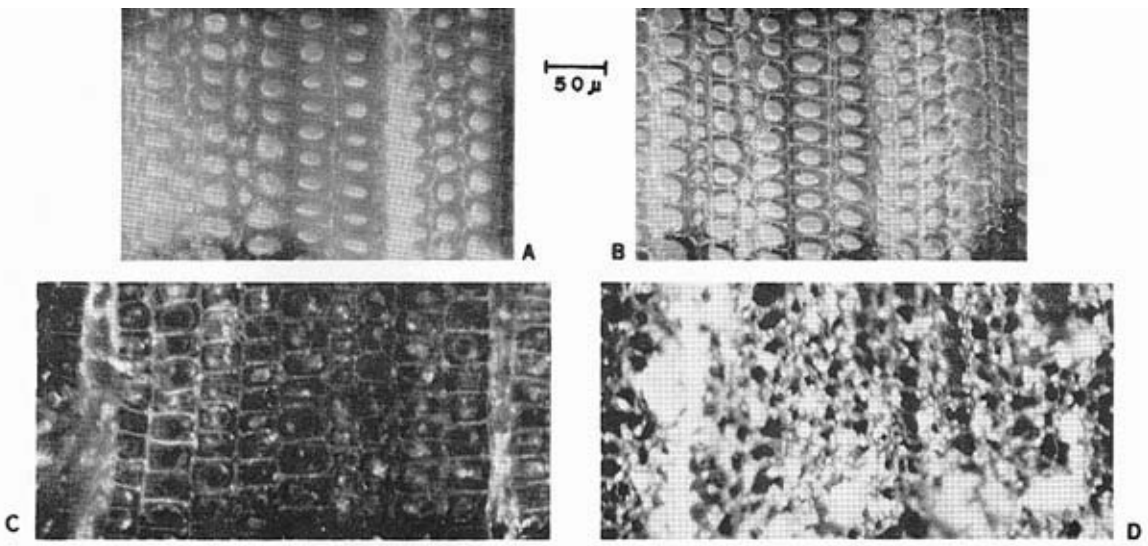


Figure 11.--Incident-light photomicrographs of cross-sectional surfaces of sound and brown-rotted pine showing effects of moisture condition and decay on cell size.

A, Block of sound pine in water-saturated condition.

B, Same block after air-drying. Size of cells has diminished only slightly.

C, Block of brown-rotted pine with residual weight of approximately 50 percent. Shows little difference in size of cells compared with those of sound pine in water-saturated condition A.

D, Same block after air-drying. Thickness of cell walls has diminished greatly as has size of cells from those in water-saturated condition and both have diminished more than in the air-dried sound block B. M 134 388

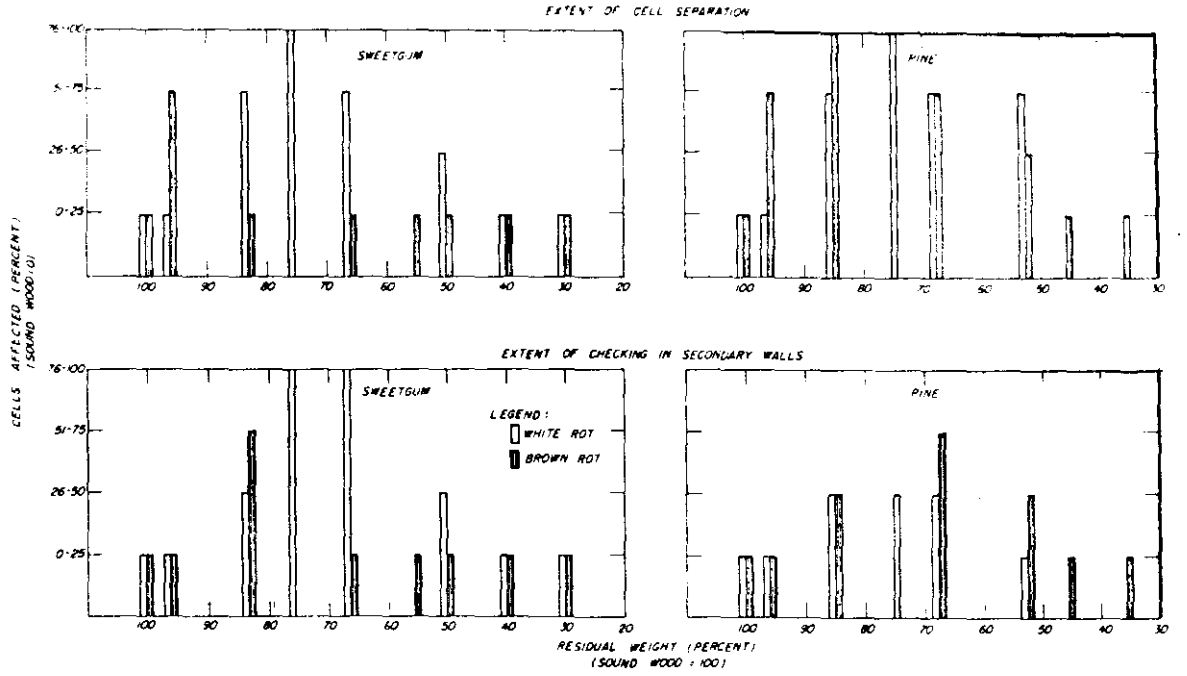


Figure 12.--Extent of cell separation and secondary wall checking in sweetgum fibers and pine latewood tracheids. (See figures 5B, 6A, 7A, 8A and B for cell separation and figures 6C, 7A, and 8B for checking.) Because of width of bars, the position of each bar on horizontal axis indicates only approximate residual weight of sample. M 127 333

in early stages of decay suggesting an early weakening of the zones near the middle lamella (figs. 5B, 6A, 7A, and 8A and B). In the white rot of both woods the number of cells showing separation was greatest in intermediate stages of decay (fig. 12). This also was true of brown rot in pine, although the number of separated cells was greater in early stages of decay than it was with white rot. In brown-rotted sweetgum cell separation was greatest in early stages and was low throughout intermediate and advanced stages.

A difference was noted between the sweetgum and the pine as to the zone in which cell separation occurred. With both types of rot, cell separation in the sweetgum occurred predominantly within the compound middle lamella or between it and the S1 layer (fig. 13A), whereas in the pine it was predominantly within the S1 layer or between the S1 and the S2 layer (fig, 13B). Therefore, it appeared that cleavage between cells often occurred in regions other than the true middle lamella. Nevertheless, in the white rot of both sweetgum and pine the compound middle lamella often was fractured so that short segments of it with attached fragments of S1 clung alternately to one and then the other of two contiguous cells that had separated.

Checking of the secondary wall was still another microscopically visible effect of decay (figs. 6C, 7A, and 8B). Checking was absent in the secondary walls of sound wood and was more prominent in intermediate stages of decay than in very early or advanced stages (fig. 12). Its occurrence at high residual weights suggested an early weakening of the secondary wall by the decay fungus.

Optically Indicated Depletion of Wood Constituents

Lignin depletion.--The major indicator relied on for evidence of changes in lignin content associated with progressive stages of decay was absorption of ultraviolet radiation at a wavelength of 2780 angstroms. Because results correlated with those obtained by staining, the as-

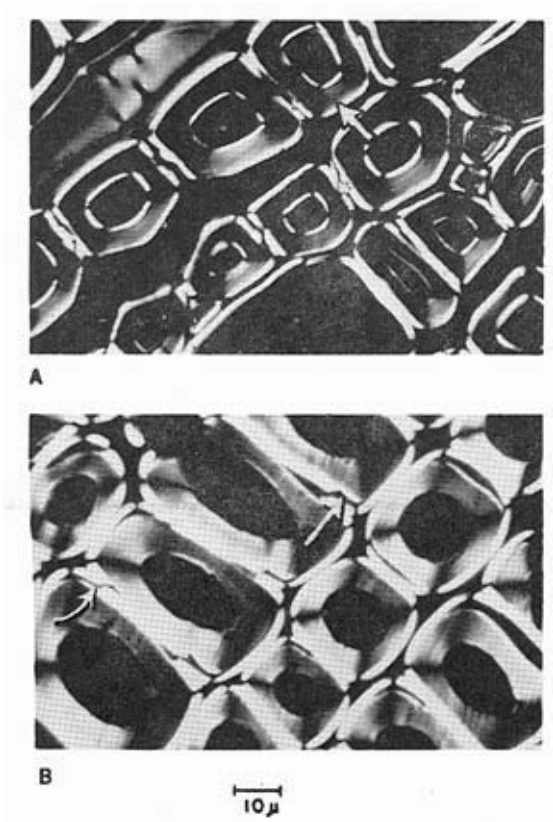


Figure 13.--Polarized-light photomicrographs of cross sections with early stages of decay indicating differences between sweetgum and pine in location of failure during cell separation. Location was similar for both types of decay.

A, Brown-rotted sweetgum; residual weight, 90 percent. S1 and S3 layers are indicated by bright regions of secondary wall (fig. 1). Cell separation is predominantly between S1 layer and compound middle lamella (⇒).

B, White-rotted pine; residual weight, 85 percent. S1 and S3 layers are indicated by bright regions of secondary wall (fig. 1). Cell separation is predominantly between S1 and S2 layers or within S1 layer (⇒). M 134 389

sumption was strengthened that the degree of opacity to ultraviolet radiation could appropriately be taken as an indicator of lignin content. The only exceptions to this correlation occurred in advanced stages of attack of both woods by the white-rot fungus. In these, at a stage when the compound middle lamella was under attack, the compound middle lamella lost some of its ability to be stained by both safranin and azure B, whereas its ultraviolet opacity did not appear to have diminished significantly.

Some of the observations of ultraviolet absorption are illustrated in figures 14 through 17. The S2 layer of cells of sound sweetgum was distinctly gray but was the least opaque of any of the cell wall layers (fig. 14A). The S1 layer was dark gray, whereas the S3 layer was almost black: however, as expected, the most ultraviolet-opaque region of the cell wall, and therefore the darkest, was the compound middle lamella. The appearance of sound pine under ultraviolet irradiation was similar to that of sweetgum but the total absorption was greater (fig. 14B). In pine the S2 layer appeared dark gray in contrast with the light gray of this layer in sweetgum Also, unlike the situation in sweetgum, the S3 layer was not distinctly darker than the S2 layer. The opacity of the S1 layer of pine appeared somewhat greater than that of sweetgum

In the decayed wood, the effects on lignin varied according to the portion of the cell wall affected and the fungus involved. In sweetgum, a distinct difference could be noted in the mode of action of the two types of decay fungi. In early stages of white rot the ultraviolet opacity of the S3 layer disappeared. As decay progressed, ultraviolet opacity of the S2 layer disappeared prior to actual destruction of this layer (fig. 15A). The S1 layer and the compound middle lamella retained their opacity. By 28 percent residual weight (fig. 15B) only the most ultraviolet opaque regions remained--the compound middle lamella and walls of the rayparenchymacells and vessels.

In the brown rot of sweetgum, no evidence was obtained of a decrease in ultraviolet opacity in advance of actual removal of wall layers as had occurred in the white rot (fig. 16A and B); rather, an increase in opacity was apparent in some regions of the residual cell wall. By 55 percent residual weight a region appeared adjacent to the compound middle lamella that was similar in opacity to the S1 layer of sound wood but approximately twice as thick, suggesting a condensation or collapse of the residual lignin in the disappearing S2 layer (fig. 16B). The distinction between this region and the compound middle lamella was lost as decay progressed (fig. 16C)

The changes in ultraviolet absorption resulting

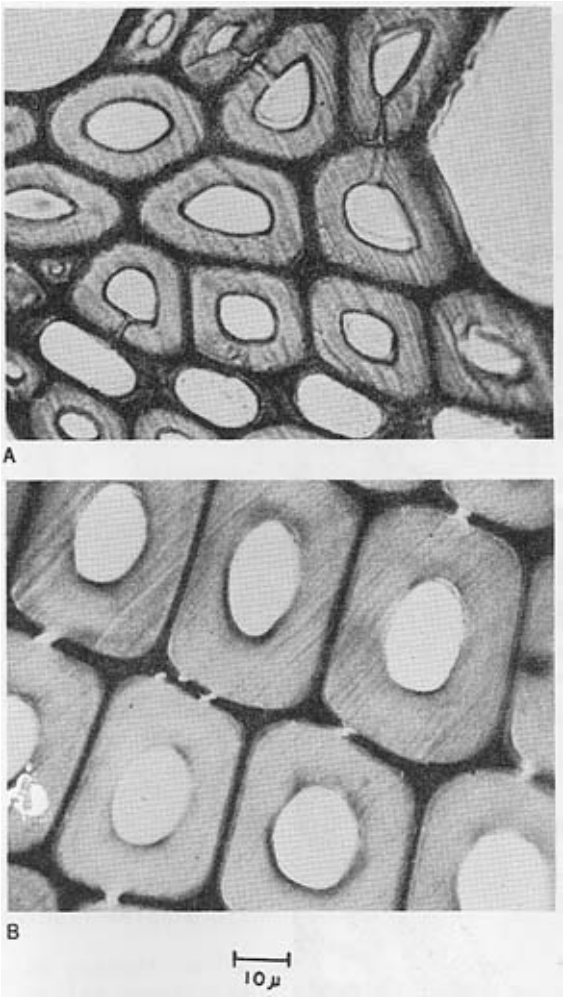


Figure 14.-- Ultraviolet-absorption photomicrograph of cross sections of sweetgum and pine in sound condition. At wavelength of 2780 angstroms, degree of absorption, indicated by darkness of image, was an indicator of lignin content.

A, Sound sweetgun.

B, Sound pine.

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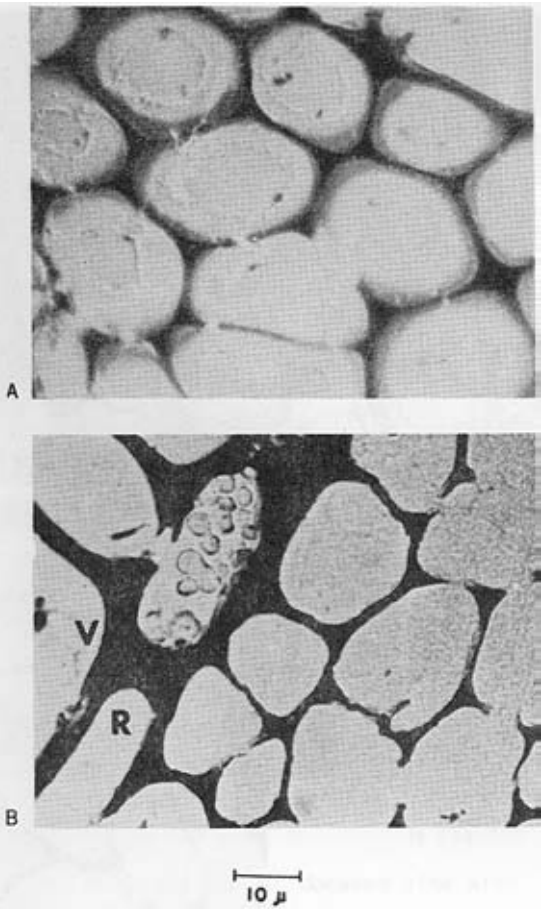

Figure 15.--Ultraviolet-absorpition photomicrograph of cross sections of white-rotted sweetgum with residual weights of:

A, 76 percent. Shows absence of S3 layer, loss of ultraviolet opacity in portions of S2 layer of many cells, and serrated appearance of portion of S2 layer bordering lumen.

B, 28 percent. Material in cell lumina is embedding matrix not removed from the section. Note high degree of ultraviolet opacity in walls of vessels V and ray parenchyma cells R that appear essentially intact.

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from decay in pine differed from those in sweet-gum In pine, action of the white-rot fungus caused no decrease in ultraviolet opacity of the cell wall layers in advance of actual destruction of the layers as was true in sweetgum (fig. 17A and B). In the brown rot, in the stages preceding the development of the collapsed or shrunken appearance of the cell walls (above 60 percent residual weight), the secondary wall appeared gray in contrast to the darkblackofthe compound middle lamella (fig. 17B). When cellwallcollapse occurred below 60 to 70 percent residual weight, the distinction under ultraviolet illumination be-tween secondary wall and compound middle lamella was lost (fig. 17D). The cells, which also were much smaller in size, had walls that uniformly possessed the high degree of opacity

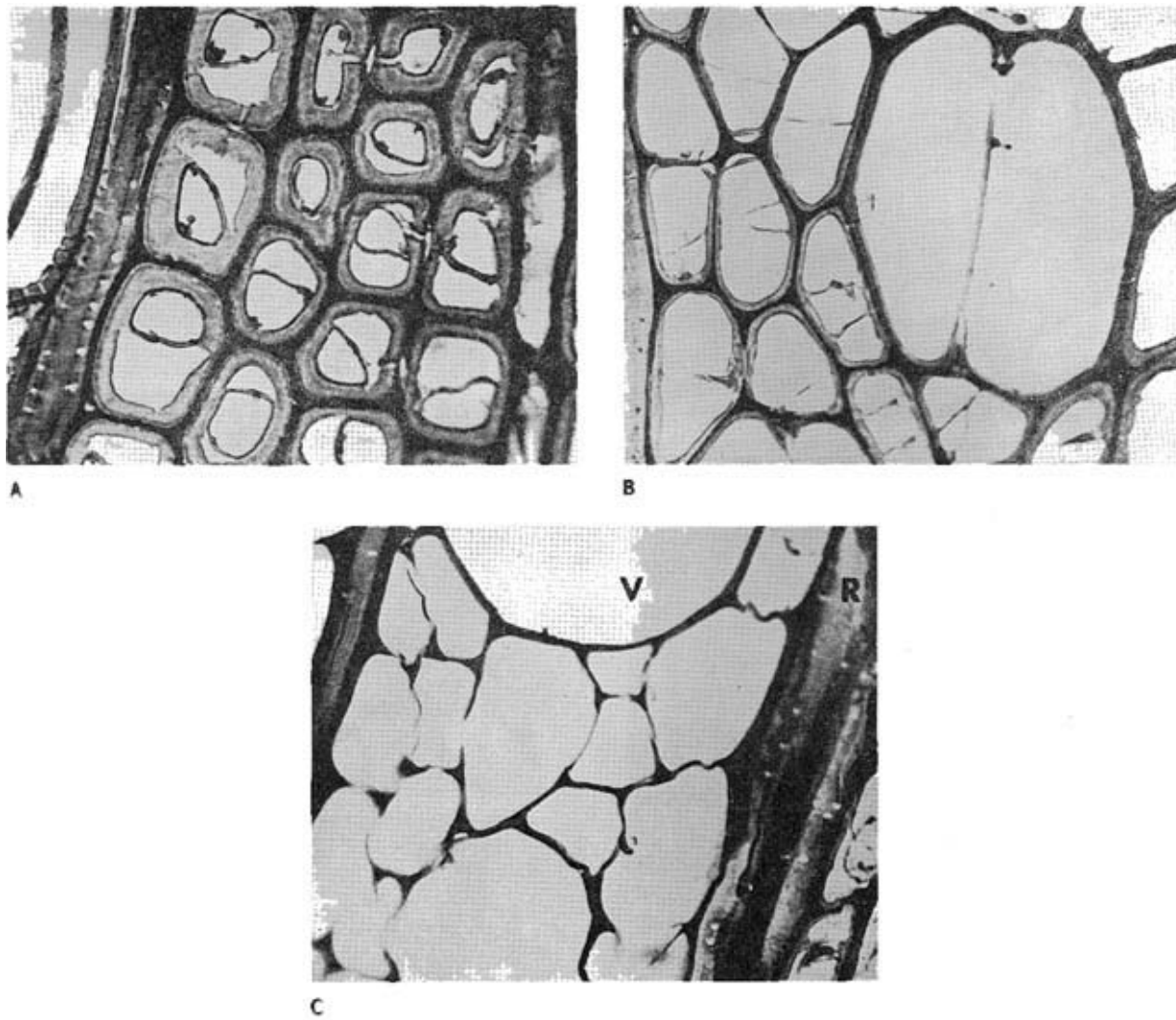

Figure 16.--Ultraviolet absorption photomicrographs of cross sections of brown-rotted sweetgum with residual weights of:

A, 81 percent. Relatively high degree of ultraviolet opacity is present in S3 layer as compared with S2 layer.

B, 55 percent. Residual portion of S2 layer appears as dark. as S1 layer making these layers indistinguishable from one another. Apparently at this stage a concentration of lignin had developed in residual S2 layer.

C, 32 percent. Shows high degree of ultraviolet opacity in walls of vessels V and ray parenchyma cells R that appear essentially intact.

present originally only in the compound middle lamella; however, the walls were many times thicker than the compound middle lamella alone. In the few pine tracheids in which separation of an intact S3 layer was observed in early stages of brown rot, a condition that predominated in the sweetgum fibers, no differences between those tracheids and those not showing this phenomenon were indicated by the ultraviolet investigation.

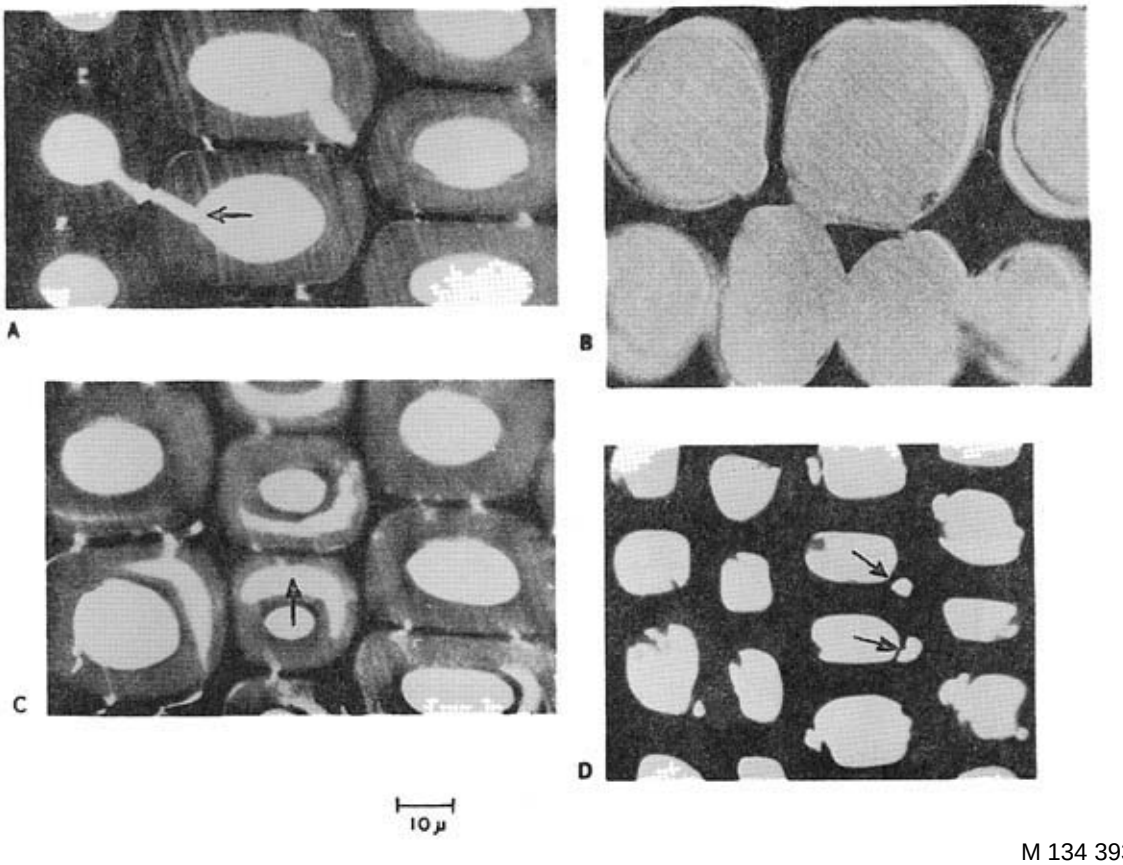

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Figure 17.--Ultraviolet-absorption photomicrographs of cross sections of decayed pine with residual weights of:

A, 85 percent (white rot). Note bore holes through cell walls (→) and retention of ultraviolet opacity in S2 layer of secondary wall.

B, 50 percent (white rot). Material in cell lumina, which has been pulled away from the residual secondary wall, is embedding matrix not removed from section. Note correspondence between loss of ultraviolet opacity in S2 layer of secondary wall and actual removal of that layer.

C, 85 percent (brown rot). Shows attack on S2 layer behind an intact S3 layer in some cells (→).

D, 35 percent (brown rot). Shows high degree of ultraviolet opacity in collapsed cell walls and lack of differentiation between secondary wall and compound middle lamella. Note discrete cavities within cell wall (→).

Examination of decayed wood under ultraviolet illumination suggested that the white-rot fungus in sweetgum was able to remove lignin from cell wall layers in advance of the actual destruction of those layers. In pine, lignin seemingly was removed concomitantly with. but not in advance of, destruction of the associated portion of the cell wall. This difference in the effects of the white-rot fungus on sweetgum and pine may have been due in part to the apparent higher lignin content of pine, which may have masked small changes in ultraviolet absorption. No evidence was obtained of removal of lignin by the brown-rot fungus from otherwise intact cell wall layers.

Cellulose depletion.--Results of observations of secondary walls under polarized light are presented in figure 18. Crystalline cellulose in the different layers is indicated by the relative brightness or birefringence of the layers when viewed between crossed polarizing filters. The data in the graphs represent the majority of

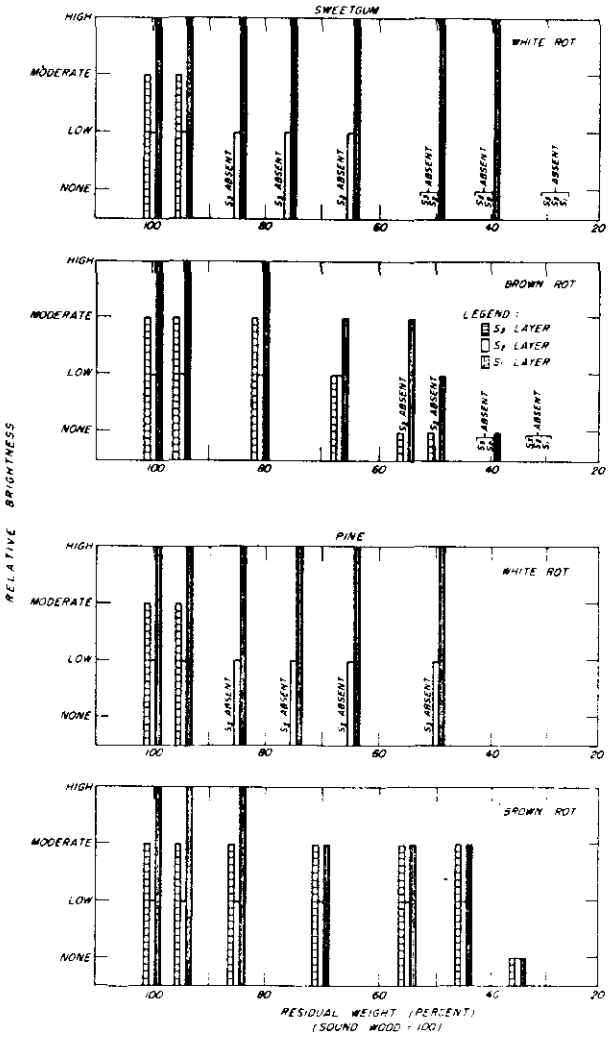

Figure 18.--Relation of residual weight to relative brightness of layers of secondary walls of sweetgum fibers and pine trachieds when examined microscopically between crossed polarizing filters. None refers to cases in which a layer was present but dark; absent, to layers of secondary wall no longer detectable. Because of width of bars, the position of each bar on horizontal axis indicates only approximate weight of sample.
M 127 332

cells and wall areas. For example, although in a number of cells of brown-rotted pine with a residual weight of 67 percent the S2 layer had been removed, observations still were recorded for this layer until it had been removed from more than 50 percent of the cells (55 percent residual weight).

Alterations in birefringence indicated further differences in the mode of action on the secondary wall between the white-rot fungus and the brown-rot fungus (figs. 19 through 22). The white-rot fungus destroyed the birefringence of each secondary wall layer in order, progressing from the S3 to the S2, and finally to the S1 layer (fig. 20A and B). This was true for pine as well as sweetgum, but in pine the S1 layer appeared to resist degradation to almost the same extent as the compound middle lamella because in areas

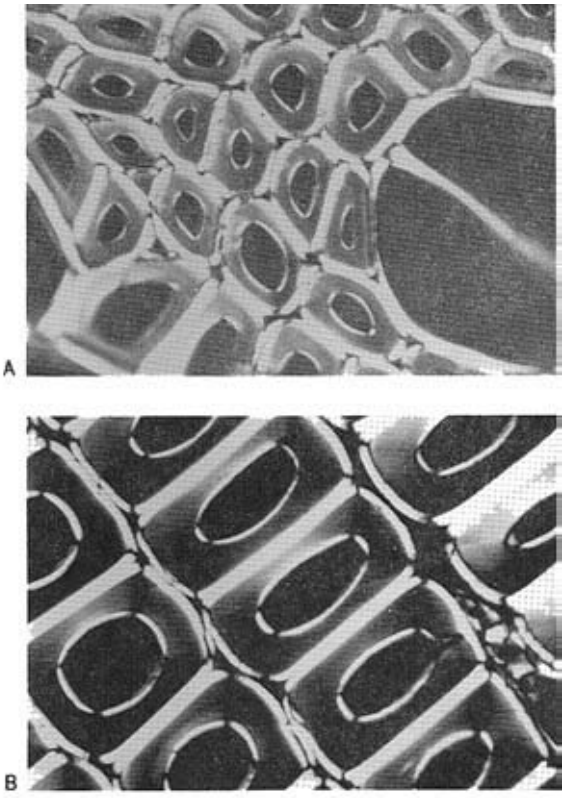

Figure 19.--Cross sections of sound sweetgum and sound pine photographed between crossed polarizing filters. The S1 and the S3 layers of the secondary walls of the fibers and tracheids appear brighter than the S2 layer because orientation of cellulose crystallites in the S1 and the S3 layer is relatively perpendicular to the path of the polarized light.

A, Sound sweetgum.

B, Sound pine. M 134 394

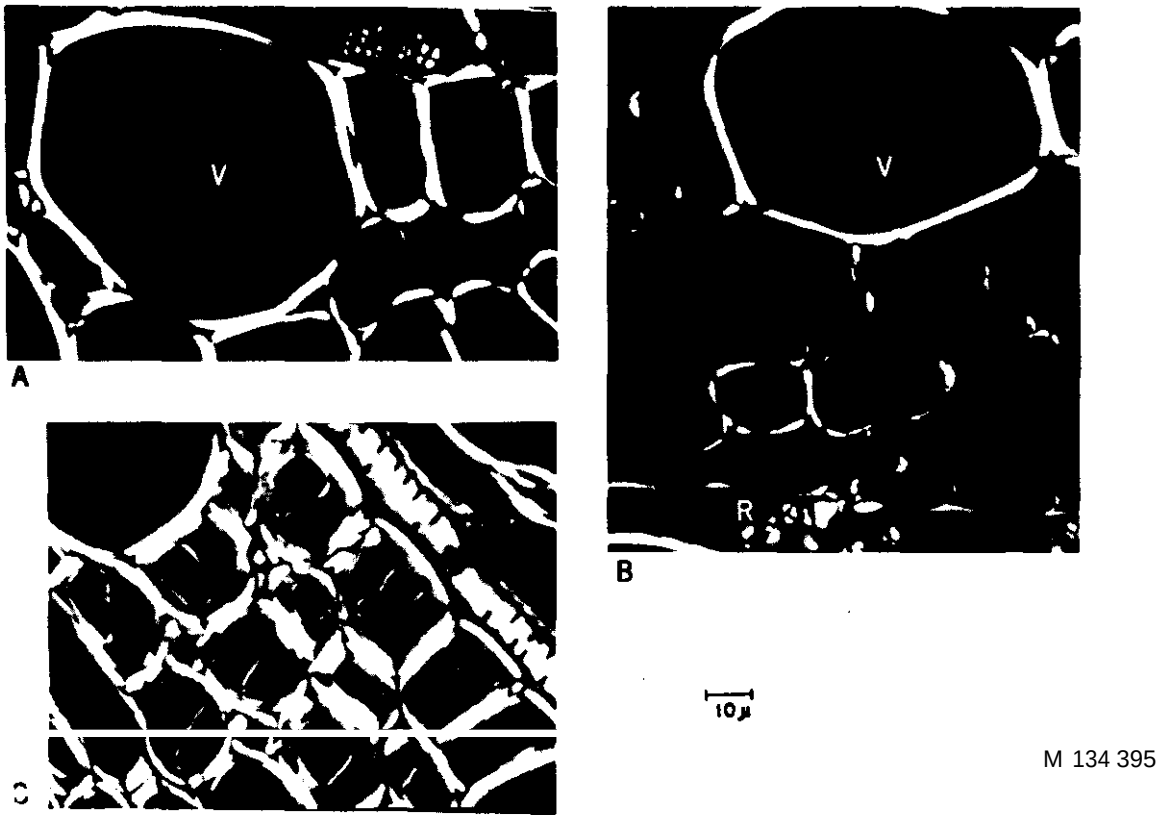

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Figure 20.--Polarized-light photomicrographs of cross sections of decayed sweetgum with residual weights of:

A, 40 percent (white rot). Shows bright S1 layer in residual secondary wall of most fiber and brightness in walls of vessels V and ray parenchyma cells R. S2 and S3 layers of secondary wall of fibers are absent.

B, 28 percent (white rot). Shows absence of all three layers of secondary wall of most fibers. Walls of vessels V and ray parenchyma cells R still appear bright.

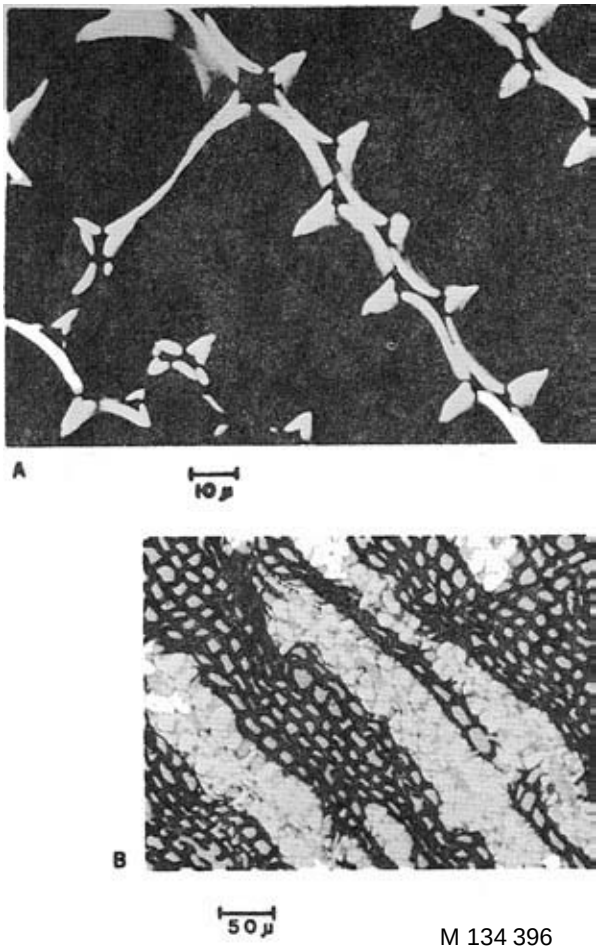
C, 81 percent (brown rot). Shows residual brightness of both Si and S3 layers of fiber secondary wall even after extensive removal of S2 layer.

where the S1 layer had been removed the compound middle lamella was absent also (fig. 21A). However, in these experiments a weight loss in the white-rotted pine high enough to remove all secondary wall layers in most cells was not obtained, and therefore is not represented in figure 18.

In the attack of the brown-rot fungus on sweetgum, although the birefringence of all three layers of the secondary wall appeared to diminish, total elimination occurred in the S2 layer first. The S3 layer retained its birefringence after becoming isolated due to the removal of the S2 layer (fig. 20C), but lost it before the S1 layer was destroyed.

In the attack of the brown-rot fungus on pine, birefringence and total wall substance disappeared in localized portions of the S2 layer of some cells in early stages of decay, as they did in sweetgum; however, the majority of tracheids showed little change prior to wall collapse. The data in figure 18 at 45 and 55 percent residual weight represent those cells in which the secondary wall had not yet collapsed. In cells in which the wall had collapsed, all birefringence of the secondary wall was lost; by 35 percent residual weight the majority of the cells were in this collapsed condition and, therefore, showed no birefringence (fig. 21B).

These data suggest that the white-rot fungus destroyed the crystalline cellulose of each wall layer in succession from the S3 to the S1 layer

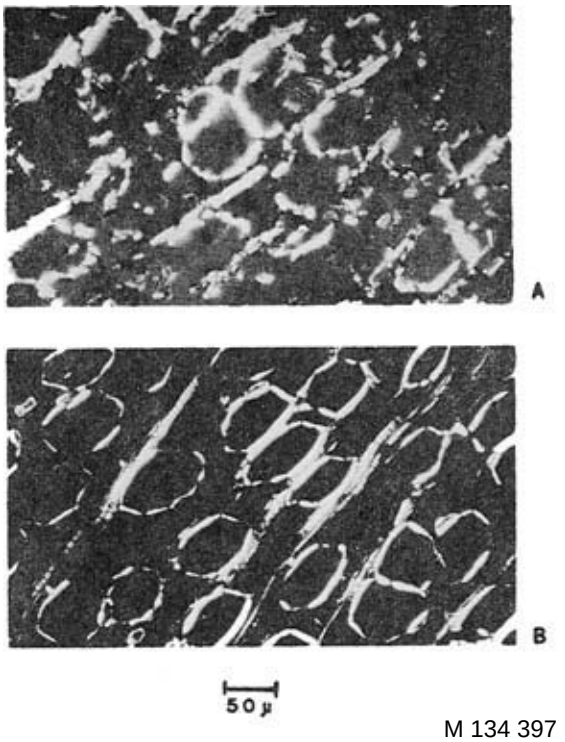


M 134 396

Figure 21.--Polarized-light photomicrographs of cross sections of decayed pine with residual weights of:

A, 50 percent (white rot). S2 and S3 layers are abient in most tracheids, and compound middle lamella is missing in regions where S1 layer has been removed in addition to S2 and S3 layers.

B, 35 percent (brown rot). Birefringence was absent in cells in which the walls had collapsed, and was retained in cells in which the walls had not collapsed. Note apparent association of long rows of uncollapsed cells with rays.



M 134 397

Figure 22.--Polarized-light photomicrographs of cross sections of sweetgum in advanced stages of decay with residual weights of:

A, 6 percent (white rot). Shows presence of relatively intact and birefringent walls of vessels and ray parenchyma cells even though most fiber walls have been completely destroyed.

B, 32 percent (brown rot). Shows presence of relatively intact and birefringent walls of vessels and ray parenchyma cells even though most fiber walls lack birefringence.

as it was exposed by removal of the preceding layer: conversely, it appeared that the brown-rot fungus destroyed crystalline cellulose in internal regions of the cell walls beforedepletion in the intervening regions was complete.

Depletion of minor wood constituents.--Some controversy has existed on the significance of pectin in the bond between cells (25, 42). In this work attempts to employ several methods recommended for detection of pectic substances (20) on sections 4 microns thick were unsuccessful. Because the specificity of histochemical procedures for detecting pectin has been questioned and because it appeared that failure within the middle lamella was not a predominant cause of cell separation, no further investigations of this kind were attempted.

A large amount of starch was present in the ray parenchyma cells of sweetgum, and the disposition of this starch through progressive stages of decay differed between the action of

the white-rot and the brown-rot fungus. The white rotter appeared to have little effect on the starch, because starch was still detectable in wood with only 28 percent residual weight. However, the starch was destroyed by the brown rotter in early stages of decay—prior to 81 percent residual weight. No starch was detected in the rays of the sound or decayed pine.

Effects of Decay on Wood Rays

Little if any decomposition of the ray parenchyma cells by either the white-rot or the brown-rot fungus occurred at any stage of decay (figs. 5, 7, 15, and 16--where rays are shown). The ray parenchyma cell walls stained red to reddish-green with safranin and fast green, and dark green with azure B, but not as dark as the compound middle lamella between fibers. When viewed from the side (as in cross sections), these cell walls had a birefringence equal to or slightly greater than that of the S2 layer of the fibers viewed in cross section. They also were highly absorbent of ultraviolet radiation but somewhat less so than the compound middle lamella between fibers. The staining and optical properties were retained into advanced stages of decay (fig. 22). These properties were presumed to indicate both the presence of considerable cellulose and a relatively high lignin content, which apparently was not depleted by either decay fungus.

The rays of the pine consisted of both ray parenchyma cells and ray tracheids. The ray parenchyma cells of pine were difficult to study. Probably because of the extreme difference in strength and hardness between the very thin-walled ray parenchyma and the thick-walled latewood tracheids, no thin sections were obtained that possessed intact ray parenchyma cells. The ray tracheids of the pine, in contrast to the ray parenchyma cells of sweetgum were attacked by the decay fungi. Also, a difference was noted in the action of the white-rot fungus and the brown-rot fungus on the walls of the pine ray tracheids. The secondary walls appeared to be completely destroyed by the white-rot fungus, whereas in the brown-rotted wood, although they had been attacked and were somewhat thinner, they were not totally destroyed. In advanced stages of brown rot, the ray tracheids had lost much of their birefringence and stained a deeper red with safranin and fast green and a deeper green with azure B, suggesting a loss in cellulose.

Effects of Decay on Vessels

The vessels of sweetgum appeared to be little affected by either the white-rot fungus or the brown-rot fungus. A slight thinning of the secondary walls of these elements may have occurred in advanced stages of decay, but it was not at the expense of a particular layer, and the walls were still intact (fig. 22). When viewed in cross section, the S1 and the S3 layers of the vessel secondary walls had a high degree of birefringence, comparable to that of the S1 and S3 layers of the fibers in sound wood. However, in the vessel secondary walls the thin S2 layer provided so little separation between the S1 and S3 layers that the wall often appeared uniformly bright when viewed in cross section between crossed polarizing filters. This birefringence was not diminished even in advanced stages of decay.

The vessel secondary walls had a high absorption of ultraviolet radiation, comparable to that of the ray parenchyma cell walls. Also, like the ray cell walls, they stained reddish-green with safranin and fast green and dark green with azure B. These optical and staining properties indicated that the vessel secondary walls contained considerable cellulose and also had a relatively high lignin content, which remained throughout the decay process.

Changes in Cross-Sectional Area

Removal of substance from the various layers of the cell wall and the resultant decrease in the amount of cell wall visible in cross section raised the question of how much of the residual weight of wood at each stage of decay could be accounted for by the respective residual areas of cell wall observable with the light microscope. This was a point of interest because it might answer a fundamental questionregardingremoval of substance from within the cell wall: Was a significant amount of material removed from the wall without being apparent in the light

Table 2.--Changes in cross-sectional area of sweetgum fibers and pine latewood tracheids[1]

Decay	Residual weight	Number of cells per sampling field	Average proportion of field occupied by single cell: Cell wall	Average proportion of field occupied by single cell: Lumen area	Relative area of: Wall	Relative area of: Lumen	Relative area of: Entire cell[2]	Wall to lumen ratio
	Percent		Percent	Percent				
SWEETGUM								
None (sound wood)	100	191	0.30	0.22	100	100	100	1.36
White rot	97	209	.27	.21	90	96	92	1.29
	85	215	.24	.22	80	100	88	1.09
	76	169	.30	.29	100	132	113	1.03
	65	190	.25	.27	83	123	100	.93
	50	228	.10	.34	33	155	85	.29
	40	179	.15	.41	50	186	108	.37
	28	224	.04	.41	13	186	87	.10
Brown rot	96	208	.25	.23	83	105	92	1.09
	81	220	.18	.27	60	123	87	.67
	67	216	.17	.30	57	136	90	.57
	55	215	.12	.34	40	155	88	.35
	50	225	.09	.35	30	159	85	.26
	40	214	.09	.37	30	168	88	.24
	32	217	.07	.39	23	177	88	.18
PINE								
None (sound wood)	100	117	0.68	0.18	100	100	100	3.78
White rot	97	138	.61	.12	90	67	85	5.08
	85	150	.46	.21	68	117	78	2.19
	75	161	.34	.28	50	156	72	1.21
	65	140	.35	.36	52	200	83	.97
	50	116	.28	.58	41	322	100	.48
Brown rot	97	143	.52	.17	77	94	80	3.06
	85	129	.54	.23	79	128	89	2.35
	70	143	.43	.27	63	150	81	1.59
	55	172	.37	.21	54	117	67	1.76
	45	230	.25	.18	37	100	50	1.39
	35	312	.19	.13	28	72	37	1.46

[1] Measured on thin sections of sound and decayed wood that were celloidin embedded, sectioned, stained, and mounted.

[2] Values below 100 indicate cell shrinkage; valves above 100 indicate cell enlargement.

microscope? It also was considered desirable to characterize each stage of decay according to the relative cross-sectional areas of residual cell wall and of lumen to provide a more quantitative basis for comparisons of wall thinning or narrowing than was previously available.

For this purpose relative cell wall and lumen areas were measured using the randomized-point sampling device developed by Ladell (26). A summary of these measurements appears in table 2. Each relative area shown is the average for five individual sampling fields within the

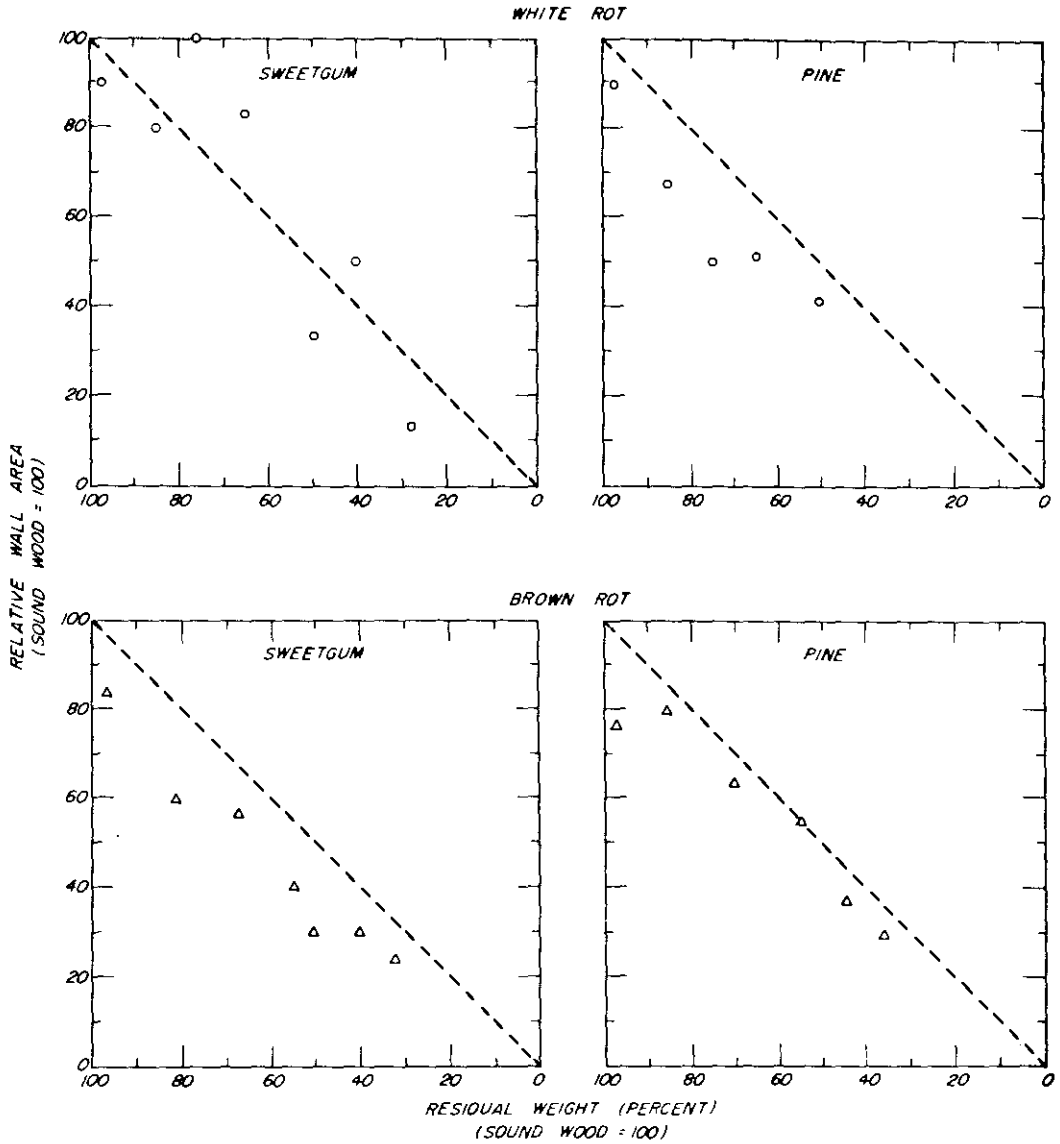


Figure 23.--Relationship between relative wall area (table 2) and residual weight. M 127 331

single Section selected earlier as being representative of each residual weight. For each residual weight approximately 1,000 cells of sweetgum and 700 of pine were included.

The relation between residual weight and relative area of visible cell wall substance (relative wall area) is shown graphically in figure 23. Theoretically, if all of the residual wall substance represented by residual weight of a decayed sample could be seen microscopically in cross section and its area measured and if the density of the residual wall substance were unchanged from that of sound wood, a straight line relationship in which all values for relative wall area would be equal to those for residual weight would be shown (fig. 23).

From a regression analysis it was determined that at a confidence level of 95 percent, the data of figure 23 were not significantly different from the theoretical relationship with the exception of the data from brown-rotted sweetgum that were significantly different. This deviation is in the direction indicating that less relative area was measured than would be expected from the residual weight. Consequently, it seems reasonable to conclude that virtually all losses

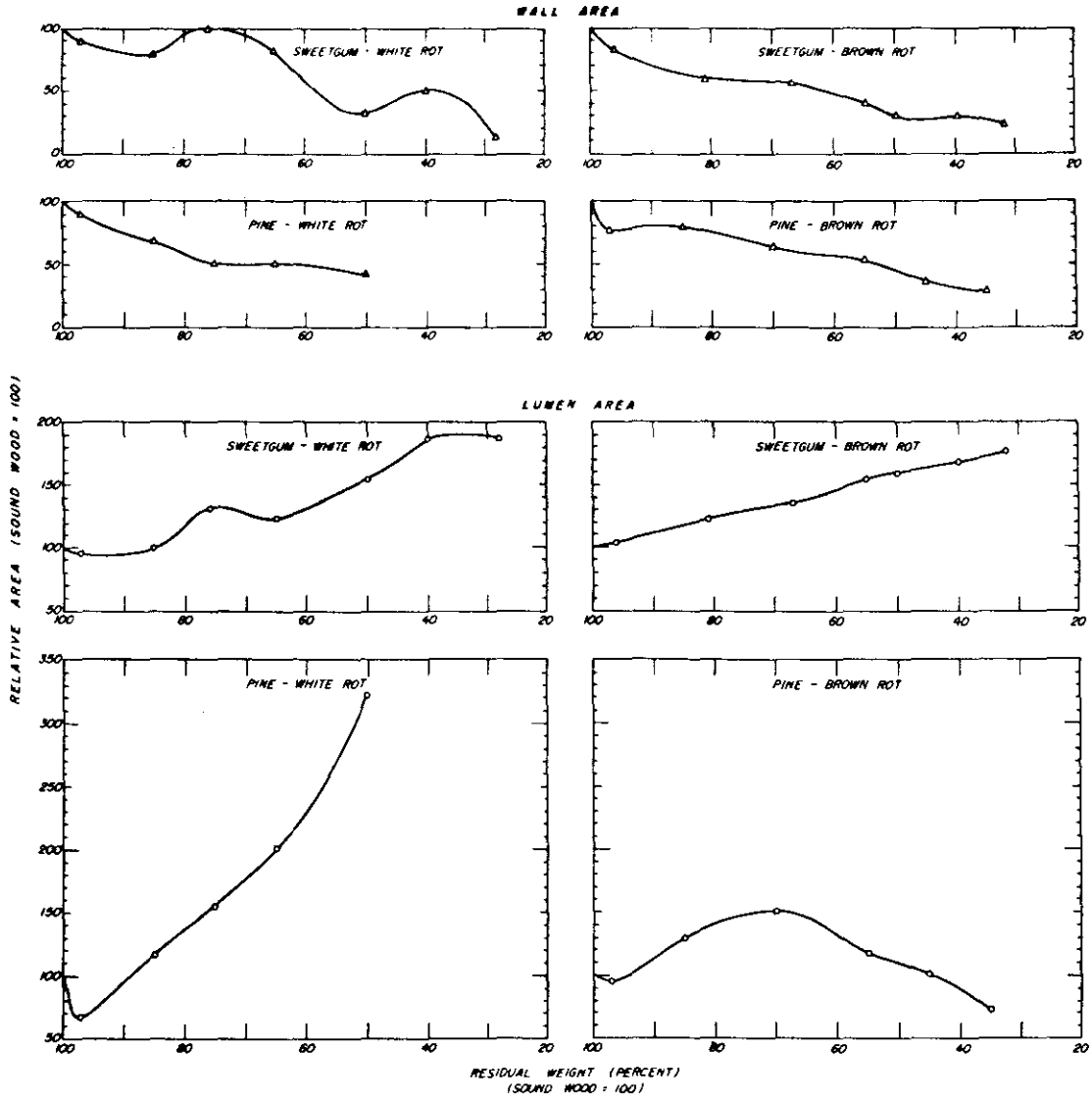


Figure 24.--Relationship of relative wall area and relative lumen area (table 2) to residual weight. The data for relative wall area are the same as those in figure 23. M 127 339

in wood weight during decay were accounted for by losses in microscopically visible cross-sectional area of dehydrated cell wall substance such as resulted from the sectioning and embedding processes used here.

Next, both the relative wall and lumen areas appearing in table 2 were plotted in relation to the residual weight so that the trends of change in areas could be visualized (fig. 24). With the white rot of both sweetgum and pine and the brown rot of sweetgum the lumen area gradually increased, and the increase was matched by a proportionate decrease in wall area. The increase in lumen area was more conspicuous in pine than in sweetgum largely because the lumina in the sound pine were much smaller than in sweetgum with the consequence that comparable increases in actual area appeared larger in pine on a percentage basis. In the early stages of the brown rot in pine the same general trend of increase in lumen area appeared. However. between 60 and 70 percent residual weight relative lumen area began to decrease significantly. The decrease apparently was the result of collapsing cell walls and the accompanying circumferential cell shrinkage.

Little significant change occurred in the total cross-sectional area of the cells of either white-

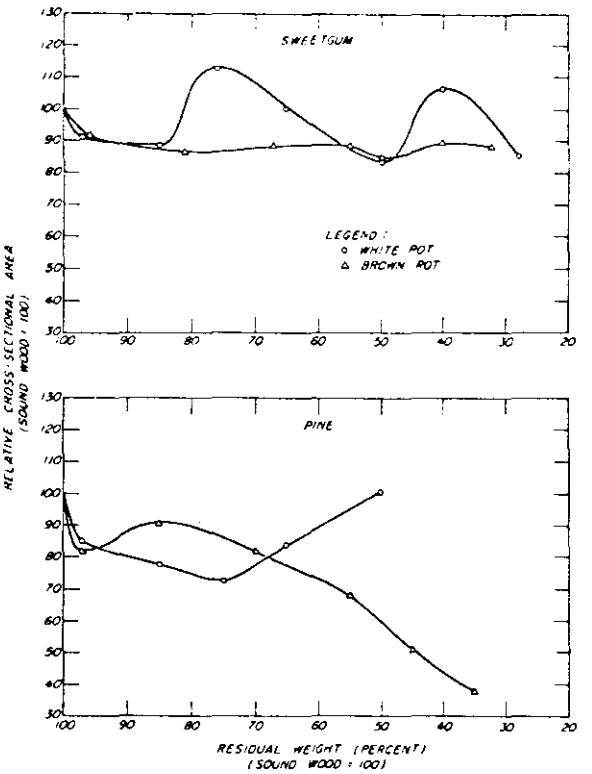


Figure 25.--Relative total cross-sectional area (table 2) in relation to residual weight. M 127 330

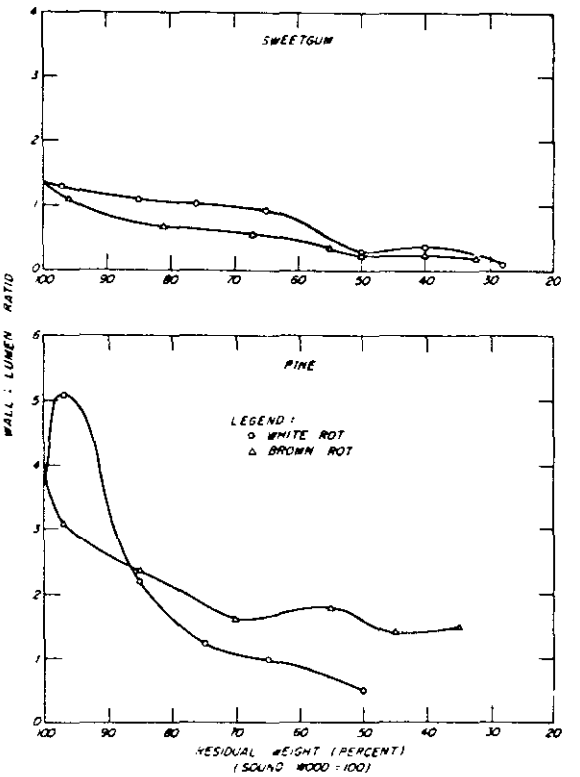


Figure 25.--Ratioof wall area to lumen area associated with removal of cell wall substance based on residual weight (table 2). M 127 329

rotted or brown-rotted sweetgum or of white-rotted pine as measured by the methods used here (fig. 25). It may be inferred that in these cases the thinning and the compaction of the cell walls were essentially all in the direction of the wall thickness and not circumferential. In the brown-rotted pine, however, there was an overall decrease in cell cross-sectional area, which, as noted, must have been brought about by a Circumferential compaction of the residual wall substance.

Plotting of the wall-to-lumen ratio (fig. 26) furnished further evidence similar to that shown in figure 25. In general, the ratio tended to decrease progressively with decreasing residual weight, reflecting again the progressive thinning of the cell wall and, in the white rot and most stages of the brown rot, the concomitant increase in lumen area. The leveling off of the curve for brown-rotted pine between 60 and 70 percent residual weight further indicated that when cell collapse occurred the decreases in thickness of the walls were largely compensated for by the compaction of the walls associated with decrease in external cell dimensions.

Synopsis of Microscopical Observations

To compare major microscopical features in pine and sweetgum undergoing decay, summarizing information is listed in tables 3 and 4.

Discussion

Artifacts of Specimen Preparation

Certain alterations produced in the specimens in their preparation for microscopical examination must be considered in interpreting the

Table 3.--Microscopically visible characteristics of decay stages in sweetgum[1] sapwood

Item observed	Early stages (80-100 percent R.W.[2])	Intermediate stages (60-80 percent R.W.[2])	Advanced stages (0-60 percent R.W.[2])
EFFECTS OF WHITE ROT BY *POLYPORUS VERSICOLOR*			
Hyphal distribution	At least several hyphae in lumina of almost all cells; large number in vessels and rays.	Decrease in number of hyphae.	Hyphae sparse in fibers and rays; still numerous in vessels.
Channels of hyphal advance from cell to cell	Advance through pits and bore holes; bore holes numerous; hyphal diameter only slightly less in bore hole than in lumen.	Bore holes and pit canals enlarged to 4 to 8 times diameter of hyphae.	Enlarged pits and enlarged bore holes no longer differentiable.
Changes in microstructure of fibers	S3 layer destroyed; checking of secondary wall and cell separation increased.	S2 layer noticeably attacked, resulting in thinning and a serrated wall-lumen boundary in many cells; checking of secondary wall remained high; cell separation decreased.	Destruction of S2 and S1 layers completed, eliminating the secondary wall; compound middle lamella attacked and removed; checking and cell separation disappeared as secondary wall was removed.
Optically indicated reductions in lignin and cellulose of fibers[3]	Lignin and cellulose removed from S3 layer of intact regions of wall and from all layers around bore holes and pit canals; much lignin of S2 layer apparently removed.	Some cellulose and the remaining lignin removed from S2 layer; also, further removal of lignin and cellulose from all layers around bore holes and pit canals.	Lignin and cellulose removed from S1 layer; lignin removed from compound middle lamella.
Effects on ray parenchyma cells	Little or no attack; starch not removed.	Same as in early stages.	Same as in early stages.
Effects on vessels	Little or no attack.	Same as in early stages.	Slight wall thinning.
Changes in cross-sectional area[4]	Progressive decrease in wall area, increase in lumen area; no significant change in cell size.	Same as in early stages.	Same as in early stages.
EFFECTS OF BROWN ROT BY *PORIA MONTICOLA*			
Hyphal distribution	Hyphae present in lumina of almost all cells very early, usually singly in fibers; total number smaller than white rot; few in rays and vessels.	Decrease in number of hyphae.	Hyphae sparse.
Channels of hyphal advance from cell to cell	Advanced mainly through pits; little or no bore hole formation.	Same as in early stages.	Same as in early stages.
Changes in microstructure of fibers	S2 layer noticeably attacked first, producing large cavities within the layer; checking of secondary wall increased; cell separation first increased then decreased.	Further attack and removal of S2 layer; with enlargement of cavities; checking of secondary wall decreased; cell separation remained low.	S1 and S3 layers removed, eliminating the secondary wall; checking and cell separation disappeared as secondary wall was removed; compound middle lamella intact.
Optically indicated reductions in lignin and cellulose of fibers[3]	Cellulose removed from S2 layer; no evidence of lignin depletion.	Further cellulose removed from S2 layer; lignin: same as in early stages.	Cellulose removed from S1 and S3 layers; lignin: same as in early stages.
Effects on ray parenchyma cells	Little or no attack on cell walls; starch removed.	Cell walls: same as early stages; starch absent.	Same as in intermediate stages.
Effects on vessels	Little or no attack.	Same as in early stages.	Slight wall narrowing.
Changes in cross-sectional area[4]	Progressive decrease in wall area and increase in lumen area; slight decrease in cell size.	Further decrease in wall area and increase in lumen area; no further decrease in cell size.	Same as intermediate stages.

[1] *Liquidambar styraciflua*.

[2] R.W. (Residual Weight) calculated by subtracting weight loss in percent from 100.

[3] The presence of lignin was indicated by absorption of ultraviolet radiation at a wavelength of 2780 angstroms and by staining with safranin and with azure B. The presence of cellulose was indicated by birefringence and by staining with fast green. Birefringence denoted only the crystalline fraction of the cellulose, but this fraction presumably was removed less rapidly by the decay fungi than the amorphous fraction; therefore, loss of birefringence was considered a valid indicator of cellulose removal.

[4] Changes in dimension include those induced by drying and solvent treatment during specimen preparation.

results. Presumably all wood constituents and breakdown products of decay that were soluble in alcohol or 2-methoxyethanol would have been removed from the sections by the time they were examined. Theproportion of such substances became significant as decay progressed, at least in the brown rot (11). Shrinkage could occur because the sections were dried on the slides prior to staining. Shrinkage could have been greater than would be found in nonembedded wood due to the removal of substances soluble in alcohol or 2-methoxyethanol. but shrinkage could also have been inhibited by the presence of celloidin in the Cell walls. It was assumed that fine openings or pores in the cell wall that might have resulted from decay could have closed during the shrinkage and the collapse of the residual wall. This was believed to be the explanation for the distinct shrinkage and change in appearance of the cell walls of the pine tracheids in advanced stages of brown rot. It is also possible. although great care was

Table 4.--Microscopically visible characteristics of decay stages in southern pine [1] sapwood

Item observed	Early stages ([illegible] percent R.W.[2])	Intermediate stages (60-80 percent R.W.[2])	Advanced stages (0-60 per cent R.W.[2])
	EFFECTS OF WHITE ROT BY POLYPORUS VERSICOLOR		
Hyphal distribution	At least several hyphae in lumina of almost all cells; at first, more numerous in rays and longitudinal resin canals than in tracheids.	Decrease in number of hyphae.	Hyphae sparse.
Channels of hyphal advance from cell to cell	Advance through pits and bore holes; bore holes numerous; hyphal diameter only slightly less in bore hole than in lumen.	Bore holes and pit canals enlarged to 4 to 8 times diameter of hyphae; hyphae appeared able to produce cavities in secondary wall and compound middle lamella in directions other than perpendicular to cell axis.	Enlarged pits and enlarged bore holes no longer differentiable.
Changes in microstructure of tracheids	S3 layer destroyed; checking of secondary wall and cell separation increased.	S2 layer noticeably attacked, resulting in thinning with a smooth wall-lumen boundary; checking of secondary wall decreased; cell separation remained the same.	S2 and S1 layers removed, eliminating the secondary wall; compound middle lamella attacked and ultimately destroyed; checking and cell separation disappeared as secondary wall was removed.
Optically indicated reductions in lignin and cellulose of tracheids[3]	Lignin and cellulose removed from S3 layer of intact regions of wall, and from all layers around bore holes and pit canals.	Lignin and cellulose removed from S2 layer; further removal of lignin and cellulose from all layers around bore holes and pit canals.	Remaining lignin and cellulose removed from S2 and from S1 layer, lignin removed from compound middle lamella.
Effects on ray tracheids	Secondary walls thinned and removed.	Most remaining secondary walls removed.	Same as intermediate stages.
Changes in cross-sectional area[4]	Progressive decrease in wall area, increase in lumen area; little change in cell size.	Same as in early stages.	Same as in early stages.
	EFFECTS OF BROWN ROT BY PORIA MONTICOLA		
Hyphal distribution	Hyphae sparse in tracheids; fairly numerous in rays.	Same as in early stages.	Same as in early stages.
Channels of hyphal advance from cell to cell	Advance mainly through pits; little or no bore hole formation.	Advance: same; hyphae appeared capable of producing cavities in secondary wall in directions other than perpendicular to the cell longitudinal axis.	Advance: same as in intermediate stages.
Changes in microstructure of tracheids	S2 and S3 layers attacked simultaneously in most cells. S2 layer attacked first in a few cells, producing large cavities within the layer; checking of secondary wall and cell separation increased.	Attack extended to S1 in some cells, accompanied by wall collapse; checking of secondary wall increased; cell separation decreased.	Wall collapse occurred in additional cells; wall residue indistinguishable from compound middle lamella which was not visibly affected; checking and cell separation disappeared as secondary wall was removed.
Optically indicated reductions in lignin and cellulose of longitudinal tracheids[3]	Cellulose removed from S2 layer in some cells, from S3 and S2 layers in others; no evidence of lignin depletion.	Cellulose further removed from S2 layer, from S1 layer also in some cells; cellulose remained in S3 layer where this layer was retained; lignin same.	Cellulose removal from secondary wall completed; residual apparently mostly lignin.
Effects on ray tracheids	Walls thinned.	Walls further thinned but not removed; some cellulose removed.	Same as in intermediate stages.
Changes in cross-sectional area[4]	Progressive decrease in wall area; increase in lumen area; little significant change in cell size.	Wall area: as in early stages; lumen area increased then decreased; little significant change in cell size.	Wall areas same; lumen area decreased; appreciable decrease in cell size.

[1] Pinus sp.

[2] R.W. (= Residual Weight) calculated by subtracting weight loss in percent from 100.

[3] The presence of lignin was indicated by absorption of ultraviolet radiation at a wavelength of 2780 angstroms and by staining with safranin and with azure B. The presence of cellulose was indicated by birefringence and by staining with fast green. Birefringence denoted only the crystalline fraction of the cellulose, but this fraction presumably was removed less rapidly by the decay fungi than the amorphous fraction; therefore, loss of birefringence was considered a valid indicator of cellulose removal.

[4] Changes in dimension include those induced by drying and solvent treatment during specimen preparation.

taken in handling the specimens, that some hyphae were removed from the decayed wood during the process of embedding in celloidin.

Hyphal Distribution

Some apparent discrepancies exist between the results of this work and those of earlier workers on hyphal distribution and channels of hyphal advance from cell to cell. It has been reported that hyphae of white rotters invaded the vessels and rays of hardwoods before other elements: however, in this study the hyphae of Polyporus versicolor were found to invade all elements of sweetgum in early stages of decay.

The observation that the hyphae of the brown rotter, Poria monticola, were widely distributed throughout the sweetgum in very early stages of decay agrees with reports in the literature of brown rotters in general, but the sparseness of hyphae in the pine does not agree with these reports. These differences. however. may have

no relevance to white rot and brown rot in general but may be merely a result of the specific fungus–wood species combinations employed. The ubiquity of the hyphae of both fungi in early stages of decay suggested that enzyme diffusion at great distances from the hyphae. such as across several cells, is not a necessary hypothesis.

Channels of Hyphal Advance from Cell to Cell

The fact that bore holes were not observed in wood decayed by the brown rotter. Poria monticola, differs from reports in the literature on brown rot, which indicate copious bore hole formation. Most of the present observations were made on cross sections, which are not well suited for surveying the prevalence of bore holes: supplementary observations of macerated brown–rotted wood also failed to reveal any bore holes, although the enlargement of pit canals was common. Thus, although it cannot be said with certainty that no bore boles were formed by the brown–rot fungus observed here, certainly far fewer were formed by this fungus than by the white–rot fungus.

The observations of longitudinally oriented cavities within the cell walls of decayed wood also correspond to earlier reports. Such cavities in the secondary wall of brown–rotted pine tracheids were considered analogous to those formed by longitudinal growth of hyphae of a brown–rot fungus within the S2 layer reported by Meier (35) and the cavities observed by Liese and Schmid (32). The small number of these observations indicated that the phenomenon was relatively uncommon and did not constitute a major means of hyphal advance in the wood. The cavities produced in the secondary wall and compound middle lamella of pine by the white–rot fungus were considered analogous to the cavities observed by Cowling (11) and by Liese and Schmid (32). These, too, were relatively infrequent. The production of longitudinal cavities within the cell wall by both the white– and the brown–rot fungi was observed only in pine and not in gum suggesting a difference between the two woods in the physical or chemical properties of the cell walls.

Chances in Microstructure and Indicated Depletion of Wood Constituents

Many of the alterations in the cell walls reported in earlier microscopical studies of decayed wood also were observed here. The observation in white–rotted wood of a progressive thinning of the cell walls that proceeded at approximately the same rate in each cell agreed with results of earlier studies. The serrated appearance of the decomposed S2 layer of sweetgum probably was analogous to the irregular attack on the cell walls reported by Cowling (11) and Meier (35) from electron microscopical studies.

The smooth and uniform appearance in the light microscope of the boundary between the lumen and the secondary wall of white–rotted pine, in contrast to the serrated appearance in sweetgum, agreed with the observations reported in most earlier light–microscopical studies of white–rotted wood. This contrast in the effects of the white–rot fungus on the two woods again suggested differences in the physical or chemical properties of their cell walls. The differences between white rot and brown rot observed in this work throughout all stages of decay are in contrast with results of Greaves and Levy (18) in which the two types of decay resembled each other in early stages.

Cell separation and checking of the secondary wall.––The absence of a significant amount of cell separation or of checking of the secondary wall in sound wood and the presence of these conditions in decayed wood suggested that they were associated withdecay. Because no embedding matrix was found within the checks or within the regions of the wall that had separated, checking and separation must have occurred subsequent to embedding. Checking and separation rarely were observed in sections that had not been dried; thus they may have resulted from wall failure due to drying stresses differentially developed between regions relatively little weakened and those more significantly weakened by fungal attack. In sound wood and in wood with early stages of decay, according to this reasoning, sufficient strength must have been present in all areas of the cell wall to resist the stresses developed during drying. The diminutionof checking and of cell separation in more advanced stages of decay may be accounted for by a more general

and a more uniform weakening of the cell walls, whereby no regions of the wall retained sufficient strength to exert the stresses necessary to cause failure.

The location of the cell wall separation was different in the two woods but was the same for the two types of decay. In sweetgum, separation occurred within the compound middle lamella or between it and-the S1 layer; in pine, it occurred between the S1 and S2 layers or within the S1 layer. According to Jurášek's photomicrographs (24) also, the cell separation found in thewhite-rotted softwood occurred to either side of, but not within, the middle lamella. The true structural basis for the separation in a given region is unknown, but each of these layers differs from the contiguous layers in the content and physical organization of lignin and cellulose.

Apparently a degree of weakness also occurs in the same regions of nondecayed wood. Previous researchers found that when sound coniferous woods were subjected to stress, cell separation occurred not within the compound middle lamella but between the S1 and the S2 layers of the secondary wall (17, 50) or between the S1 layer and the compound middle lamella (50).

Other researchers studying thetensile strength of spruce and birch found that in water-swelled untreated wood, failure under tensile stress occurred at either side of the cell wall or sometimes between the S1 and the S2 layers (6, 27). Elevated temperature or alkali treatment increasd the amount of separation that occurred between the S1 and the S2 layers. When the wood was partially delignified, however, failure also occurred within the middle lamella, and the amount of failure increased with the degree of delignification. Wardrop (51) found a preponderance of separation within the true middle lamella in wood that had been subjected to a chemical pulping process, with some failure occurring within the S1 layer or between the S1 and S2 layers. In pulping processes that rely on mechanical as well as chemical action, separation between the S1 and S2 layers became predominant.

The data from these various studies suggest a correlation between the zone of cell wall separation and the content or condition of lignin in the cell wall. In the previous studies, it was found that cell wall separation occurred closer to the middle lamella when the wood had been subjected to a treatment that could alter or destroy lignin than it did in untreated wood. If this is typical, it could explain why wall separation occurred in the pine farther from the middle lamella than it did in the sweetgum, because the pine presumably had a higher initial lignin content. However, it does not account for failure occurring in the same region with both white rot and brown rot for each type of wood, unless it is assumed that both types of decay similarly affect the cell-bonding action of the lignin even though they differ greatly in the extent to which lignin is removed.

Order of attack on the different cell wall layers.--A consistency was observed intheorder in which the layers of cell walls of fibers and tracheids and the walls of other wood elements were removed by decay. During attack by the white-rot fungus on sweetgum the order of removal was as follows:

S3 layer
S2 layer
S1 layer
Compound middle lamella
Ray parenchyma and vessel cell walls

The order of removal of the cell wall layers of pine tracheids was the same as that for sweetgum fibers. Vessels were, of course, not present. The ray tracheids exhibited little resistance to degradation, less than the S2 and S1 layers of the longitudinal tracheids. These findings agree with those of Meier (35) for the same fungus, with the additional observation of high resistance to attack possessed by the rays and vessels of sweetgum. This order of removal in the walls of fibers and tracheids is the same as that in which the layers occur in the cell wall, beginning at the lumen. Therefore, it appears that the white-rot fungus destroyed the cell wall progressively from the lumen outward. The evidence of this progressive destruction applies only to the removal of significant proportions of the cell wall constituents, quantities sufficiently large that their disappearance could be detected microscopically. This need not, however, imply that no enzyme activity occurred within interior wall layers prior to complete destruction of the intervening layers.

This progression of destruction seemed to be based on the order of accessibility of the wall layers rather than on differences in susceptibility to decomposition. The smooth, uniform enlargement of bore holes and pits suggested that all layers of the cell walls of fibers and tracheids could he decomposed simultaneously if equally accessible to the fungal enzymes. Only

the walls of the ray parenchyma cells and vessels of sweetgum appeared to have genuinely superior resistance to degradation.

Attack by the brown-rot fungus on the cell wall layers was somewhat different from that by the white-rot fungus. The order in which the layers of sweetgum fibers and the walls of other wood elements were removed was as follows:

S2 layer
S1 layer
S3 layer
(Compound midd1e lamella and the walls of ray parenchyma cells and vessels were not removed)

These findings for the order of destruction of secondary wall layers agree in general with those of Jurášek (22) and Meier (35), except that Meier reported that the S3 and the S1 layers were not completely degraded during brown rot. This difference might be due to the examination, in this study, of specimens in more advanced stages of decay, because it was only in very late stages that the S1 and S3 layers disappeared.

The pronounced resistance of the ray parenchyma and the vessel cell walls to brown rot corresponded to the high resistance of these elements to white rot.

The observation in this study that the vessel walls in sweetgum were resistant to the action of both the white rotter and the brown rotter contrasted with the work of Greaves and Levy (18) in which they found that the walls of the vessels in birch and beech were attacked by both a white-rot fungus and a brown-rot fungus.

The occurrence of cell wall collapse in brown-rotted pine made it impossible to follow the order of removal of the secondary wall layers in this wood as precisely as with the sweetgum Nevertheless, it seemed that the order of destruction was more variable. In some tracheids the S2 layer was removed first, and in others the S3 layer appeared to be attacked prior to or concomitantly with the S2 layer. The S1 layer retained its birefringence somewhat longer than either the S2 or the S3 layer. Degradation of any of the wall layers was visible in at least some cells in each section. The lack of uniformity in the order of attack on the secondary wall layers suggested that there was less difference in decay resistance between the three layers in pine than in sweetgum

optically indicated order of attack on Cellulose and lienin.--The results obtained from assaying by several methods for the presence of lignin (by means of absorption of ultraviolet radiation and by staining with safranin and with azure B) and for the presence of cellulose (by means of birefringence under polarized light and by staining with fast green) were in closeagreement as to changes in the content of these components in the cell walls undergoing decay. Therefore, although each method may not have been highly specific for the given component, the agreement in the results from all methods materially strengthened the conclusions drawn from the microscopical observations for changes in these components.

The methods indicated for the sound wood of both sweetgum and pine a high cellulose content in all three layers of the secondary wall and a high lignin content in the compound middle lamella. From the work on absorption of ultraviolet radiation, the lignin content of the S3 layer in sweetgum appeared to be only slightly less than that of the compound middle lamella. The S1 layer seemed to have a greater lignin content than the S2 layer but it was slightly less than the S3 layer. All assay methods indicated a lignin content of the S2 layer in pine considerably higher than that in sweetgum Also, analysis by ultraviolet absorption indicated that a small number of pine tracheids had a slightly higher lignin content in the S3 layer than in the S2 layer, but in most cells these two layers appeared similar in lignin content. By all methods the S1 layer was shown to have a higher lignin content than the S2, although the content was considerably less than that of the compound middle lamella. All methods indicated a fairly high lignin content in the walls of the ray parenchyma cells and vessels of sweetgum and the ray tracheids of pine.

From analyses of the data on depletion of the major constituents of the cell walls, differences in the decomposition of cellulose and lignin by the two types of decay fungi were apparent. In white rot, most of the microscopically detectable removal of cellulose from the secondary wall occurred progressively outward from the lumen. In bore holes and pit canals also, the action radiated outward from the opening, apparently because of decomposition of the exposed surface. The microscopical data from staining and observation under polarized light gave no indication of cellulose degradation within the wall behind the exposed surface, except for the infrequent

localized interior dissolution in pine, presumably caused by growth of hyphae longitudinally within the cell wall.

From this information and the order in which the cell wall layers were attacked, it was concluded that most of the decomposing action of the cellulolytic enzymes of the white-rot fungus was restricted to wall layers or regions of the wall adjacent to the lumen or connecting cavities. This type of restriction also was hypothesized by Cowling (11) from chemical analyses of decayed wood.

The course of activity of the lignin-destroying enzymes of the white-rot fungus in sweetgum suggested that their action was not entirely restricted to wall layers or regions adjacent to the lumen, however. Ultraviolet absorption indicated that in early stages of decay of sweetgum, lignin was removed not only from the S3 layer but also from the S2 layer, although the S2 layer was still intact and possessed normal birefringence. This observation implied an ability of the lignin-destroying enzymes of the white-rot fungus to act on microscopically detectable amounts of lignin within the cell wall prior to complete decomposition of the intervening layers. This type of ability also was postulated by Cowling (11) from chemical analyses of decayed wood. The existence of this property in the lignin-destroying enzyme systems of the white-rot fungus would explain how the relative proportions of residual lignin and cellulose remained fairly constant throughout the decay of sweetgum by the same white-rot fungus, as found by Cowling (11) and Scheffer (44), even though attack on the cellulose apparently occurred progressively from the lumen outward on only the exposed cell wall surfaces. This advance lignin decomposition did not visibly extend into the S1 layer and the compound middle lamella until the S3 and the S2 layers were destroyed.

A similar removal of lignin in advance of cellulose decomposition was not observed in the white rot of pine, even in the early stages of decay. But this does not necessarily establish that there had been no advance lignin removal. The S2 and the S3 layers of pine both appeared to contain considerably more lignin than the S2 layer of sweetgum Therefore, it is likely that advance lignin degradation could have occurred without detection within the relatively high lignin S2 and S3 layers of pine.

It is interesting that the structures in the sweetgum that displayed the greatest resistance to degradation by the white-rot fungus (walls of the ray parenchyma cells and vessels and the compound middle lamellae of all cells) appeared to have a high lignin content. It is possible that lignin content was related to resistance to the action of the white-rot fungus even though this fungus was capableof decomposing lignin.

The high concentration of lignin in the S2 and the S3 layers of pine might also provide an explanation for the relatively slow attack on the pine by the white-rot fungus. If it is possible that the lignin incrustations between the microfibrils of cellulose in the secondary wall would impede the entrance of cellulolytic enzymes, the difference in rate of attack by the white-rot fungus between sweetgum and pine could be explained.

In sweetgum the lignin was removed in early stages of decay from the S3 and the S2 layers, thereby perhaps allowing the cellulolytic enzymes to act progressively through these regions. However, in pine, possibly due to the high lignin content in both the S2 and the S3 layers, early delignification of the secondary wall was not so complete. Therefore, it is conceivable that decomposition of cellulose would be retarded until sufficient delignification had occurred to allow the penetration of cellulolytic enzymes. The pine was not immune to attack by the white-rot fungus; the attack simply proceeded very slowly in early stages of decay.

White rot fungi in general, at least in aboveground exposures, display a greater tendency to attack hardwoods than softwoods (10, 14, 45). This is true even when nondurable softwoods, such as the pine sapwood employed in this study, are involved. Attempts have been made to correlate the apparent resistance to white rot of nondurable softwoods with extractable materials in the wood, but with little or no success (41, 48). It is possible that this difference between hardwoods and softwoods is related instead to differences in lignin content or distribution within the cell walls.

The cellulolytic enzymes of the brown-rot fungus, unlike those of the white-rot fungus, apparently were able to diffuse throughout the secondary wall in both sweetgum and pine. In both woods this ability was indicated by loss

in birefringence and the formation of cavities in the S2 layer behind the apparently intact S3 layer. Nevertheless, a degree of localization of activity in the general vicinity of the hyphae may have occurred This was suggested by more advanced dissolution of the S2 layer in some cells in a region adjacent to a hypha that was appressed to the lumen surface of the S3 layer.

Because the brown rotter, Poria monticola, is unable to utilize a significant amount of lignin (11), it was considered possible that the order of the deterioration of the layers of the secondary walls of sweetgum fibers was substantially determined by lignin content. This conclusion was strengthened by the observation that the order of removal of the wall layers by the brown-rot fungus was the same as the order of increasing lignification as determined from the literature and the methods employed here. Furthermore, staining and ultraviolet absorption data from this work indicated that the layers of the secondary wall in pine tracheids were more similar in lignin content than those of sweetgum fibers; this could explainthegreater uniformity among these layers in pine in their resistance to degradationby thebrown-rot fungus.

Meier (35) found that when wood was macerated and delignified until the separated elements were practically pure cellulose and then were subjected to various types of decay fungi, many of the differences among wall components and cell types in susceptibility to decomposition disappeared. The rate of destruction also was similar for all decay fungi used in his study. These relationships strongly suggest the hypothesis that the higher the lignin content of the cell wall layer, the greater is its resistance to degradation by brown-rot fungi. However, another explanation also is possible.

Conceivably, the presence of relatively resistant hemicelluloses could be an additional factor in the resistance of cell walls and wall layers to degradation by a brown-rot fungus. Lange (28) indicated that the distribution of lignin and of hemicellulose were positively correlated. Meier (36) reported at least some of the hemicelluloses, those possessing relatively high molecular weights, may occur in the form of microfibrils and may possess a high degree of birefringence. In addition, at least one layer of the secondary wall (S3 layer) may consist largely of hemicellulose (38). Cowling (11) reported a significant accumulation of high molecular weight hemicelluloses (6-cellulose) in the residue of brown-rotted sweetgum having residual weights ranging down to approximately 70 percent. With further decay these residues were only slowly removed. This significant build-up of hemicellulose, he felt, could be dueentirely to a more rapid production of low molecular weight carbohydrates from the depolymerization of 5-cellulose than the fungus was capable of utilizing. However, it might also indicate an inability of the fungus to appreciably utilize, during early stages of decay, hemicelluloses originally present in the cell walls. If this were true and if those layers found to possess a high lignin content might also contain considerable hemicellulose, it could be concluded that hemicellulose content might be a factor in determining the resistance to attack by a brown-rot fungus.

Changes in Cross-Sectional Area and Dimensions

The conspicuous, characteristic shrinkage of brown-rotted wood was attributed to collapse and shrinkage of the walls of the individual cells. The walls apparently did not shrink, however, until they dried to the stage where further drying involved the removal of water from within the wall structure. At 60 to 70 percent residual weight in brown-rotted pine. apparently enough carbohydrate had been removed from the secondary wall so that when the wood was dried the lignin residue and carbohydrate depolymerization products lacked sufficient strength to maintain the integrity of the cell. It might be expected that removal of some of these residues, in addition to water, would increase the extent of cell wall collapse and shrinkage. The supplementary experiment in which a block of brown-rotted pine was leached with 2-methoxyethanol bore this out. In this connection it is pertinent that Cowling (11) reported with brown rot an accumulation of the decomposition products soluble in 2-methoxyethanol, which continued to accumulate as decay advanced.

The absence of significant shrinkage in white-rotted wood as it dried could be explained by the absence of shrinkage and collapse of the individual cells. Cowling (11) attributed the differences in shrinkage characteristics between white- and

brown-rotted wood to differences in the cellulose structure of the residual cell wall. The wall in advanced brown rot contained structurally weak depolymerization products of cellulose, whereas in white rot it contained cellulose molecules still possessing high molecular weight and strength.

The data derived from measurement of cross-sectional area at various stages of decay showed quantitatively the cell wall thinning that has long been qualitatively observed in white-rotted wood. A reduction in wall thickness also was apparent in the brown rot of sweetgum and in early stages of the brown rot of pine prior to cell wall collapse but this was not thinning in the same sense as that characteristic of white rot.

Two distinct terms were used to describe the decrease in cell wall thickness for the two types of rot to emphasize the difference in processes by which the two types of fungi degraded the cell walls. In the white rot, decomposition occurred layer by layer progressively outward from the lumen, an action that appropriately may be described as "thinning," corresponding to the earlier concept of "corrosion." In the brown rot. decomposition occurred in internal layers of. or throughout, the secondary wall accompanied by subsequent shrinkage or collapse of the residual cell wall substance, a process here termed "narrowing" that corresponds to the earlier concept of "destruction."

Both wall thickness and lumen area changed as decay advanced, but the trend of change was not the same in the white rot as in the brown. The difference can be ascribed chiefly to the difference between the two kinds of rots in cell wall collapse and cell shrinkage. Because essentially no collapse or shrinkage occurred in the white rot, the cell wall became progressively thinner and the lumen area correspondingly larger with increasing degrees of decay.

The pattern was similar in the brown rot of sweetgum a circumstance that will be commented on. In the brown rot of pine, however, the circumferential shrinkage of cells caused by wall collapse tended to thicken the walls--by compaction of the residual material--and, consequently, to make the lumina smaller. The wall collapse and resulting circumferential shrinkage opposed the reduction in wall thickness and enlargement of lumina, the net result depending on the factor dominant at a given stage of decay. This explained the otherwise anomalous observations of a relatively constant rate of decrease in wall area accompanied by a decrease in lumen area.

Although in later stages of brown rot the residual secondary walls of sweetgum fibers appeared to undergo a slight collapse similar to that encountered in pine tracheids, there was no great shrinkage in cell cross-sectional area as in the pine. Because the same fungus was involved in the decay of both species of wood, again a difference between the two woods in the physical or chemical properties of the cell walls is indicated.

The measurements of relative cell wall area showed that losses in wood weight resulting from decay could be fully accounted for by losses in the amount of cell wall substancevisible in cross sections determined by the methods employed here. In fact, the reductions in cell wall area predominantly indicated a greater weight loss than had actually been incurred. For only one set of data--brown-rotted sweetgum—regressionanalysis indicated that the difference from the straight line representing an equality relationship between residual weight and relative wall area was statistically significant. Nevertheless, it seems indicative that most of the points (22 out of 25) relating wall area to residual weight for both decay fungi and both woods are on or below this equality line. Therefore, it is probable the reductions observed in relative wall area in excess of those expected from a consideration of residual weight were artifacts produced during specimen preparation. perhaps involving increased shrinkage associated with removal of soluble degradation products.

The observation that essentially all loss in weight could be accounted for by loss in cross-sectional area of the cell wall differs from that by Cowling (11) on similarly decayed wood. In Cowling's material, only 6 percent of the total loss in weight of wood could be accounted for by loss in cell wall substance visible with the light microscope: furthermore, the entire 6 percent loss was represented by substance removed during bore hole formation.

A possible explanation of the difference in these two sets of data is that Cowling's specimens may have been observed in a more swollen state than those of this work because of the different methods of embedding and section preparation. Cowling also found submicroscopical voids

in the cell walls of his specimens, whereas the close correlation between residual weight and cross-sectional area in this work suggests that little void space was present in the cell walls examined in the shrunken or collapsed condition.

Relationships with Observations of Jurášek

Because some of the objectives ,of a study by Jurášek (24) were similar to those of this study, it is interesting to compare the results and the conclusions. Both works showed that white rot involved a thinning of the cell walls that progressed from the lumen outward. Both also disclosed radial cracks in the cell walls of the decayed wood. Jurášek considered the cracks cavities of decomposition, direct results of decay, and used them as evidence of the ability of the fungal enzymes to act within the cell wall in advance of complete destruction. This study led to a different interpretation: Checks in both types of decay were shown to be only secondarily a result of the decay because they did not form until the cell walls were dried. It still must be concluded, of course, that although the checks were not cavities formed directly by the action of the fungi, they were nevertheless indicative of some change brought about within the cell walls.

Both studies indicated that in white rot the lignin-destroying enzymes of the fungus acted within the cell wall in advance of the carbohydrate-destroying enzymes. Jurášek's strongest basis for this conclusion was the chemical information that lignin and polysaccharides were decomposed proportionately during decay whereas most of the lignin was contained in the inner wall layers. Lignin decomposition in localized areas deep within the cell wall also was reported from results of certain staining reactions. Thepresent study produced evidence through ultraviolet-absorption microscopy of a uniform progressive decomposition of lignin within the cell wall in advance of complete wall destruction.

In both studies, an increase in the size of pit cavities was observed as a result of white rot, beginning in intermediate stages of decay and continuing as decay progressed. In the white-rotted hardwoods, the S1 layer and the compound middle lamella were retained to somewhat lower residual weight with Jurášek's wood and fungus than was true in this study. The white-rotted softwood examined by Jurášek reportedly exhibited little change from sound wood other than enlargement of pit cavities and slight cell separation, whereas the material of this study showed significant microstructural change.

Both studies reported a certain amount of narrowing of the cell walls from brown rot in the hardwoods; however, the separation of a resistant S3 layer at the center of the cell while decomposition of the S2 progressed, observed in this study, was not reported for the hardwood investigated by Jurášek Furthermore, Jurášek reported little lignin in the S3 layer of the hardwood in contrast to the results of this work The separation of a resistant S3 layer was reported in the softwood examined by Jurášek but was not observed in the softwood here. In both investigations, resistance of the S3 layer to degradation was attributed to its degree of lignification. Also, in both studies the form of the secondary wall of the brown-rotted softwood was maintained by the residual material, and in advanced stages of decay the individual layers of the cell wall became indistinguishable.

Jurášek reached two major conclusions. First, wood with a high lignin content may be more resistant to decay than less lignified wood; this conclusion also was reached here, and there was evidence that the concept may also apply to cells or regions of cells having higher lignin contents than the surrounding tissues. Second, the decay produced by a certain fungus basically should be similar regardless of the species of wood under attack. This conclusion was contradicted by the experimental evidence of this investigation.

Summary

The sequence of changes observed microscopically that occurred in wood throughout successive stages of decay was studied in the sapwood of a hardwood, sweetgum (Liquidambar styraciflua L.), and of a softwood, southern pine (Pinus sp). The fungi used were Polyporus versicolor L. ex Fr., a white-rot fungus, and Poria monticola Murr., a Inown-rot fungus. Light microscopy, plus the techniques of polarization and ultraviolet-absorption microscopy, was used to make the observations on sections 4 microns thick cut from celloidin-embedded specimens.

The hyphae of both fungi were widespread in both types of wood in early stages of decay. Hyphae of the white rotter were much more numerous than those of the brown rotter. Although the white-rot fungus formed abundant bore holes, none were observed in brown-rotted wood. Both fungi enlarged pit canals to the extent that they could not be distinguished from true bore holes in all but early stages of decay.

The white-rot fungus removed each cell wall layer of both woods in sequence from the S3 layer to the compound middle lamella. The brown-rot fungus removed the S2 layer before the S1 and the S3 layers in sweetgum; the same pattern of removal was found in some cells of pine, but in others the S2 and the S3 layers were attacked simultaneously. It was concluded that most action of the cellulolytic enzymes of the white-rot fungus was limited to exposed cell wall layers, whereas the lignin-destroying enzymes were capable of penetrating and acting upon layers within the cell wall. The cellulolytic enzymes of the brown-rot fungus, however, apparently were capable of penetrating and acting on layers within the secondary walls. The degree of lignification, or some factor directly associated with lignin content, was correlated with resistance of wood cell walls to brown rot. It was possible to account for the amount of cell wall substance removed during decay by measuring weight loss and by measuring losses in cell wall cross-sectional area by a microscopical randonuzed point sampling procedure. So change occurred in the average cross-sectional area of cells of either wood attacked by the white-rot fungus or of sweetgum attacked by the brown-rotfungus; however, in the cells of the pine, attack by the brown-rot fungus caused a significant shrinkage in the cell cross-sectional area.

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