Lignin Distribution in Cell Walls of Birch Wood Decayed by White Rot Basidiomycetes

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ABSTRACT

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Sound wood and wood decayed by various white rot fungi were brominated and analyzed by Energy Dispersive X-ray Analysis (EDXA-STEM) to determine lignin distribution within the cell wall. Lignin was distributed throughout the cell wall in sound wood with the highest concentration in the middle lamella and in cell corners. Wood decayed by *Coriolus versicolor*, a simultaneous white-rotter, had eroded cell walls with all cell wall components degraded. Lignin was removed from the secondary wall beneath hyphae and around the circumference of the lumen before cellulose loss was detected. Erosion troughs and thinning of the cell walls were evident. Wood decayed by white-rotters that selectively delignified wood, *Dichomitus squalens*, *Heterobasidion annosum*, *Phellinus pini*, *Phlebia tremellosus*, and *Poria medulla-panis*, had extensive amounts of lignin removed from throughout the cell wall layers. Lignin was removed in the secondary wall from the lumen toward the middle lamella. The middle lamella between cells was degraded only after extensive lignin depletion in the secondary wall. In advanced stages of decay, lignin was removed from throughout the cell wall layers but not from the cell corners. After delignification, only *D. squalens* and *H. annosum* had noticeable amounts of cellulose removed from the secondary wall. Wood fixed with $KMnO_4$ and chemical analyses of lignin and wood carbohydrates confirmed these patterns of lignin degradation from the cell walls.

White rot basidiomycetes are a large, diverse group of fungi that cause different types of cell wall attack (21). These fungi are able to degrade all cell wall components, but great variation can be found in the types of decay produced. Cellulose, hemicellulose, and lignin may be degraded simultaneously, each component may be degraded at a different rate from the others, or a preferential attack on lignin and hemicellulose can occur. Recently, a large number of white rot fungi were shown to selectively remove lignin from a variety of woods (6). Preferential lignin degradation can occur in localized areas of wood resulting in a white-pocket rot or in larger, less restricted areas typical of a mottle-rot (3,7,21). In vitro studies have shown that extensive loss of lignin can also occur throughout the substrate (2,8,24).

Micromorphological studies of wood in advanced stages of delignification have demonstrated a loss of middle lamella from the cell walls and a defibration of the wood (5–7). Ultrastructural investigation of lignin loss from the cell walls of aspen and birch wood decayed by *Phlebia tremellosus* confirmed the extensive loss of lignin from the secondary wall as well as the compound middle lamella (8). The process of lignin removal from various morphological regions of the cell wall is not completely understood. Knowledge of these basic wood decomposition mechanisms is essential to improve our understanding of wood biodegradation and for potential application of white rot fungi for industrial use (e.g., biopulping, biobleaching of pulps, lignin removal for release of polysaccharides, ruminant animal feeds, etc.).

Methods to determine precisely the quantity and distribution of lignin in wood cell walls using a bromination technique and subsequent analysis with scanning electron microscopy coupled with energy dispersive X-ray analyses (SEM-EDXA) have recently been developed (27,28). The bromine concentrations (Br-L series) in different morphological regions of the cell wall have provided an accurate measurement of lignin distribution. This method has also been used successfully to monitor lignin loss during the chemical delignification of wood in various pulping processes (29). The objectives of the present study were to determine the lignin distribution in birch wood that had been decayed by various white rot fungi using SEM-EDXA and observe how lignin is sequentially degraded from the different morphological regions of the cell wall.

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MATERIALS AND METHODS

Cultures of *Coriolus versicolor* (L.:Fr.) Quél (Mad 697-R), *Dichomitus squalens* (Karst.) D. Reid (Syn. *Polyporus anceps*) (RAB-83-12), *Heterobasidion annosum* (Fr.) Bref. (Syn. *Fomes annosus*) (RAB-83-9), *Phlebia tremellosus* (Schard: Fr.) Nakas. Burds. (Syn. *Merulius tremellosus*) (PRL-2845, Forintek Canada strain A-350), *Poria* (*Perenniporia*) *medulla-panis* (Pers.) Bres. (RAB-82-5), and *Phellinus pini* (Thore.:Fr.) A. Ames (Syn. *Fomes pini*) (RAB-83-19) were grown in glass vials (height, 7 cm; diameter, 2 cm) containing 5 ml of malt yeast broth (15 g of Difco malt extract, 2 g of Difco yeast extract, 1,000 ml of distilled water) for 3 wk before they were used to inoculate wood blocks. Wood blocks ($1.6 \times 1.6 \times 0.7$ cm) were cut from a single length of birch wood (*Betula papyrifera* L.), dried for 72 hr at 60 C and weighed to determine dry weight. They were then placed in 30-ml glass bottles (height, 5.0 cm; diameter, 3.5 cm) containing 10 ml of vermiculite. Five milliliters of distilled water was added to each bottle. Bottles were loosely capped, autoclaved for 30 min at 121 C, cooled, and inoculated. Each wood block was inoculated with a mat of mycelium from the culture vials. Bottles with inoculated wood blocks were loosely capped and incubated in the dark at 27 C and 90% relative humidity.

Ten blocks inoculated with *Phlebia tremellosus* were removed for analysis after 1 and 3 mo and 10 blocks inoculated with *D. squalens*, *Phellinus pini*, *Poria medulla-panis* or *C. versicolor* were analyzed after 3 mo. Ten uninoculated control blocks were also removed for comparison at these times. Mycelium was carefully removed from the decayed blocks; blocks were then dried for 72 hr at 60 C and reweighed to determine weight loss.

To determine the distribution of lignin in cell walls, areas of the decayed and control wood blocks were cut with a razor blade into

small pieces (1 × 1 × 7 mm) and brominated in chloroform using the method of Saka and Thomas (27,28). After embedding wood pieces in a modified Spurr's (30) epoxy resin as previously published (8), transverse sections (0.2 μm) were cut with a diamond knife mounted in a Reichert Ultracut E ultramicrotome. Transverse sections were placed on a Formvar coated copper slot grid and coated with a thin layer of nickel in a Kinney KSE-2A-M vacuum evaporator. Grids were attached to a carbon specimen post using spectroscopically pure carbon paint and observed with a Philips 500× scanning electron microscope in the transmitted electron mode (STEM). Micrographs of areas analyzed for X-rays resulted from electrons transmitted through the 0.2-μm sections. X-ray analyses were performed using an EDAX 711-F Si (Li) energy dispersive spectrometer in conjunction with the SEM.

To standardize some of the variables inherent in X-ray detection the distance of the X-ray detector from the specimen was set at 1 cm, the tilt angle was set to 0°, and specimen height and takeoff angle remained constant at 21 mm and 20°, respectively. To standardize the illuminating electron beam current, a copper standard was attached to the carbon specimen post and a copper k-alpha X-ray count was generated scanning a small raster at 60× using an accelerating voltage of 12 kV, condenser aperture of 400 μm, emission setting of 3, and spot size adjusted to give a stored count rate of 1,200 counts per second after detector saturation. Under these conditions, the electron beam spot size was approximately 190 nm. The beam current resulted in 400–600 counts per second from the sections. To compensate for variation in section thickness, point analysis of the wood cell wall was performed using an integral preset of 2,000 counts in a background window (4.92–5.08 keV). The window for Br-L series X-rays was set at 1.40–1.56 keV. To compare spectra the net peak areas with automatic background subtraction for thin specimens were used (EDAX Corporation's FDEM Software, THIN program).

Additional samples were also cut into 1-× 1-× 4-mm pieces and fixed in 2.0% $KMnO_4$ in distilled water for 3 hr at 4 C. During fixation, samples were placed in a chamber under low vacuum to aid infiltration. Samples were dehydrated through a graded acetone series and embedded in a modified Spurr's hard consistency embedding medium (30) consisting of 10.0 g of vinyl cyclohexene dioxide, 4.5 g of diglycidyl ether of polypropylene glycol (DER 736), 26.0 g of nonenyl succinic anhydride, and 0.2 g of dimethyl amino ethanol. Polymerization was done at 70 C for 16 hr.

Lignin and wood sugar analyses were done using techniques previously described (11,23).

RESULTS

Sound birch wood fixed in $KMnO_4$ had cells with intense staining of middle lamella in cell corner regions and between adjacent cells (Fig. 1A). The secondary wall was moderately

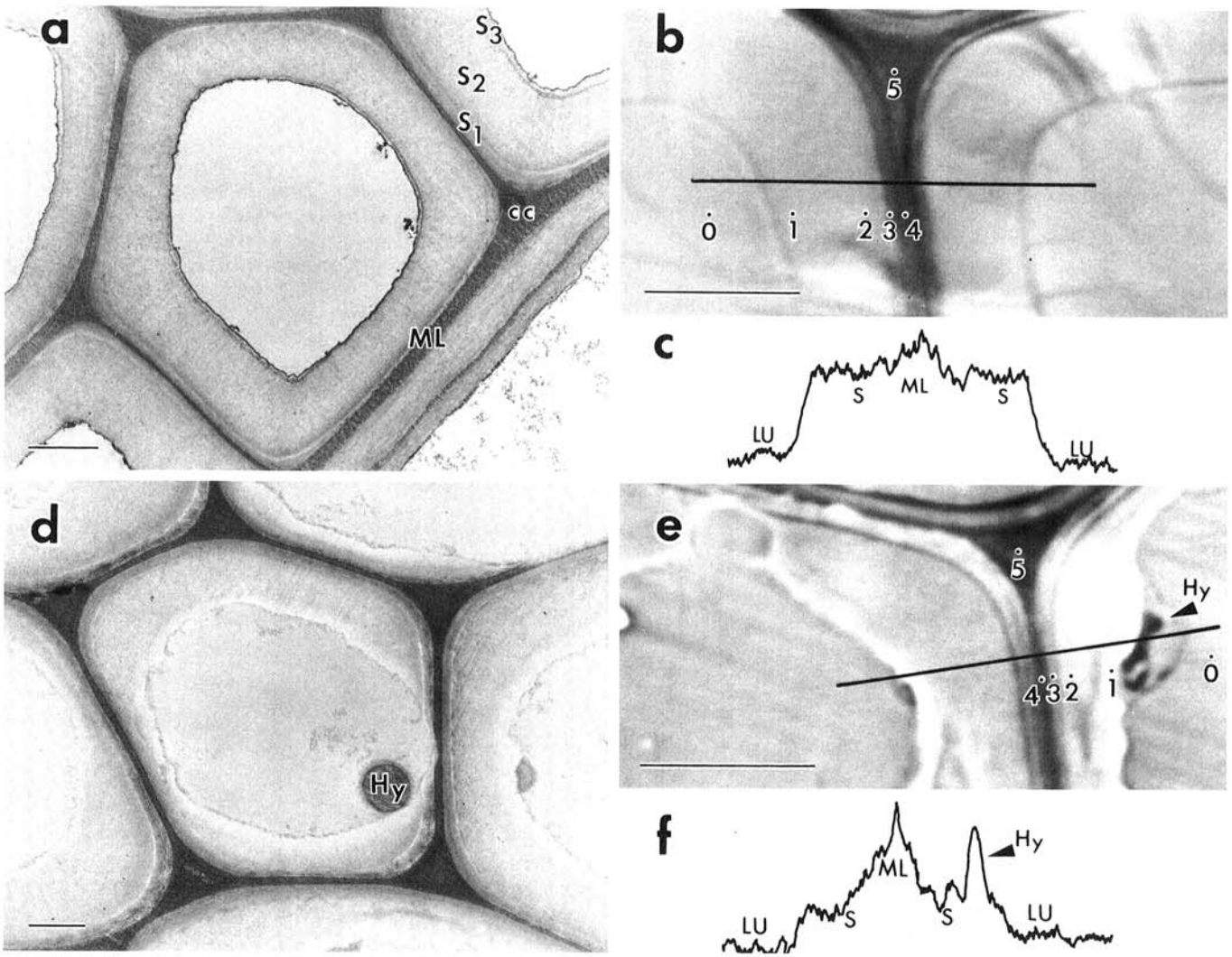


Fig. 1. A, Noninoculated sound birch wood cells fixed with $KMnO_4$ showing the various cell wall layers (ML = middle lamella, cc = cell corner, S_1, S_2, and S_3 = secondary wall layers. **B,** STEM micrograph of brominated sound wood. Scan line and point analyses locations (see Table 1) are indicated. **C,** Br distribution profile from area of scan line in Fig. 1B (LU = lumen, S = secondary wall, ML = middle lamella). **D,** Cells decayed by *Coriolus versicolor* for 12 wk and fixed with $KMnO_4$. Erosion of secondary wall is evident (HY = hypha). **E,** STEM micrograph of brominated wood decayed by *C. versicolor*. Scan line passes through two fiber cells and hypha (HY) in cell lumen. **F,** Br distribution profile from area of scan line in **E** showing secondary wall with low concentrations and high levels in middle lamella and at the site of the hypha (HY).

stained and had good definition of the S_1 and S_2 layers. A line scan (Fig. 1C) of two fiber cells from brominated wood (Fig. 1B) provided a distribution profile of lignin in the cell wall. The highest peak of the profile corresponded to the middle lamella region and decreasing amounts were present in the secondary wall. Point analysis at various locations (Fig. 1B, 0–5) showed the highest amounts in the cell corner and middle lamella between cells (Table 1). Bromine concentrations were not as great in the secondary wall, and the lowest counts were found in the secondary wall layer nearest the cell lumen. The mean Br-L X-ray counts for various morphological regions of several different cells are presented in Table 2. Although some variation occurred, similar distribution patterns were observed.

Wood decayed by *C. versicolor* for 12 wk had cells with severely eroded secondary wall layers (Fig. 1D). In some cells, stages of incipient decay were present and the secondary wall was degraded immediately beneath fungal hyphae, whereas others had stages of advanced decay and entire secondary walls were removed. The compound middle lamella was degraded only at lysed zones beneath the hyphae. Localized erosion troughs extended through all cell wall layers. Line scan and point analyses of Br-L X-rays from wood decayed by *C. versicolor* showed a loss of lignin in secondary wall layers near fungal hypha and adjacent to eroded parts of the cell (Fig. 1E and F, Tables 1 and 2). In advanced stages of decay, lignin was also removed from the entire circumference of the secondary wall bordering the cell lumen. The compound middle lamella between cells and in cell corners did not have different Br-L X-ray counts than sound wood, indicating that lignin was not attacked (Table 2). High concentrations of Br were frequently found in the cell lumen at sites of fungal hyphae (Fig. 1F).

Wood decayed by *Phlebia tremellosus* for 4 wk had stages of incipient decay present. The secondary wall adjacent to hyphae was less electron dense than cell walls from other areas (Fig. 2A). The middle lamella was not altered and intense staining was maintained. Br concentrations in the secondary wall regions beneath hyphae were substantially lower than values from sound wood indicating a loss of lignin (Fig. 2B and C). Bromine in the middle lamellae and secondary walls of adjacent noncolonized cells was not changed at this early stage of decay. Advanced stages of decomposition from wood decayed for 12 wk by *Phlebia tremellosus* had poorly stained secondary walls and completely degraded middle lamellae (Fig. 2D). The cell corner regions of the middle lamella, however, persisted and showed little alteration. Low Br counts from throughout the remaining secondary walls indicated extensive depletion of lignin. The cell corner region was the only area where lignin remained in quantities similar to those in sound wood.

Extensive delignification was also found in wood decayed for 12 wk by *Phellinus pini*. The S_1 and S_2 layers of the secondary wall were poorly stained but still discernible, and the compound middle lamella was no longer evident (Fig. 2G). The cell corner regions appeared to be in various stages of degradation and frequently were completely removed. The distribution profile of Br was reduced to only the background level that occurred in cell lumens (Fig. 2H and I). Point analyses also showed the low Br concentrations throughout the wood (Tables 1 and 2).

A comparison of Br-L X-ray counts in sound and decayed wood is illustrated in Figure 3. *Phlebia tremellosus* and *Phellinus pini* caused extensive delignification of the cell wall. Incipient stages of decay showed similar Br distribution patterns for these two fungi. Lignin was removed progressively from the lumen toward the middle lamella. The delignification of the secondary wall preceded loss of lignin from the middle lamella. In advanced stages of decay, *Phellinus pini* was able to degrade some of the cell corner regions, whereas *Phlebia tremellosus* generally did not. Wood decayed by *C. versicolor* also had less Br in the S_1 and S_2 layers than sound wood but amounts were greater than those found in wood decayed by *Phlebia tremellosus* or *Phellinus pini*.

Extensive delignification was also found in birch wood decayed for 12 wk by *Poria medulla-panis*, *H. annosum*, and *D. squalens* (Fig. 4A–D). Br profiles were similar to those presented in Figure

TABLE 1. Bromine-L X-ray counts for point analysis locations in different morphological regions of the cell wall on Figures 1B and E and 2B, E, and H

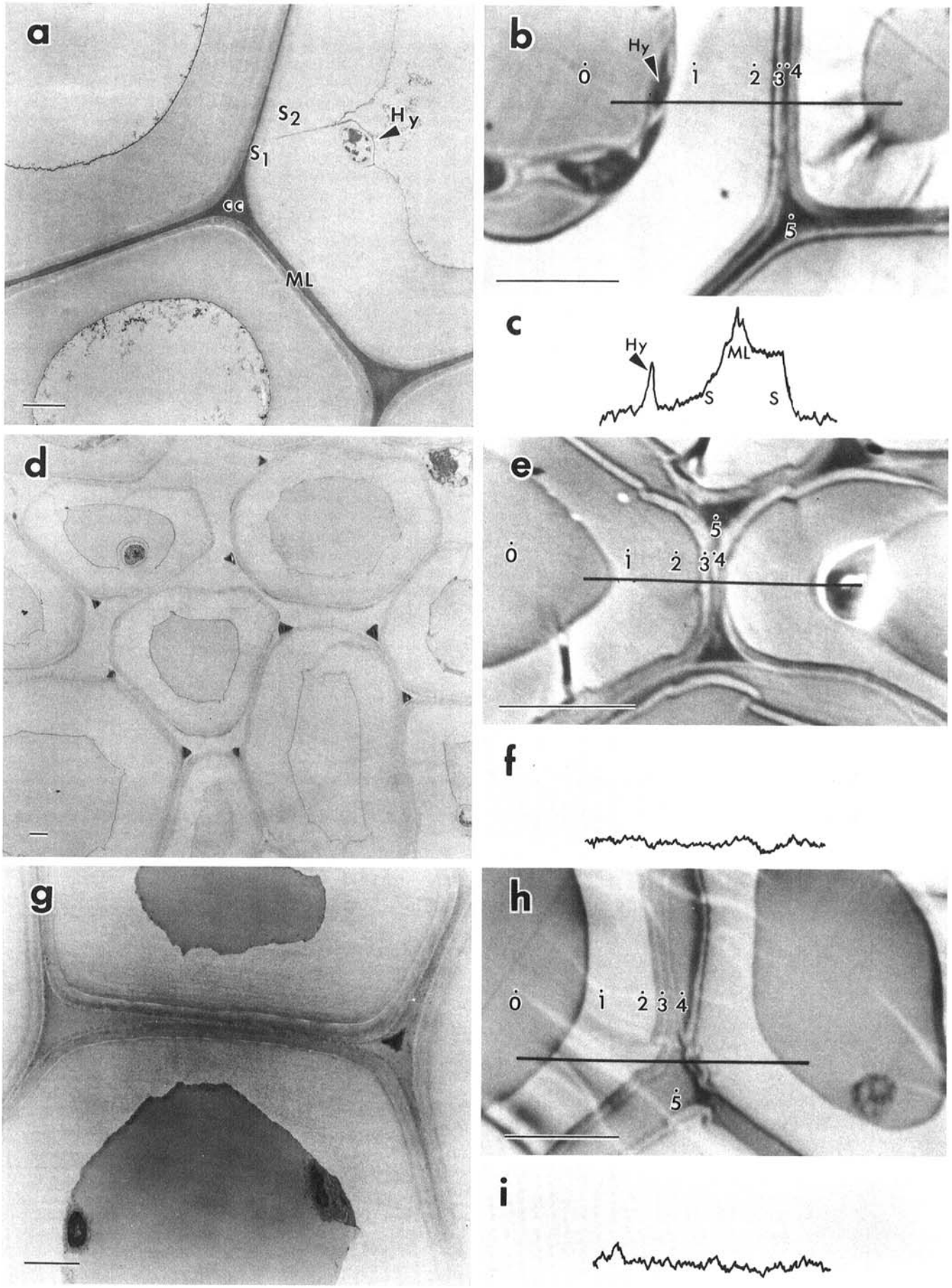
	Point location on micrographs					
					Middle lamella	
Specimen	Lumen (0)[a]	S_2(1)	S_2(2)	S_1(3)	Between cells (4)	Cell corner (5)
Sound birch (Fig. 1B)	279	5,972	7,402	6,859	7,608	9,435
Coriolus versicolor (Fig. 1E)	236	3,760	3,044	5,066	5,702	11,589
Phlebia tremellosus 1 mo (Fig. 2B)	42	1,149	2,847	6,524	8,042	9,029
Phebia tremellosus 3 mo (Fig. 2E)	279	326	269	284	402	10,659
Phellinus pini (Fig. 2H)	295	561	611	367	48	138

[a] Numbers in parentheses refer to the location of point analyses on Figures 1 and 2.

TABLE 2. Mean ± standard deviation Br-L X-ray counts for five point analysis locations in different morphological regions of the cell walls of sound and decayed wood

	Lumen	S_2	S_2'	S_1	Middle lamella	Cell corner
Sound birch	182 ± 67	6,732 ± 497	7,209 ± 654	7,294 ± 1,074	7,597 ± 532	11,063 ± 1,399
Coriolus versicolor	238 ± 84	3,726 ± 1,191	4,428 ± 1,154	6,252 ± 1,465	9,445 ± 1,952	11,074 ± 2,208
Phlebia tremellosus (1 mo)	163 ± 65	1,543 ± 134	1,724 ± 242	5,324 ± 1,753	9,547 ± 1,845	12,255 ± 601
Phlebia tremellosus (3 mo.)	148 ± 130	431 ± 175	437 ± 126	996 ± 632	1,680 ± 1,701	11,207 ± 3,509
Phellinus pini (incipient)	152 ± 104	757 ± 324	1,650 ± 1,084	5,846 ± 1,333	8,105 ± 2,005	12,595 ± 785
Phellinus pini (advanced)	151 ± 138	434 ± 101	493 ± 69	299 ± 115	250 ± 63	134 ± 86

Fig. 2. A, Cells decayed by *Phlebia tremellosus* for 4 wk and fixed with $KMnO_4$. Secondary wall of fiber cell with hypha (HY) in lumen was less electron dense than surrounding cells. **B,** STEM micrograph of brominated wood decayed by *P. tremellosus* for 4 wk. Scan line passes through hypha (HY) and two fiber cells. **C,** Br distribution profile from area of scan line in **B** showing low concentrations in secondary wall of cell with hypha (HY). Br in middle lamella and secondary wall of adjacent cell was not different than concentrations in sound wood. **D,** Cells decayed for 12 wk by *P. tremellosus* and fixed with $KMnO_4$. Secondary walls are free of electron dense stain and middle lamella between cells is degraded. Cell corner regions are not completely degraded. **E,** STEM micrograph of cells decayed for 12 wk by *P. tremellosus*. **F,** Br distribution profile from area of scan line in **E**. Very low concentrations of Br are found throughout the scan line. **G,** Cells decayed by *Phellinus pini* for 12 wk and fixed with $KMnO_4$ showing degraded middle lamella and secondary walls with little electron dense stain. **H,** STEM micrograph of cells decayed by *Phellinus pini* for 12 wk. **I,** Br distribution profile from area of scan line in **H**. Very low concentrations of Br are evident.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2F and I. Decay by *Poria medulla-panis* resulted in removal of lignin from the secondary wall and middle lamella (Fig. 4A). The cell corners were not completely degraded. Lignin loss from cells decayed by *H. annosum* followed similar patterns to those observed for *Poria medulla-panis*, however, some cells showed a localized erosion of the secondary wall (Fig. 4B). In wood decayed by *D. squalens*, cells were delignified and secondary walls were severely degraded (Fig. 4C and D). Erosion troughs were frequently observed in the cells and many exhibited a thinning of the entire secondary wall layer. In parts of wood blocks less severely attacked, the cells were depleted of lignin and the secondary walls were not eroded.

Lignin and individual wood sugar analyses of wood blocks decayed for 12 wk confirmed the preferential degradation of lignin by *H. annosum*, *Phlebia tremellosus*, *Phellinus pini*, and *Poria medulla-panis* (Table 3). When lignin was removed a relatively small percentage of glucose and larger percentages of xylose and mannose were lost. Wood blocks decayed by *D. squalens* had extensive lignin losses but also substantial amounts of wood sugars removed. In contrast, *C. versicolor* removed large amounts of all wood components after 12 wk of decay.

DISCUSSION

Lignin was found throughout the cell wall layers of sound birch wood with the greatest concentrations in the middle lamella between cells and in cell corners. The greater intensity of $KMnO_4$ stained cell wall layers corresponded to areas of increased Br concentrations. These results confirm those previously published (9) indicating $KMnO_4$ specifically stains lignin in wood. Fungi that caused a preferential degradation of lignin removed lignin from the secondary wall before degrading the middle lamella (Tables 1 and 3). Lignin was removed from throughout the secondary wall with as few as one fungal hypha evident in the cell lumen. The loss of lignin occurred first at the secondary wall near the lumen followed by a progressive attack through the secondary wall toward the middle lamella. Although degraded lignin products have been reported to be more reactive to $KMnO_4$, causing intense staining around decayed zones (18,25), the results presented here show delignified cell walls with less electron density. Advanced stages of decayed wood did not have lignin degradation products present that reacted with $KMnO_4$. Other investigators have also noted the loss of electron density in the cell wall as decay progressed (20,26). Bromine-L X-ray analyses showed extensive loss of lignin from the entire cell wall and also suggested that lignin degradation products are not present or do not react with Br.

During stages of incipient decay by *C. versicolor*, a localized loss of lignin from the secondary wall was evident before erosion troughs formed. The delignification was restricted, however, to the secondary wall immediately beneath the hyphae. In these specific areas of the cell wall, lignin degradation precedes cellulose degradation. The loss of lignin in advance of cellulose by *C. versicolor* has been postulated by previous investigators (10,31). The techniques used in our study confirm these previous investigations. As decay by *C. versicolor* progressed, the middle lamella was degraded in areas where substantial erosion of the secondary wall occurred (Fig. 4C and D). A similar attack of the middle lamella by *C. versicolor* in sweetgum wood was found only after the S_2 layer was destroyed (31). These decay patterns observed for *C. versicolor* also corresponded to decay by *Trametes hirsuta* (Wulf.:Fr.) Pilát and other white rot fungi capable of degrading all cell wall components (7,19). In the study presented here, lignin was also partially removed from the entire circumference of the secondary wall in advanced stages of decay. A subsequent loss of cellulose resulted in a thinning of the secondary wall.

Wilcox (31) observed, in advanced stages of decay by *C. versicolor* (50–94% weight loss), that the thickened areas of the middle lamella at the cell corners resisted degradation the longest. Fungi used in our study, such as *Phlebia tremellosus* and *Poria medulla-panis*, and other preferential lignin degraders, removed lignin from throughout the cell wall but were slow to degrade the cell corner region. Although lignin was more selectively removed from cell walls by these fungi, the cell corners persisted the longest. The resistance of the cell corner regions could be due to the type and quantity of lignin present. Hardwood species with greater proportions of syringyl to guaiacyl lignin are more susceptible to attack by white rot fungi (10,12). One factor responsible for the relatively small number of white rot fungi that attack conifer species in nature may be the guaiacyl-type lignin found in coniferous woods (10,15). In birch wood, Fergus and Goring (13) suggested that the secondary wall lignin is predominantly syringyl lignin, whereas the cell corner regions have greater amounts of guaiacyl lignin. In the study reported here, *Phellinus pini* (a fungus found in nature only on conifers), appeared to degrade the cell corners of birch wood more rapidly than isolates of fungi from wood of deciduous species. These results suggest that conifer isolates of white rot fungi appear to grow rapidly in wood of deciduous tree species and are efficient decomposers of syringyl and guaiacyl lignin. Further screening of isolates from wood of coniferous species should be done to evaluate delignification potential in deciduous substrates.

Bromine concentrations in the middle lamella of sound wood (Tables 1 and 2) may underestimate the amount of lignin. Differences in the reactivity of Br to guaiacyl and syringyl lignin have been reported (17). If less Br reacts with guaiacyl lignin than syringyl and the middle lamella contains a greater percentage of guaiacyl units (14), the Br counts obtained may not reflect a correct representation of lignin concentration. The small differences between counts for the middle lamella between cells and the

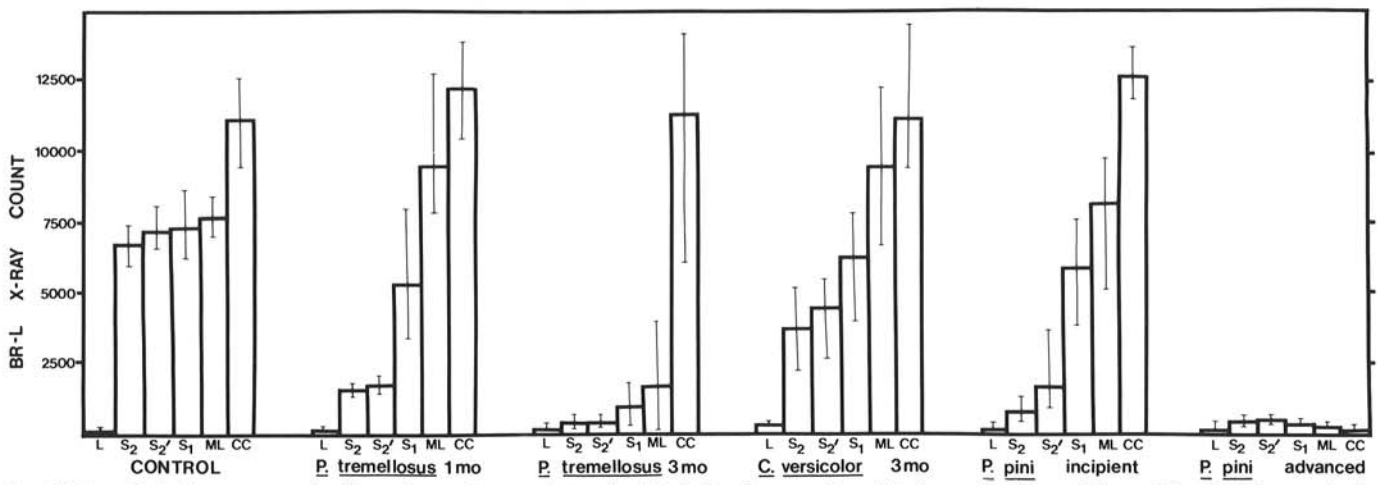

Fig. 3. Mean Br-L X-ray counts for five point analyses and standard deviation from noninoculated wood and wood decayed by *Coriolus versicolor*, *Phellinus pini*, and *Phlebia tremellosus*. L = lumen, S_2, S_2', S_1 = secondary wall layers from lumen toward middle lamella respectively, ML = middle lamella, CC = cell corners.

secondary wall may also be due to inadequate resolution of the relatively thin middle lamella during point analysis.

The high concentration of lignin in the cell corners and minimal amount of carbohydrate associated with it may contribute to the resistance of cell corners to degradation. When lignin is degraded a concomitant loss of carbohydrate occurs (1,16). Fungi that preferentially degrade lignin also remove considerable amounts of hemicellulose (Table 3). Wood sugar analyses of advanced stages of wood decayed in the field by preferential lignin degrading fungi showed extensive removal of hemicellulose when lignin was removed (5,7). The close spatial and chemical arrangement of lignin and hemicellulose within the cell walls of wood may be responsible for lignin and hemicellulose being degraded without significant loss of cellulose (5,22). The high concentration of lignin and reduced ratios of hemicellulose in the cell corners could result in a slower enzymatic attack.

Cellulose utilization differed among the white rot fungi investigated. Evidence of cellulose degradation by *C. versicolor* was observed at the cell wall where lignin had been partially removed. The erosion of the secondary wall caused the loss of all cell wall components from these localized areas. In advanced stages of decay, lysed zones formed holes from one cell lumen to another and the entire secondary walls were thinned. Preferential lignin degrading fungi, such as *Phlebia tremellosus*, *Phellinus pini*, and *Poria medulla-panis* do not cause extensive cellulose loss accompanying lignin degradation. It is only after cells are void of lignin that initial evidence of cellulose degradation is apparent. Cellulose loss may be minimal with only scattered cells showing

TABLE 3. Percent weight, lignin, and individual wood sugars lost after 12 wk of birch wood decay by white rot fungi

Fungus	Percent loss				
	Weight[a]	Lignin[b]	Glucose[b]	Xylose[b]	Mannose[b]
Coriolus versicolor	54.1 ± 10.6	61.2 ± 2.9	51.9 ± 11.8	57.8 ± 11.5	54.8 ± 28.9
Phlebia tremellosus	38.0 ± 3.3	77.6 ± 4.8	9.7 ± 7.0	45.0 ± 5.6	19.0 ± 7.8
Phellinus pini	16.3 ± 1.4	53.1 ± 4.9	4.0 ± 1.3	12.3 ± 1.4	12.4 ± 5.2
Poria medulla-panis	31.8 ± 1.9	77.1 ± 5.0	0.1 ± 0.2	34.7 ± 6.6	27.7 ± 18.8
Heterobasidion annosum	23.8 ± 1.4	58.2 ± 7.7	6.6 ± 3.7	26.5 ± 1.7	34.2 ± 16.3
Dichomitus squalens	45.1 ± 3.2	78.4 ± 8.8	33.9 ± 8.7	47.1 ± 5.7	55.3 ± 13.9

[a] Mean of 10 replications ± standard deviation.
[b] Mean of 3 replications ± standard deviation.

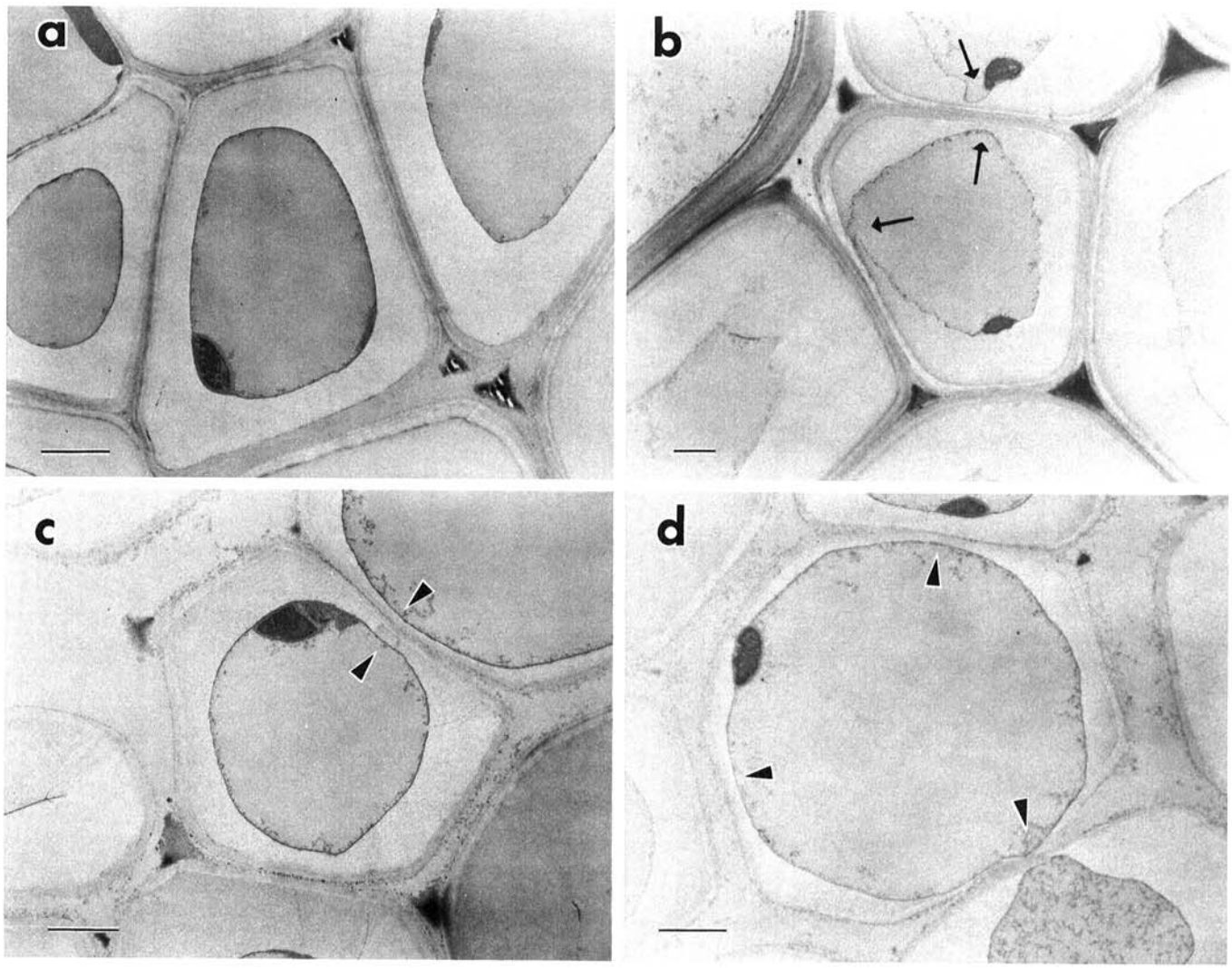

Fig. 4. A, Cells delignified by *Poria medulla-panis* for 12 wk and fixed with $KMnO_4$ showing no middle lamella in areas between cells and secondary walls with little electron dense stain. **B,** Delignified cells decayed by *Heterobasidion annosum* for 12 wk and fixed with $KMnO_4$ showing cells with eroded secondary walls (arrows). **C and D,** Cells delignified by *Dichomitus squalens* with thin secondary walls (arrowheads).

eroded secondary walls as observed in this study and in a previous investigation (8) or more extensive loss as observed for *D. squalens* (Fig. 4C and D). In nature, wood remaining after selective delignification consists primarily of cellulose (5). The cellulose appears to be in a crystalline form because brown rot fungi were not able to decompose the substrate (4). The nature and characteristics of the cellulose remaining after selective delignification by white rot fungi require additional study.

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