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# Micromorphology of Degradation in Western Hemlock and Sweetgum by the White-Rot Fungus *Coriolus versicolor*

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## Summary

The micromorphological changes in sweetgum and western hemlock sawdust caused by the white-rot fungus *Coriolus versicolor* were studied by transmission electron microscopy (TEM). Degradation by *C. versicolor* differed in sweetgum and hemlock. Sweetgum was attacked by hyphae from both the lumen and the cell corners. Hemlock was attacked only from the lumen. The compound middle lamella of hemlock was not attacked until removal of the secondary wall was completed; the cell corners were particularly resistant to degradation. In sweetgum, the middle lamella and cell corners were severely degraded without appreciable degradation to adjacent cell wall material; delignification of the cell wall occurred without substantial structural alteration of the remaining cell wall. In contrast, in hemlock, lignin was degraded with the simultaneous destruction of the cell wall. These results support earlier findings that, because of the slower degradation of softwood lignin, *C. versicolor* degrades softwoods slower than it degrades hardwoods.

## Introduction

*Coriolus versicolor* is an important wood-decay fungus. It is classified as a simultaneous white-rotter because it degrades and metabolizes lignin and carbohydrates at approximately the same rate (Cowling 1961; Liese 1970). In most hardwoods *Coriolus versicolor* decay is considerably faster than in softwoods (Cowling 1961; this study, Fig. 1). The slower decay of softwoods by some white-rot fungi has been attributed to the higher lignin content of softwoods (Takahashi 1976). However, hardwoods and softwoods differ structurally in the chemistry of their lignins (Adler 1977; Freudenberg 1968). Recent evidence indicates that the chemical nature of the lignins in softwood and hardwood may be more important than their amount (Faix *et al.* 1985; Highley 1982).

*Coriolus versicolor* has been studied extensively with respect to its biochemistry of degradation and microscopy at the light microscopic level (Cowling 1961; Greaves and Levy 1965; Wilcox 1968, 1970). Light microscopy studies show that it degrades wood in a manner typical of white-rotters, starting the attack from the $S_3$-layer and progressing through the middle lamella. Little information is available at the ultrastructural level (Cowling 1961; Wilcox 1970).

The goal of the present research was to determine the ultrastructural changes produced by *C. versicolor* in sweetgum and hemlock, and establish whether differences exist in the degradative patterns which might be helpful in explaining its faster attack on hardwood substrates.

## Materials and Methods

### Decay tests

Blocks, 1.3 × 1.3 × 0.32 cm, the small dimension in the grain direction, were cut from the sapwood of sweetgum (*Liquidambar styraciflua* L.) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) They were decayed by *C. versicolor* (L.: Fr.) Quel. (Mad. 697) and *Ganoderma applanatum* (Pers: S.F. Gray) Pat. (MAD 708) using the soil-block procedure (ASTM 1971). The blocks were removed at 4, 6, 8, and 12 weeks for determination of weight loss. Percentage weight loss, as a measure of decay, was calculated from the weights of the decayed blocks after they were brought to equilibrium at 70% relative humidity and 27°C.

### Transmission electron microscopy

For transmission electron microscopy (TEM), sawdust from hemlock and sweetgum was degraded by *C. versicolor* using the soil-block procedure (ASTM 1971). After 6 weeks, the degraded sawdust was removed from bottles and fixed in formaldehyde-glutaraldehyde mixture in 0.1 M phosphate buffer, pH 7.2, postfixed in 1.33% $OsO_4$ in s-collidine buffer, pH 7.2, and stained "in block" with 1.5% uranyl acetate. Another series of sawdust samples was fixed in 2.5% aqueous $KMnO_4$. All samples were dehydrated in acetone series, followed by propylene oxide. Embedment was in araldite, epon, and dodecenyl succinic anhydride in proportions 1:1:3. Polymerization was carried out at 80°C, and micrographs were taken with RCA EMU 3G microscope.

[1] The Laboratory is maintained in cooperation with the University of Wisconsin.


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## Results and Discussion

The weight losses produced by *C. versicolor* and *G. applanatum* in hemlock and sweetgum are given in Fig. 1. In agreement with previous findings (Cowling 1961; Highley 1982), *C. versicolor* produced considerably greater weight losses in the hardwood substrate (sweetgum) than in the softwood substrate (hemlock). *Ganoderma applanatum* also produced greater weight losses in sweetgum than in hemlock, but the differences were not nearly as great.

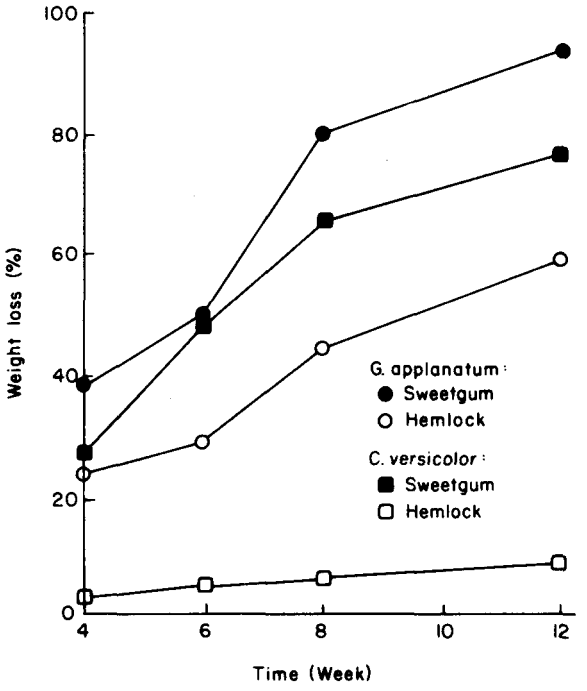


Fig. 1. Weight loss caused by *Coriolus versicolor* and *Ganoderma applanatum* in sweetgum and hemlock. (ML86 5114)

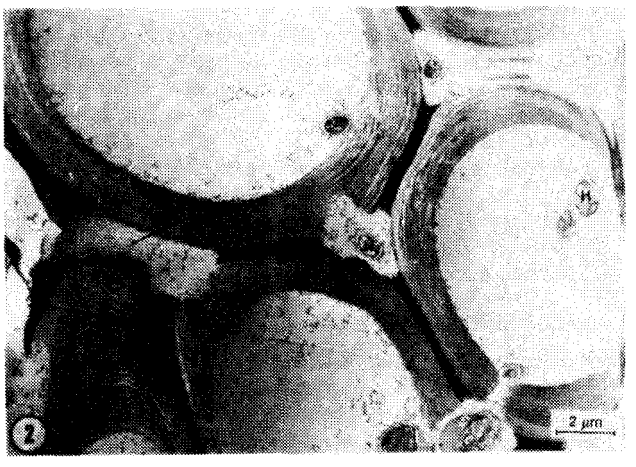


Fig. 2. Cell wall comers and adjacent middle lamellae of sweetgum removed (arrow points to degradation product), but parts of wood cell wall and middle lamellae not degraded. Hyphae (H) in cell lumina and cell corners. $KMnO_4$. 3,400× (MU6 0073)

Distribution of hyphae and initiation of attack by *C. versicolor* differed in sweetgum and hemlock. In

Fig. 3. Higher magnification of sweetgum showing light-colored, delignified areas in wood cell wall; attack from cell corners and lumen; cell wall thinning (arrow) on one side of middle lamella with opposite cell wall not degraded. Dark granular material present in lumen and cell wall corners. $KMnO_4$. 6,000× (ML86 0074)

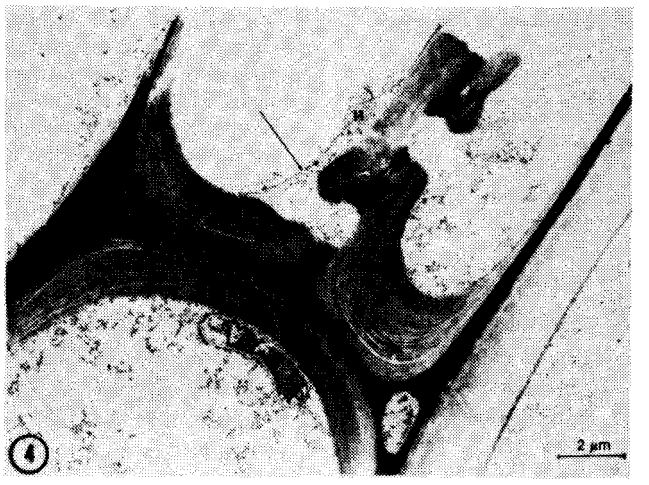


Fig. 4. Hypha (H) attached to sweetgum cell wall by sheath (arrow). Cell wall degraded beneath sheath and away from hypha toward middle lamella. Note localized degradation at cell corners and at erosion troughs. $KMnO_4$. 4,100× (ML86 0075)

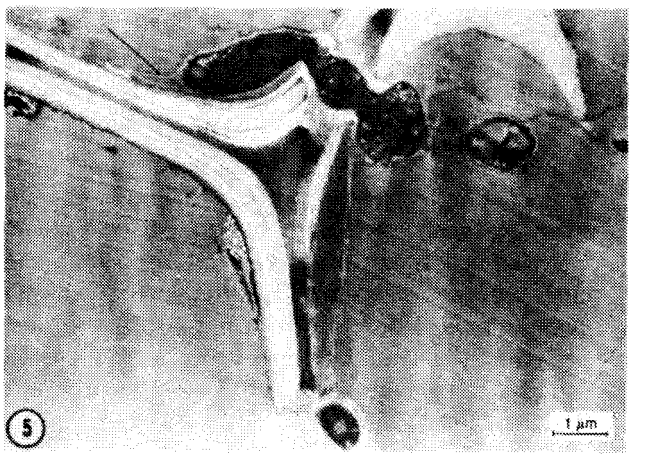


Fig. 5. Hypa surrounded by sheath (arrow) penetrating through sweetgum cell wall. Note constriction of hypha in bore hole and localized degradation of middle lamella around hypha. Cell wall on right mostly degraded. $OsO_4$. 6,300× (ML86 0076)

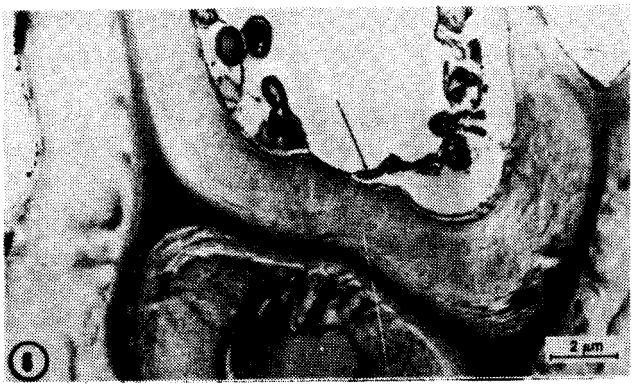


Fig. 6. Hyphae surrounded by sheath (arrow) attacking hemlock from lumen in early stage. $OsO_4$. 4,100× (ML86 0077)

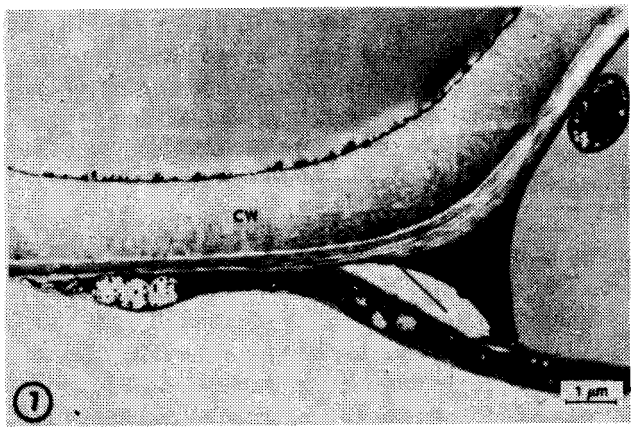


Fig. 7. Thinning of cell wall in hemlock proceeds toward middle lamella and cell corner (arrow). Adjacent cell wall (CW) not degraded; cell corner resistant to attack. $OsO_4$. 6,000 × (ML86 0078)

Fig. 8. Degradation of hemlock by hyphae (H) confined to the outer border of the cell wall. $OsO_4$. 8,500 × (ML86 0079)

Fig. 9. Tracheids separated at $S_1$ area due to hypha (H) invasion; middle lamella (ML) and secondary wall in hemlock appear unattacked. $KMnO_4$. 8,080× (MU6 0080)

Fig. 10. Entire middle lamella and the tracheid wall at right totally degraded in hemlock. Hypha (H) attached to the remaining undegraded cell wall. $KMnO_4$. 11,780 × (MU6 0081)

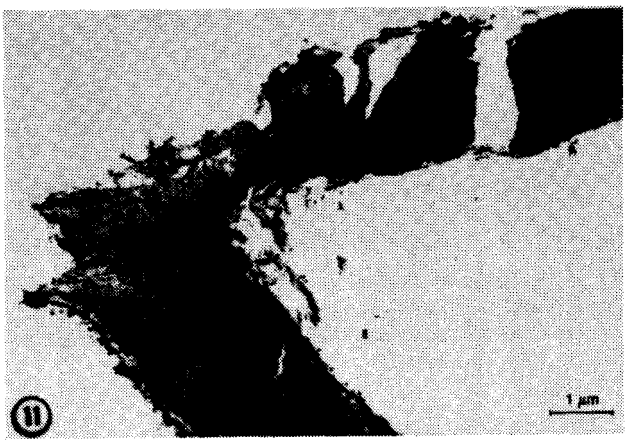


Fig. 11. Very advanced cell wall degradation of hemlock showing areas of total and partial removal of cell wall materials. Adjacent $S_2$ and middle lamella completely removed. $KMnO_4$. 7,630 × (ML86 0082)

sweetgum, C. versicolor attacked the cell wall both from within the lumen and from the cell corners (Figs. 2–4). We also observed hyphae boring through the cell wall (Fig. 5). Hyphae penetrating the cell wall were constricted at the point of entry, a phenomenon apparently characteristic of penetrating hyphae (Highley *et al.* 1985). Growth of hyphae within the cell walls of sweetgum was predominantly localized in the cell corners (Figs. 2–4).

In hemlock attack was from the lumen only. We did not observe boring hyphae or hyphae in the cell walls or corners (Figs. 6–11). In a previous study (Murmanis *et al.* 1984), we reported that the whiterotter *Ganoderma applanatum* attacked the cell walls of hemlock primarily by hyphae growing in the lumen, but perforation hyphae or microhyphae (fine hyphae penetrating directly into the cell wall) were also formed. Cell wall invasion by perforation hyphae is

reported in other white-rot fungi (Liese 1970). *Ganoderma applanatum* degrades softwood substrates in laboratory tests at a much faster rate than *C. versicolor* (Fig. 1). The ability of *G. applanatum* to form microhyphae that penetrate the cell walls of hemlock may facilitate degradation.

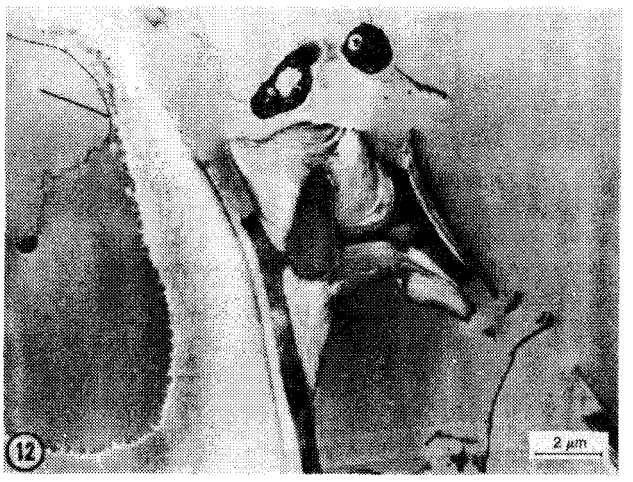


Fig. 12. Serrated inner cell wall surface at left, and thinning of wall beneath sheath (arrow) in sweetgum. At right, middle lamella and the rest of the cell wall removed. $OsO_4$. 4,100× (ML86 0083)

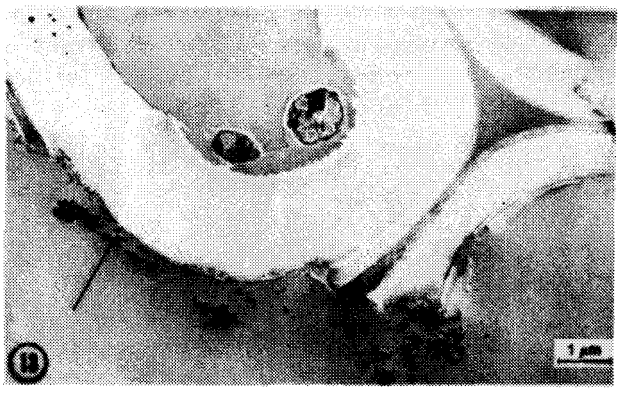


Fig. 13. Electron dense particles (arrow) associated with sweetgum cell wall. Cell wall thinning proceeds toward middle lamella. Inner cell wall surface serrated beneath hyphae (H) within sheath. $OsO_4$. 6,150× (ML86 0084)

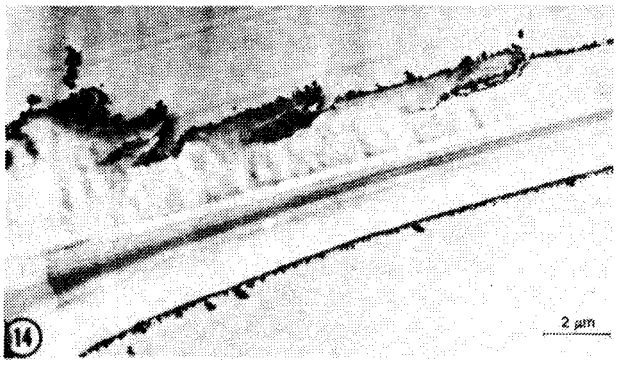


Fig. 14. Electron dense particles on inner surface of hemlock cell wall and around hypha. $OsO_4$. 4,100× (ML86 0085)

Hyphae attacking from the lumen in both sweetgum and hemlock were often surrounded by a hyphal sheath (Figs. 4–6, 12, 13). The sheath extended some distance from the hyphae, and cell walls were degraded beneath it (Fig. 4, 12, 13). Similar degradation activity of the hyphal sheath on the cell wall in the absence of hyphae was observed previously with *G. applanatum* (Murmanis *et al.* 1984).

The degraded cell wall surface adjacent to the lumen in sweetgum was occasionally irregularly eroded (Figs. 4, 12, 13), and in some cells it appeared serrate (Figs. 12, 13). In hemlock, the surface adjacent to the lumen appeared smooth, and the thinning of the cell wall was fairly uniform (Figs. 6–10). Wilcox (1968), using light microscopy, found similar differences in attack of sweetgum and pine by *C. versicolor*. In both sweetgum and hemlock we observed one-half of the cell wall entirely degraded or only remnants left (Figs. 3–5, 7). This type of attack resembles that in pine decayed by *Trametes hirsuta* in a TEM study by Messner and Stachelberger (1984).

Degradation of spruce by the white-rot fungus *Sporotrichum pulverulentum* is confined to the hyphae (Ruel *et al.* 1981). However, Messner and Stachelberger (1984) found that decay of pine cell walls by *T. hirsuta* was not limited to the area around hyphae but occurred over the entire lumen surface and resulted in a thinning of walls toward the middle lamella. In the present study, degradation by *C. versicolor* occurred over the entire lumen surface in hemlock and resulted in thinning of walls toward the middle lamella (Figs. 7–11, 14). However, in sweetgum, in addition to the thinning of cell walls, there was also locally restricted total degradation (Figs. 2–5, 12).

In hemlock there was no evidence of attack on the compound middle lamella until removal of the secondary wall was complete (Figs. 7–10, 14). The middle lamella at the cell corners of hemlock appeared particularly resistant to degradation (Figs. 6, 7). This was not the case with sweetgum. Although rich in lignin of a more reticulate nature (Yanz and Goring 1980), the middle lamella of sweetgum was extensively degraded (Figs. 2–5, 12). The cell corners were especially susceptible to degradation (Figs. 2–4, 12). Degradation was locally restricted but was very intense (involving all wall layers), and was presumably caused by direct contact with hyphae. The cell wall adjacent to the intensely degraded areas often was not noticeably degraded. This observation conflicts with that of Wilcox (1968). He reported that *C. versicolor* did not degrade the compound middle lamella of sweetgum until the secondary wall was completely removed; the cell corners were the last to be destroyed.

Occasionally, electron dense particles were present in $OsO_4$-fixed samples of both woods (Figs. **13, 14).**

When present, they occurred on the lumen surface or surrounded the hyphae. Messner and Stachelberger (1984) found electron dense particles (they use the term "osmiophilic" to describe the particles) in all stages of decay by the white-rot fungus *Trametes hirsuta.* As in our observations with *C. versicolor,* the particles occurred on the intact or degraded lumen or in places of intensive wood decay. These authors propose that the particles represent enzymes or products of decay. The infrequent appearance of the particles in our study makes them difficult to interpret.

Sweetgum fixed with $KMnO_4$ (contrasting the lignin) showed that lignin can be removed throughout the cell wall without substantial alteration of the cell wall; the lighter areas reflect occurrence of delignification (Fig. 3). Hemlock fixed with $KMnO_4$ showed that the lignin was not removed by *C. versicolor* prior to destruction of the cell wall (Figs. 9–11). In Fig. 9, the $S_1$-layer is totally degraded without attack of the middle lamella or the adjacent $S_2$. Dark granular material was present in the lumen and in intensively degraded areas of the sweetgum cell wall (Figs. 2–4). We assume that this is degraded cell wall material (Fig. 2, arrow).

Using ultraviolet radiation as an indication of changes in lignin content, Wilcox (1968) suggested that *C. versicolor* in sweetgum is able to remove lignin from cell wall layers without destruction of the wall layers. However, in pine, lignin was apparently removed concomitantly with, but not in advance of, destruction of the associated portion of the cell wall. Cowling (1961) obtained similar results by chemical analysis of decayed wood. Recent evidence suggests that some white-rot fungi are better adapted to utilize syringyl lignin residues than guaiacyl residues (Faix *et al.* 1985; Highley 1982; Leightley 1984). Hardwood lignin contains both guaiacyl and syringyl lignin residues, but softwoods contain only guaiacyl residues. Highley (1982) found that degradation by *C. versicolor* was similar in a hardwood containing coniferous (guaiacyl) lignin and in softwoods. Faix *et al.* (1985) found that the white-rot fungus *Phanerochaete chrysosporium* preferentially degraded syringyl lignin residues. Using $^{13}C$ CP/MAS NMR analysis of decayed softwoods, Leightley (1984) found that white-rotters and soft-rotters did not readily degrade the guaiacyl-type lignin, and suggested that this may account for the slower attack by white-rot and soft-rot fungi in softwoods.

Thus, the inability of *C. versicolor* to produce an early delignification of the secondary wall in hemlock, as supported by our micrographs, may result from difficulty in utilization of the guaiacyl lignin. Cellulose decomposition would also be expected to be retarded until sufficient delignification had occurred to allow the penetration of cellulolytic enzymes. In a previous study Highley (1982) found that in hemlock C. *versicolor* removed carbohydrate at about the same rate as lignin or slightly faster. In sweetgum, particularly in the early stages, *C. versicolor* removed lignin considerably faster than carbohydrate. Hemlock and other softwoods are not immune to the attack by *C. versicolor;* the attack simply proceeds very slowly in early stages of decay. This slower attack apparently results from the nature of the lignin composition of softwood cell walls.

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