

# Mechanism of Brown-Rot Decay: Paradigm or Paradox<sup>1</sup>

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Interest in understanding how brown-rot fungi degrade wood has received increasing attention in recent years because of a need to identify novel targets that can be inhibited for the next generation of antifungal wood preservatives. Brown-rot fungi are unique in that they can degrade holocellulose (cellulose and hemicellulose) in wood without first removing the lignin. Furthermore, they degrade holocellulose in an unusual manner, causing a rapid decrease in degree of polymerization at low weight loss. Despite increased research effort, the mechanism of brown-rot decay remains unclear. Furthermore, this research has not pointed to biochemical targets for inhibition and development of new wood preservatives. In reviewing the brown-rot literature, it became apparent that many beliefs about brown-rot decomposition of wood are based more on tradition or conjecture than on facts. In some cases, these misconceptions have become near dogma. They cloud our understanding of brown-rot decay and as a result may contribute to a misdirection of research efforts. The purpose of this paper is to attempt to identify and clarify some of these misconceptions. © 1997 Published by Elsevier Science Limited

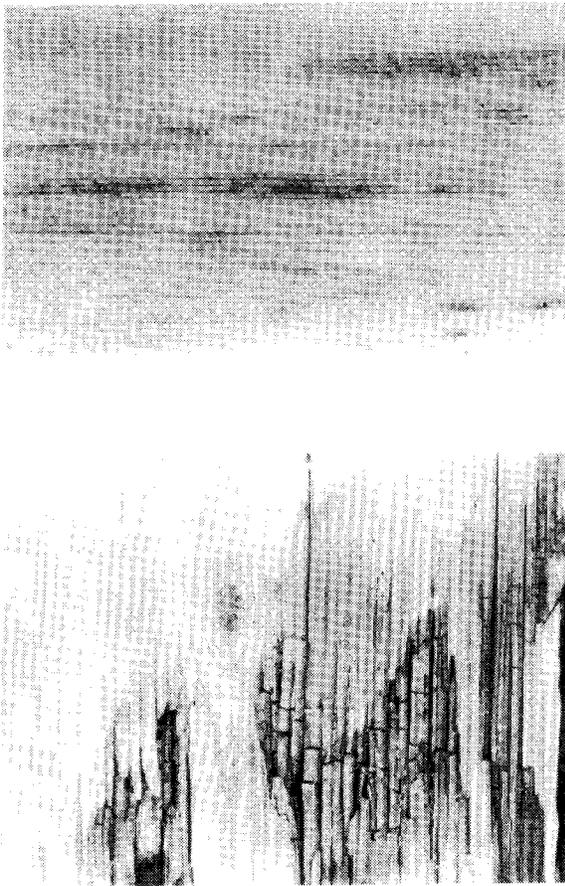
## INTRODUCTION

The most serious kind of microbiological deterioration of wood is caused by fungi because they can cause rapid structural failure. Brown-rot decay is the most common and most destructive type of decay of wood in use. Brown-rot fungi utilize the hemicelluloses and cellulose of the cell wall, leaving the lignin essentially undigested, albeit modified by demethylation and oxidation. The wood darkens, shrinks, and breaks into brick-shaped pieces that crumble easily into a brown powder (Fig. 1). Brown-rot fungi, rapidly depolymerize holocellulose, and the degradation products are produced faster than they are utilized (Cowling, 1961). The rapid depolymerization of the wood carbohydrates is reflected by the substantial increase in alkali volatility products and the

rapid decrease in strength properties of brown-rotted wood. Chemical analysis of brown-rotted white pine (*Pinus monticola*) and hard maple (*Acer rubrum*) shows that the hemicellulose glucomannan is removed considerably faster than cellulose or xylan (Highley, 1987a). Xylan is usually depleted faster than cellulose. Because hemicelluloses form an encrusting envelope around the cellulose microfibrils, further degradation and removal of depolymerized cellulose may depend on prior removal of the hemicelluloses. Thus, hemicellulose utilization may be a critical initial step in establishment of brown-rot fungi in wood.

The traditional wood preservatives (e.g., chromated copper arsenate, pentachlorophenol creosote) are effective in controlling brown-rot decay, but the safety of these preservatives is a concern. The move to less toxic methods for the preservation of wood could be facilitated if the process was better understood. Then, it would be possible to devise natural screening procedures to find inhibitors of novel targets in the fungi. Presently, in spite of much research,

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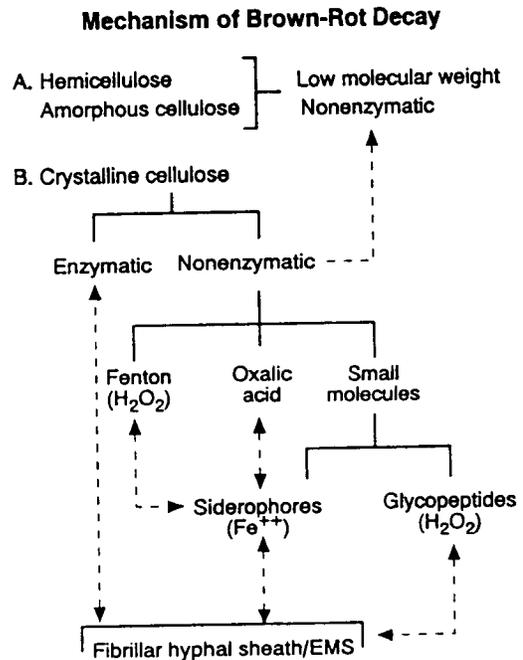
**Fig. 1.** Stages of brown-rot decay: (top) early stage-discoloration in surface and end grain (left); (bottom) late stage-cracked and collapsed wood (M 124 928).

the precise mechanism of brown-rot decay remains unclear (Fig. 2), and research has uncovered few specific biochemical targets for inhibition. In reviewing the literature, it became apparent that many convictions concerning brown-rot degradation of wood are based more on tradition or convenience than on facts. These 'half-truths' tend to obscure our understanding of brown-rot decay mechanisms and, as a result, may contribute to a misdirection of research effort. The purpose of this paper is to identify and clarify some of these misconceptions about brown-rot decay.

## PARADIGM OR PARADOX

### The rapid decrease in wood strength early in brown-rot decay reflects rapid cellulose depolymerization

Brown-rot fungi affect several strength properties of wood before significant weight loss is detected (Wilcox, 1978). Richards (1954) found a 75% loss



**Fig. 2.** Proposed mechanisms of brown-rot decay. EMS—extracellular membranous structures.

in toughness by the time 1% weight loss had occurred. Toole (1971) found that brown rot caused about 18% loss in compressive strength perpendicular to the grain at 2% weight loss. Similarly, Mizumoto (1965) found a 10% reduction in compressive strength parallel to the grain at 2% weight loss.

The drastic reduction in strength of wood early in decay is often attributed to depolymerization of the cellulose (Cowling, 1961). However, hemicelluloses form an encrusting envelope around cellulose microfibrils. Thus, the brown-rot fungi must first penetrate the hemicelluloses to access the cellulose. Brown-rot fungi remove hemicellulosic sugars (xylose and mannose) before cellulosic sugars (glucose) (Kirk & Highley, 1973; Highley, 1987a). Strength loss (MOR) is correlated with hydrolysis of hemicellulose during incipient decay by *P. placenta* (Green *et al.*, 1991). Recently, Winandy & Morrell (1993) found strength decreased dramatically in brown-rotted wood with low cellulose utilization. However, degradation of hemicellulose components was clearly related to wood strength losses. Thus, they concluded that although depolymerization of cellulose by brown-rot fungi probably accounts for some strength loss, a significant portion of the initial effect of brown-rot fungi on wood strength is a direct result of hemicellulose decomposition.

### Brown-rot fungi degrade cellulose by hydroxyl radicals or equally potent metallo-oxygen species generated by a Fenton system

Cowling & Brown (1969) recognized more than 25 years ago that even the smallest cellulases are too large to penetrate the pores of the wood. Also, cellulases do not mimic the action of brown-rot fungi in generating cellulose crystallites (Chang *et al.*, 1981; Phillip *et al.*, 1981). Cowling & Brown thus proposed that a nonenzymatic oxidative agent might be involved in depolymerization of cellulose by brown-rot fungi. They also noted that Halliwell (1965) had described the degradation of cotton cellulose by Fenton's reagent ( $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ ), which generates hydroxyl radical or a similar oxidant reagent (Halliwell & Gutteridge, 1988). Based on these observations, Halliwell was the first to propose the possibility of the existence of a nonenzymatic cellulolytic system involving peroxide and iron. Subsequently, Koenigs (1972a,b, 1974a,b, 1975) demonstrated that cellulose in wood can be depolymerized by Fenton's reagent, that brown-rot fungi produce extracellular hydrogen peroxide, and that wood contains enough iron to make Halliwell's hypothesis reasonable.

In an attempt to gain direct evidence for a nonenzymatic decay mechanism, Cobb (1981) grew liquid cultures of *Gloeophyllum trabeum* in an apparatus in which [ $^{14}\text{C}$ ] uniformly labeled cellulose was separated from the fungus by an ultrafiltration membrane. The membrane was reported to prevent the passage of enzymes between the two chambers. Under these conditions, [ $^{14}\text{C}$ ]  $\text{CO}_2$  was detected and radioactive water-soluble products accumulated in the growth chamber. These observations support the hypothesis that a nonenzymatic cellulose decay mechanism is being employed by *G. trabeum* (Cobb, 1981; Eriksson *et al.*, 1990).

Enoki *et al.* (1989) reported the ability of brown-rot fungi to oxidize 2-keto-4-thiomethylbutyric acid (KTBA) to ethylene; KTBA is converted to ethylene by one-electron oxidants such as hydroxyl radical. Ethylene production was correlated with weight loss but not cellulose depolymerization. Recently, Enoki *et al.* (1990) reported the isolation of an extracellular protein from cultures of *G. trabeum* requiring  $\text{H}_2\text{O}_2$  and capable of KTBA oxidation. These authors partially purified the protein and reported it to be an iron-containing glycoprotein of  $M_w$ , ~1600 or

2000kDa. Based on their work, Enoki and co-workers suggested the existence of "A unique wood-component degrading system that participates directly or indirectly in the fragmentation of cellulose as well as of lignin in wood and oxidizes KTBA to give ethylene." However, it is yet to be established whether the  $\text{H}_2\text{O}_2$ -dependent KTBA-oxidizing ability of this protein is related to cellulose depolymerization. Similar glycoproteins were isolated from white-rot and soft-rot fungi (Enoki *et al.*, 1991), raising questions about their role in wood decay.

Although much work has been done on the chemistry of Fenton's reagent with D-glucose, the action of  $\text{H}_2\text{O}_2$  and ferrous salts on cellulose has received relatively little attention (Moody, 1963, 1964). In an acidic medium and in the presence of  $\text{Fe}^{2+}$ ,  $\text{H}_2\text{O}_2$  will oxidize a primary hydroxyl in monohydric alcohols to an aldehyde or carboxyl group (Ivanov *et al.*, 1953). In polyhydric alcohols, primary hydroxyl groups will react first, followed by secondary hydroxyl groups. Presumably, if this mechanism was operative in brown-rot decay, attack would occur in the amorphous region of the cellulose and the reactivity of the primary hydroxyl group would not be diminished by hydrogen bonding, as would be expected of the primary hydroxyl groups in the crystalline region. Thus, uronic acid residues would be expected in the brown-rotted cellulose, but none has been reported (Highley, 1977; Kirk *et al.*, 1991). In their examination of the reaction of cellulose with  $\text{H}_2\text{O}_2$  and  $\text{FeSO}_4$  at pH 4.6, Ivanov *et al.* (1953) concluded that oxidation proceeds dramatically in acid medium (pH < 4), that primary hydroxyls are oxidized to aldehyde or carboxyl groups and that secondary hydroxyl groups are oxidized to ketone groups with eventual rupture of the ring to form two aldehyde groups, which may be further oxidized to carboxyl groups.

An important component of Fenton chemistry is  $\text{H}_2\text{O}_2$ . After more than two decades of research regarding  $\text{H}_2\text{O}_2$  production by brown-rot fungi, it has not definitely been established that these fungi synthesize extracellular  $\text{H}_2\text{O}_2$  (Highley & Flournoy, 1994). (Koenigs, 1972a,b, 1974a,b) first reported  $\text{H}_2\text{O}_2$  production by brown-rot fungi. Later, Koenigs (1974a or b) observed  $\text{H}_2\text{O}_2$  production with cellulose depolymerization. The  $\text{H}_2\text{O}_2$  assay (catalase-aminotriazole) employed in the 1974 investigations was subsequently demonstrated by Highley (1991) to be an invalid

method for measuring  $H_2O_2$  by wood decay fungi. Since then, a variety of investigators (Highley, 1982, 1978; Highley & Murmanis, 1985a, 1985b; Highley *et al.*, 1988; Illman *et al.*, 1989; Veness & Evans, 1989; Ritschkoff and Viikari, 1991) utilizing diverse assay procedures (*O*-dianisidine and titanium tetrachloride, diaminobenzidine, the ABTS reagent or an oxygen electrode/catalase) have re-examined  $H_2O_2$  production by wood-decay fungi (Flournoy, 1994; Highley & Flournoy, 1994). These studies have yielded conflicting findings.

Presumably, the conflicting reports of whether wood decay fungi produce extracellular  $H_2O_2$  can be explained by the transient appearance of  $H_2O_2$  in culture and the lack of a selective assay for the reagent.

The presumptive role of hydrogen peroxide is the generation of the hydroxyl radical in a reaction with a metal or a metal chelate. Hydroxyl radical has been detected in liquid media, agar media, or wood by various methods, including *p*-nitrosodium methylaniline (Highley, 1982), desilvering (Veness & Evans, 1989), electron spin resonance (Illman *et al.*, 1989) and chemiluminescence (Backa *et al.*, 1992). The hydroxyl radical would need to be formed at its site of action because it is very reactive with a very short lifetime and therefore would not diffuse into wood. Kremer *et al.* (1993) proposed that  $Fe^{2+}$ -oxalate diffused into wood and was then oxidized to create  $H_2O_2$  and reaction with the remaining  $Fe^{2+}$  then yielded the hydroxyl radical. Flournoy (1994) noted that because most reports concerning free radicals are phenomenological in nature, it is difficult to assign any meaning to them and little can be concluded regarding their significance to brown-rot decay.

Finally, oxalic acid, an important physiological metabolite of brown-rot fungi, may be produced at concentrations inhibitory to the Fenton reaction. Oxalic acid was proposed by Schmidt *et al.* (1981) to play a role in reduction of  $Fe^{3+}$  to  $Fe^{2+}$ , which increased cellulose decomposition by the Fenton reaction. However, Schmidt *et al.* (1981) and Tanaka *et al.* (1994) reported that at higher concentrations of oxalic acid, cellulose degradation by the Fenton's system is inhibited. *Postia placenta* and *Serpula incrassata* were shown to accumulate oxalic acid in wood (Green *et al.*, 1992 b). Furthermore, Schmidt *et al.* (1981) found additional oxalic acid following oxidative breakdown of cellulose.

In summary, considering the inhibitory effects of oxalic acid on the Fenton reaction and that the effects of Fenton's reagent on wood components and model compounds are not duplicated by brown-rot fungi in many respects (Flournoy, 1994), the Fenton system does not appear to represent an adequate model for brown-rot decay in all brown-rot species.

#### **Extracellular oxidase production by brown-rot fungi is necessary to generate hydrogen peroxide required for metal-catalyzed reactions**

The source of  $H_2O_2$  required for a Fenton-type reaction to operate in fungal degradation of wood is usually thought to be via an extracellular oxidase, such as glucose oxidase, produced by the decay fungus. Extracellular oxidase production by brown-rot fungi has rarely been reported, and as discussed in the previous section, reports of  $H_2O_2$  production by these fungi are quite controversial. It is possible, however, that a metal-catalyzed system could be involved in breakdown of wood cell wall components independent of an external source of  $H_2O_2$  provided by the decay fungus. Kenten and Mann (1953) reported that manganooxalate autoxidizes to form  $H_2O_2$ . This results in the formation of manganic manganese ( $Mn^{3+}$ ), either during the autoxidation or in a peroxidase-catalyzed oxidation of the manganooxalate by the  $H_2O_2$  produced. Koenigs, (1972a,b) suggested that  $H_2O_2$  formed from oxalic acid in such a system could be involved in brown-rot degradation of wood. Hyde and Wood (1995) recently proposed a model for attack at a distance from the hyphae of the brown-rot fungus, *Coniophora puteana*, based upon the formation of  $H_2O_2$  by autoxidation. *C. puteana* produces cellobiose dehydrogenase, which reduces  $Fe^{3+}$  to  $Fe^{2+}$ . Diffusion of  $Fe^{2+}$  away from the hyphae in a low pH environment promotes conversion to  $Fe^{2+}$ -oxalate and autoxidation with  $H_2O_2$  as the product. The critical  $Fe^{2+}/H_2O_2$  combination is therefore formed at a distance.

#### **Brown-rot fungi degrade cellulose by an oxidative mechanism**

Koenigs (1974a) reported that the hypothesis involving Fenton chemistry in brown-rot decomposition of cellulose is reasonable, pending proof that brown-rot fungi oxidize cellulose. Oxidative changes in brown-rotted lignin are

firmly established, resulting primarily in hydroxylation and demethylation of the lignin polymer (Kirk & Adler, 1969, 1970). Similar support for an oxidative system in brown-rot degradation of cellulose was sought in several studies that chemically characterized brown-rotted cellulose.

Highley (1977) compared the oxidative properties of brown-rotted cellulose and cellulose treated with oxalic acid or  $\text{H}_2\text{O}_2/\text{FeSO}_4$ . In conducting these studies, Highley compared the copper number, weight loss in alkaline boil, pH in 10% NaCl, and ion exchange capacity of the various celluloses. Brown-rotted cellulose increased copper number, increased weight loss on boiling in sodium hydroxide, decreased pH in 10% NaCl, and increased ion-exchange capacity relative to native cellulose. Brown-rotted cellulose also gave a positive test with ferrous sulfate-potassium ferricyanide, indicating the presence of oxycellulose. An increase in uronic acid content of the brown-rotted cellulose was not detected. Similar results were obtained from cellulose treated with 1%  $\text{H}_2\text{O}_2$  and ferrous sulfate. Infrared spectra showed that carbonyl groups had been introduced into brown-rotted cellulose. The spectra of brown-rotted cellulose were similar to the spectra of cellulose treated with  $\text{H}_2\text{O}_2/\text{FeSO}_4$ .

Later, Highley *et al.* (1988, 1989) produced infrared spectra (obtained by diffuse reflectance) of undegraded cotton cellulose that showed a low intensity broad carbonyl band centered at  $\sim 1740\text{cm}^{-1}$ . This finding is consistent with the presence of a reducing-end group at the end of each cellulose molecule. In brown-rotted cellulose, this band has greatly increased intensity and is centered at a slightly lower wavenumber. Reduction of the brown-rotted cellulose sample with sodium borohydride resulted in a decrease in the intensity of the carbonyl band but did not eliminate it completely. This led to the observation of several unidentified acids on HPLC profiles. No uronic acid was reported, and it was suggested that based on the methylene blue determination of 0.5% mole carboxyls, there was approximately one carboxyl moiety per cellulose chain in the depolymerized sample.

Brown-rotted cellulose depolymerization products were further chemically characterized and compared with other depolymerized cellulose samples (Kirk *et al.*, 1989, 1991). In these studies, the following four depolymerized cellulose samples were prepared from pure cotton cellulose: (1) acid-

hydrolyzed (HC1) to the limit DP; (2)  $\text{H}_2\text{O}_2/\text{FeSO}_4$  oxidized (Fenton-oxidized); (3)  $\text{HIO}_4/\text{Br}_2$  oxidized; and (4) brown-rotted (*Postia placenta*). These four samples were characterized as to molecular size distribution, yield of glucose on complete acid hydrolysis, carboxyl content, uronic acid content, carbonyl content, and sugar acids released on acid hydrolysis. Consistent with earlier results, the Fenton system, but not the other oxidation system, mimicked the brown-rot system in nearly all measured characteristics. The acid-hydrolysed sample also possessed similar characteristics. The following sugar acids were identified by GC/MS in the hydrolysates of the brown-rotted and Fenton-oxidized samples: glyceric, erythronic, arabonic, and gluconic. These results were consistent with the depolymerizing agent being related to the Fenton system, but Flournoy (1994) noted that the authors did not establish that the fungi employ such a system. Shimada (1993) stated that "The identification of these aliphatic acids does not always provide proof of the involvement of Fenton's system in physiological wood decay processes, since enzymatic evidence for the production of these acids has not yet been offered. Furthermore, Fenton's system yields such a destructive OH radical to living matter as to degrade their own cell polymers." The Fenton's system is powerful enough to decompose cellulose to carbon dioxide under the appropriate conditions as long as hydrogen peroxide is continuously supplied (Schmidt *et al.*, 1981). Flournoy (1994) discussed several pitfalls in these studies (Highley, 1977; Kirk *et al.*, 1991). They did not establish a correlation between the oxidation of cellulose and depolymerization. We do not know, for instance, whether oxidation of the cellulose precedes depolymerization, whether oxidation is a result of post-depolymerization modification, or whether oxidation and depolymerization are coupled. The cellulose used in these studies was highly degraded. Samples from early decay were not examined; these will be the samples of most interest in understanding the depolymerization mechanism. In addition, the fungus-decayed samples in one study (Kirk *et al.*, 1991) were washed with 0.1N NaOH at 50°C for 3h prior to analysis, which resulted in only 70% recovery of the original sample.

Flournoy (1994) suggested that in light of the alkaline lability of oxidized cellulose (Davidson, 1934, 1936, 1940) and the high weight losses

observed, that we re-examine the carboxyl content of brown-rotted cellulose with particular attention to analysis of samples of low weight loss (early decay). Also, the cellulose should not be exposed to alkali prior to analysis, and brown-rotted cellulose should be further characterized to determine whether formation of carboxyl groups is correlated with depolymerization of cellulose. Flournoy (1994) points out that a shortcoming of techniques currently employed for carboxyl group analysis work is their lack of sensitivity. At low weight loss, the expected yield is very low (< 0.5%), and such a small number of carboxyls may easily escape detection. Definitive analysis will depend on the development of more sensitive techniques based on radiochemical labeling (Flournoy, 1994).

In summary, although the literature indicates that chemical changes in brown-rotted lignin are oxidative in nature, oxidative changes in cellulose as a result of brown-rot decay have not been unequivocally demonstrated.

#### Brown-rot fungi cannot utilize isolated cellulose

It is often stated in the literature that brown-rot fungi cannot degrade or utilize isolated cellulose. Thus, other constituents in wood may be required to induce the cellulolytic system of these fungi (Highley, 1977; Nilsson, 1974). However, given the right cultural conditions, brown-rot fungi can degrade and utilize isolated cellulose.

Highley (1988) and Highley *et al.* (1989) studied the degradation of cellulose by brown-rot fungi in liquid and over an agar or solid medium. Cellulose saturated with water by placement in contact with agar-medium or in liquid culture was utilized by *Coniophora puteana*, *Leucogyrophana arizonica*, and *L. olivescens* (conio-phoroid brown rotters), indicating that they produced the full-cellulase-degrading complement. The nonconio-phoroid brown-rotters (*Gloeophyllum trabeum*, *Lentinus lepideus*, and *Postia placenta*) were unable to utilize cellulose under these conditions, which indicates that they produced a deficient cellulose-degrading system. However, when cellulose was placed over agar soil medium so that it did not become saturated with water, all the brown-rot fungi tested were capable of degrading and utilizing cellulose (Table 1).

The previously noted conio-phoroid brown-rotters could also solubilize dyed microcrystalline cellulose (water saturated) over an agar medium,

**Table 1.** Weight Loss of Cellulose Exposed to Brown-rot Fungi in Absence of Wood in Soil-block and Agar Tests<sup>a</sup>.

Fungi	weight loss (%) in 12 weeks			
	soil		Agar	
	-Gl <sup>b</sup>	+Gl	-Gl	+Gl
<i>Postia placenta</i>	0	55	0	47
<i>Gloeophyllum trabeum</i>	41	66	15	17
<i>Coniophora puteana</i>	41	32	27	63
<i>Leucogyrophana arizonica</i>	47	41	33	64

<sup>a</sup>Highley (1988).

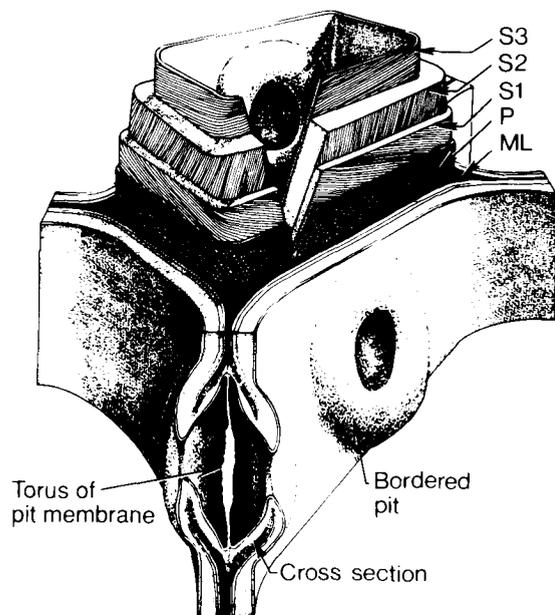
<sup>b</sup>+ or - glucose.

indicating that they produced the full cellulolytic enzyme complex (endo- and exoglucanase) (Highley, 1988). The non-members of this family were unable to solubilize dyed microcrystalline cellulose but solubilized cellulose azure, a regenerated cellulose, which indicates that under these test conditions they produced a deficient cellulolytic system. Collett (1984) found that some isolates of *Gloeophyllum* were able to solubilize dyed microcrystalline cellulose. Hegarty *et al.* (1987) reported that most brown-rot fungi isolates tested were capable of degrading dyed microcrystalline cellulose, but some strains had only weak activity. Thus, the ability of the brown-rot fungi to produce enzymes capable of degrading microcrystalline cellulose (exoglucanase activity) evidently varies with the particular strain of the species used. Production of exoglucanase is not related to the capacity of brown-rot fungi to degrade wood (Hegarty *et al.*, 1987), which means that the enzyme may not be required for cellulose decomposition by these fungi.

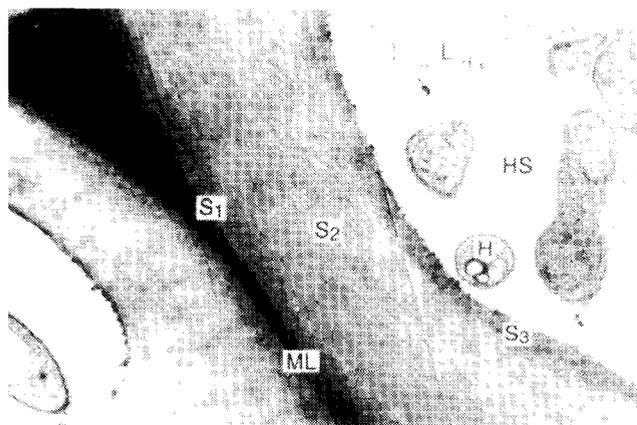
#### The S<sub>3</sub> layer of the tracheid cell wall remains intact during brown-rot decay

Brown-rot fungi move from cell lumen to cell lumen within wood by means of both pit apertures and bore holes. All wood cells are colonized early in the decay process. The decay process is thought to affect the S<sub>2</sub> layer of the wood cell wall first, and it is widely accepted that the S<sub>3</sub> layer of the wood cell wall remains intact until the late phases of decay (Fig. 3 and Fig. 4) (Kuo *et al.*, 1988). Furthermore, the porosity of the S<sub>3</sub> remains relatively unchanged during the decay process, excluding polysaccharidases (Flournoy *et al.*, 1991).

The evidence for the perseverance of the S<sub>3</sub>



**Fig. 3.** Bordered pit structure and its relationship to the layers of the wood cell wall of tracheids. Note that the bordered pit membrane is not aspirated. Cell wall layers: middle lamella (ML), primary wall (P), S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>.



**Fig. 4.** Early stage of brown-rot degradation of hemlock. Hyphal sheath (HS) of hyphae (H) growing in lumina (L) is attached to cell wall. Primary (S<sub>1</sub>), secondary (S<sub>2</sub>), and tertiary (S<sub>3</sub>) wall layers and middle lamella (ML) appear intact. Aldehyde-O<sub>5</sub>O<sub>4</sub> fixation, TEM, 8200× (M89 01 67).

layer of the tracheid cell wall comes from electron microscopic studies of brown-rotted wood. These studies show the hyphae positioned in the cell lumen, while the S<sub>2</sub> layer of the wood cell wall gradually disintegrates (Highley & Murmanis, 1987) (Fig. 4 and Fig. 5). Scanning electron microscopic studies revealed the presence of an extracellular hyphal sheath or matrix completely covering the S<sub>3</sub> layer of the wood cell wall (Larsen & Green, 1992, Green *et al.*, 1992a, 1989), which is represented in Fig. 6. If the wood cell wall is occluded by the hyphal sheath, it increases the

possibility that the inner layers of the wall are depolymerized by low molecular weight chemical agents released through the S<sub>3</sub> and that low molecular weight sugars are transported by diffusion to the S<sub>3</sub>/hyphal sheath interface for uptake and utilization by the fungi.

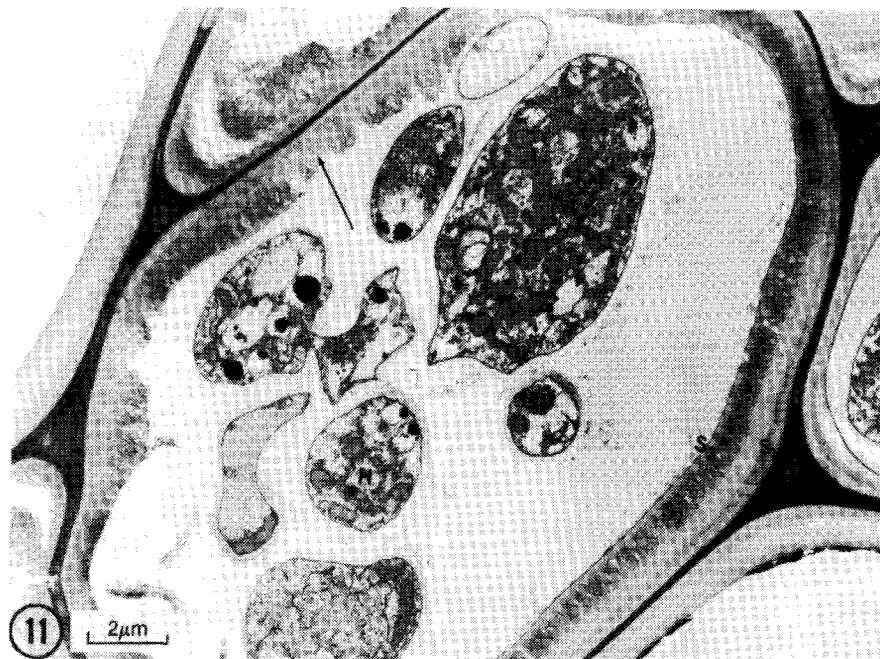
Evidence is accumulating that oxalic and other organic acids can depolymerize hemicellulose and cellulose by acid catalysed hydrolysis (Green *et al.*, 1991; Winandy & Morrell, 1993). Dilute acids have also been shown to open up the porosity of the wood cell wall (Grethlein, 1985; Beck-Anderson, 1987; Ucar, 1990). Does the hyphal sheath cover the S<sub>3</sub> and protect it from depolymerization?

Although the S<sub>3</sub> is high in lignin content, there is little cause to suspect that the S<sub>3</sub> can resist depolymerization by brown-rot agents able to decompose the S<sub>2</sub>. Thus, we hypothesize that the S<sub>3</sub> is not excluded from direct attack until late in decay. We also propose that more accurate porosity data could be generated on the S<sub>3</sub> if the hyphal sheath were removed by solubilization (detergents) or hydrolysis (enzymes).

How is the S<sub>2</sub> layer of the wood cell attacked? The most simple model would predict from the lumen inward-but no experimental evidence currently exists. Attack might also occur from the compound middle lamellae (CML) into the S<sub>2</sub> layer across the S<sub>1</sub> and primary wall. Heavy metals have been shown to diffuse through the edges of the pit apertures into the CML, (Yata, 1983). Large gaps in the cell corners of the CM are commonly observed by TEM during brown-rot decay. With immunolabeled monoclonal antibodies to xylanase from *Postia placenta*, labelling was first observed in the CM before crossing the S<sub>3</sub> (Srebotnik & Clausen, unpublished). Also, in an SEM study of brown-rot decay, we observed early weakening of the S<sub>1</sub>-S<sub>2</sub> interface during incipient decay (Green *et al.*, 1989). In both cases, longitudinal leakage of depolymerizing agents could not be excluded.

#### **Brown-rot fungi do not produce oxalate decarboxylase**

Oxalic acid (OA), the strongest organic acid (p<sub>k</sub> = 1.23), has been implicated directly and indirectly in the brown-rot decay process (Beck-Anderson, 1987; Green *et al.*, 1992b; Schmidt *et al.*, 1981; Shimada *et al.*, 1994). Takao (1965) screened Basidiomycetes for organic acid



MC84 9011

Fig. 5. Advanced cell wall degradation in sweetgum by hyphae (H) in lumen. At left (arrow), entire wall attacked, At right, the  $S_2$  layer appears to be attacked first, and  $S_1$  and  $S_3$  relatively unattacked. Aldehyde-OsO<sub>4</sub> fixation. 3900 × (MC 84 9011).

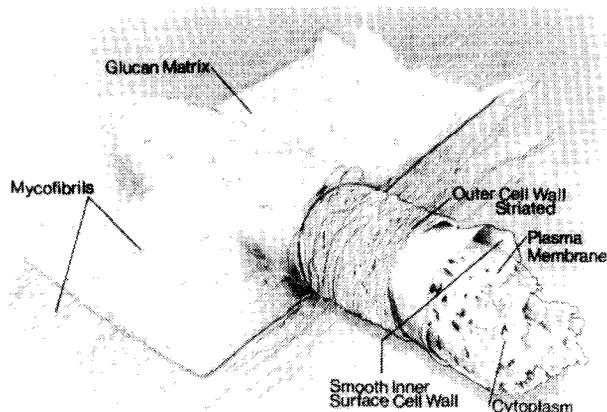


Fig. 6. Schematic representation of hypha and hyphal sheath structures as observed on inert substrates.

production and concluded that there were two main groups. In general, brown-rot fungi accumulate oxalic acid during growth and white-rot fungi do not unless supplemented with calcium carbonate. White-rot fungi fail to accumulate oxalate as a result of the activity of oxalate decarboxylase, first described by Shimazano (1955), which degrades oxalate to formate and CO<sub>2</sub>.

One apparent exception among the aggressive brown-rot fungi is *Gloeophyllum trabeum*, which accumulates very low levels of oxalic acid and

causes relatively small reductions in wood pH (Akamatsu *et al.*, 1994; Espejo & Agosin, 1991; Green *et al.*, 1992 b). Another exception is the senescent strain of *Postia placenta* (ME20), which does not lower the pH of wood or cause weight loss (Green *et al.*, 1992b; Micales & Highley, 1989).

Recently, Micales (1995) has shown that brown-rot fungi do produce oxalate decarboxylase (ODC), especially under low pH conditions. *P. placenta* (ME20) produces 10 times more ODC than *P. placenta* (MAD-698), thus hydrolyzing all OA produced. Thus, the role of oxalate decarboxylase appears related to pH, under normal conditions, to protect the fungus from self-destruction (circa pH 2.2), and to have no direct role in cellulose depolymerization as proposed by Espejo and Agosin (1991) for *G. trabeum*. Nevertheless, isolates of *G. trabeum* decay wood with the same efficiency as high OA accumulators, e.g., *P. placenta* (MAD 698) and *S. incrassata* (MAD 563), suggesting that *G. trabeum* employs other mechanisms to lower wood pH (unlike ME20). Koenigs (1975) confirmed that isolates of *G. trabeum*, which decreased the pH of wood to 3.2–3.5, were able to degrade wood more rapidly than those that increased the pH. *P. placenta* (MAD 698) has been shown to accumulate OA and lower the pH of wood to 1.7

**Table 2.** Weight Loss Produced by Brown-rot Fungi in Soil-block Tests<sup>a</sup>

Wood species	Percentage weight loss produced by			
	<i>Gloeophyllum trabeum</i>	<i>Gloeophyllum saepiarium</i>	<i>Postia placenta</i>	<i>Neolentinus lepideus</i>
<i>Softwoods</i>				
Douglas-fir	58	57	57	37
Southern pine	55	45	51	41
Western hemlock	53	49	53	26
Western white spruce	33	51	58	34
<i>Hardwoods</i>				
Balsam poplar	62	49	62	37
Red alder	44	39	48	37
Sweetgum	42	–	67	26
Maple	57	–	60	–

<sup>a</sup>Highley (1978).

in 7 days, thus further underscoring species differences in the brown-rot decay fungi.

### Brown-rot fungi preferentially degrade softwoods

Because brown-rot attack in nature is most often associated with softwood structures, it is frequently stated that these fungi possess a greater capacity to degrade softwoods than hardwoods (Cowling, 1961). However, laboratory decay tests demonstrate that many brown-rot fungi can equally decay softwood and hardwood species (Table 2).

### CONCLUSIONS

The diffuse nature of wood decomposition by brown-rot fungi and the rapid decrease of strength at low weight loss suggest that agents smaller than enzymes are involved. Enzyme preparations from brown-rot fungi have not been able to duplicate these effects on cellulose or wood, nor have preparations from powerful cellulolytic organisms, such as *Trichoderma*. To accomplish these changes in wood by brown-rot fungi, a Fenton system is most often given credit. However, as discussed in this paper, the evidence to date is largely circumstantial, and the effects of the Fenton's reagent on wood components are not duplicated in many respects, creating doubt about its representation as an adequate model for brown-rot decay. It is noteworthy that brown-rot decay and thermally induced acid hydrolysis by fire retardants attack the hemicellulose components of wood at the onset of strength loss (LeVan *et al.*, 1990; Green *et al.*, 1991; Winandy & Morrell, 1993). These

authors also noted a remarkable similarity in appearance and texture between fungus-induced and acid degradation of fire-retardant-treated wood. In both cases, strength loss initially occurred before corresponding weight loss. Is oxalic acid production a key to the initial stages of brown-rot decay and could the hydronium ion be the diffusible, low-molecular-weight 'decay agent'? Remarkably, Hawley & Campbell (1927) offered a similar hypothesis almost 70 years ago. Schmidt *et al.* (1981) reported that oxidation of oxalic acid by Fe<sup>3+</sup> releases two hydronium ions. Whether or not the hydronium ion is the diffusible agent produced by brown-rot fungi to depolymerize cellulose, it is becoming evident that production of organic acids, such as oxalic acid, are important in initiation of brown-rot decay. Green *et al.* (1995a,b) proposed that oxalic acid participates in incipient brown-rot decay by chelating calcium, thus facilitating pectin utilization in the pit membranes (Fig. 3), which helps to initiate fungal metabolism and promote the spread of fungal hyphae in wood.

Many notions about brown-rot decay have become dogma, but to put it quite simply, certain conclusions about brown-rot decay are invalid and unproven. Too frequently these hypotheses are based upon research on a single brown-rot species. However, brown-rot fungi are not monolithic, and some generally accepted ideas are not applicable to another brown-rot species. Future advances in our understanding of brown-rot decay processes will require new paradigms and experimental models that encompass more than one brown-rot species. Certain systematic methods of scientific thinking may produce much more rapid progress than others (Platt, 1964).

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