DEVELOPMENT OF LONGITUDINAL SPLIT FAILURE IN WHITE-ROTTED ASPEN (*POPULUS TREMULOIDES* MICHX.)

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ABSTRACT

Longitudinal splits and associated smooth fracture planes were often noted along the growth ring boundaries of aspen, *Populus tremuloides* Michx., which were impact-loaded on the tangential plane, after decay by *Trametes versicolor* (L.: Fr.) Pilat, and *Bjerkandera adusta* (Willd.: Fr.) Karst. To characterize this failure pattern, scanning (SEM) and transmission (TEM) electron microscopy were employed. Results showed that this failure is a result of longitudinal fracture lines that cut through the parenchyma cell-wall layers (*transwall* failure) and opened the lumens. These parenchyma cells were preferentially invaded by fungal hyphae early (weight loss = 10%) in the degradation process. Prominent on the fracture planes was evidence of parenchyma cross walls perpendicular to the fiber axis, fungal hyphae, and associated hyphal sheaths. Localized fracturing along the parenchyma cells suggests that fungal invasion and degradation patterns influence the development and morphology of longitudinal fracture in wood.

Keywords: Smooth, split, transwall, *Populus tremuloides, Trametes versicolor, Bjerkandera adusta,* parenchyma, SEM, TEM.

INTRODUCTION

It is important to understand fully how decay fungi affect failure morphology in wood. Early researchers on wood-decay interactions recognized an abrupt "transverse" brash failure type resulting from advanced (e.g., >60% weight loss) fungal decay (Panshin and De Zeeuw 1980). Another failure type occurring at lower levels of stress is the longitudinal split failure (Fig. 1a) associated with more incipient stages of decay. Observations on broken samples of *Populus tremuloides* Michx., decayed to 10% weight loss by *Trametes versicolor* (L.: Fr.) Pilat and *Bjerkandera adusta* (Willd.: Fr.) Karst. revealed evidence of longitudinally split failure having smooth and planar fracture surfaces. The term "split" essentially describes a lengthwise separation due to cleavage along the grain (Gove 1961). Visual evaluation of the longitudinal split failure planes suggested that the surface areas are smaller than those of their sound wood counterparts.

Failure is known to occur along the weakest area in wood (Debaise et al. 1966). The weak zone may be related to the anatomical arrangement of the tissues (Kucera and Bariska 1982) or may be caused by a degrading factor like fungal decay. The unique appearance of the split fracture plane encouraged an ultrastructural characterization of the nature of interaction between the test fungi and wood anatomical elements. The underlying objective was to identify the processes leading to longitudinal split failure in white-rotted aspen. Both scanning (SEM) and transmission (TEM) electron microscopy were employed for this research.

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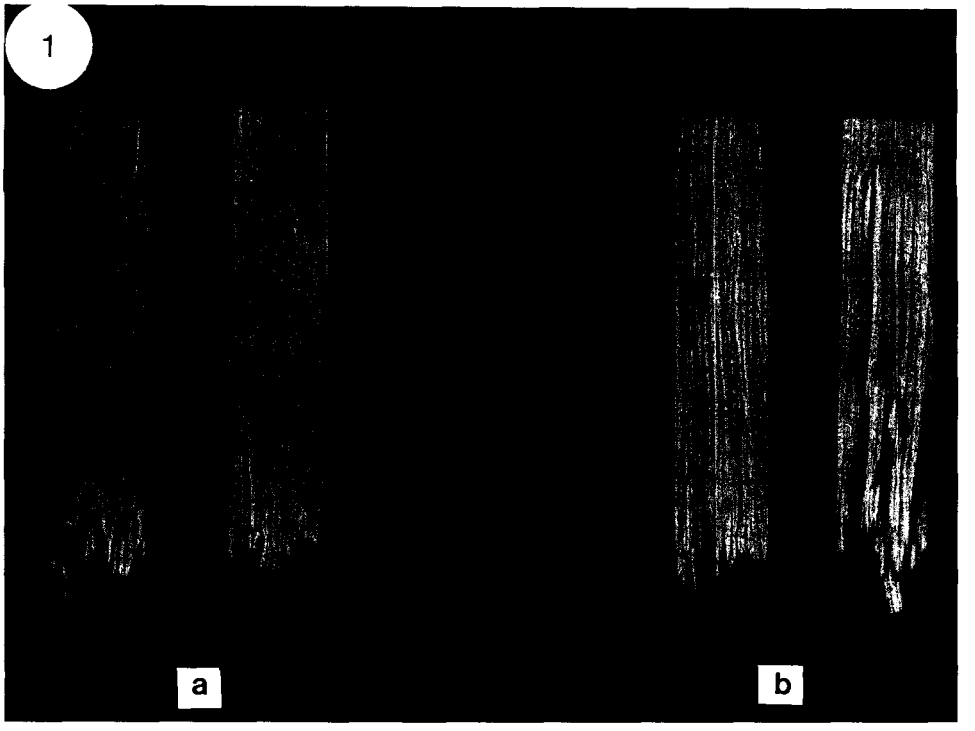

FIG. 1. Comparison of a smooth longitudinal split fracture plane (arrow on 1a) typical of the white-rot degradation with a rough shear fracture plane (arrow on 1b) in sound wood. Identical smooth split fracture planes were obtained after failing wood samples decayed by *Trametes versicolor* and *Bjerkandera adusta* to 10% weight loss.

MATERIALS AND METHODS

Wood specimens

Defect-free specimens (1.3 cm × 1.3 cm × 15.2 cm longitudinal) were machined from freshly kiln-dried sapwood of *Populus tremuloides*. One hundred and twenty specimens (thirty per fungus and sixty more for two sets of controls) were utilized. Control specimens were divided into two sets to run the different incubation periods anticipated for the two fungi. Test specimens were all straight-grained and designed to fit into the decay chambers. Their configuration was such that they could be impact-loaded to failure using the Forest Products Laboratory (FPL) toughness testing machine. Sapwood was utilized so that decay was achieved at a relatively fast rate; and the choice of wood was based on availability, decay susceptibility, and a noticeable lack of adequate research on hardwoods. *P. tremuloides* is widely distributed in the United States, and in view of current efforts to find structural uses for hardwoods, its choice for this study is justified. Oven-dry weights of the test specimens were taken prior to decay experiments.

Decay procedure

An agar-block method was employed. Fresh cultures of *Trametes versicolor* (L.: Fr.) Pilat (SUNY ESF 42), which can cause a simultaneous white-rot, and *Bjerkan-*

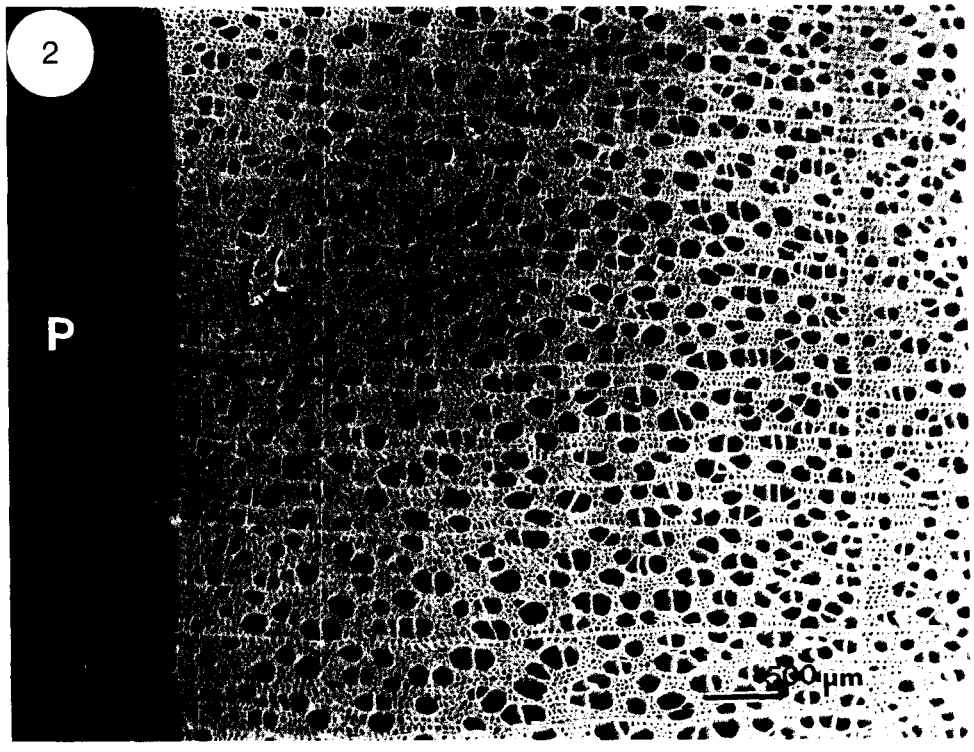

FIG. 2. Scanning electron micrograph showing that the fracture plane **P** associated with a longitudinal split failure (shown in Fig. 1a) was along the growth ring boundary (**grb**). The wood specimen examined here was decayed by *Trametes versicolor*.

dera adusta (Willd.: Fr.) Karst. (SUNY ESF 58), which can cause selective delignification (Blanchette 1984) were obtained from the wood microbiology laboratory at SUNY, ESF, Syracuse, NY. Forty decay chambers (32-oz French square bottles) were needed to accommodate the test wood specimens—ten for each set. Other supplementary chambers were set up to monitor weight losses as decay progressed. The same procedure was used to set up all the chambers except for fungal treatment. Malt extract agar medium (MEA) was prepared and added to the chambers by using 1.5% agar and 2.0% malt extract in distilled water. The mixture was sterilized in loosely capped chambers, which were autoclaved at 121 C, 15 lb/in.^{2} pressure for 20 minutes. After autoclaving, the chambers, still capped, were laid on their side for the medium to cool and gel. No contamination is tolerable at this stage. The chambers were left for a week to allow any contaminant to manifest itself and the few contaminated chambers were discarded and replaced. Ten chambers (3 specimens laid down horizontally per chamber) were maintained throughout the experiment for each of the two decay trials and the two sets of controls. Preliminary experiments indicated that sufficient longitudinal split failures would be obtained at 10% weight loss, which was chosen as the target. Weight loss estimates were progressively monitored from supplementary chambers exposed to identical experimental conditions. Decay test specimens and their matching

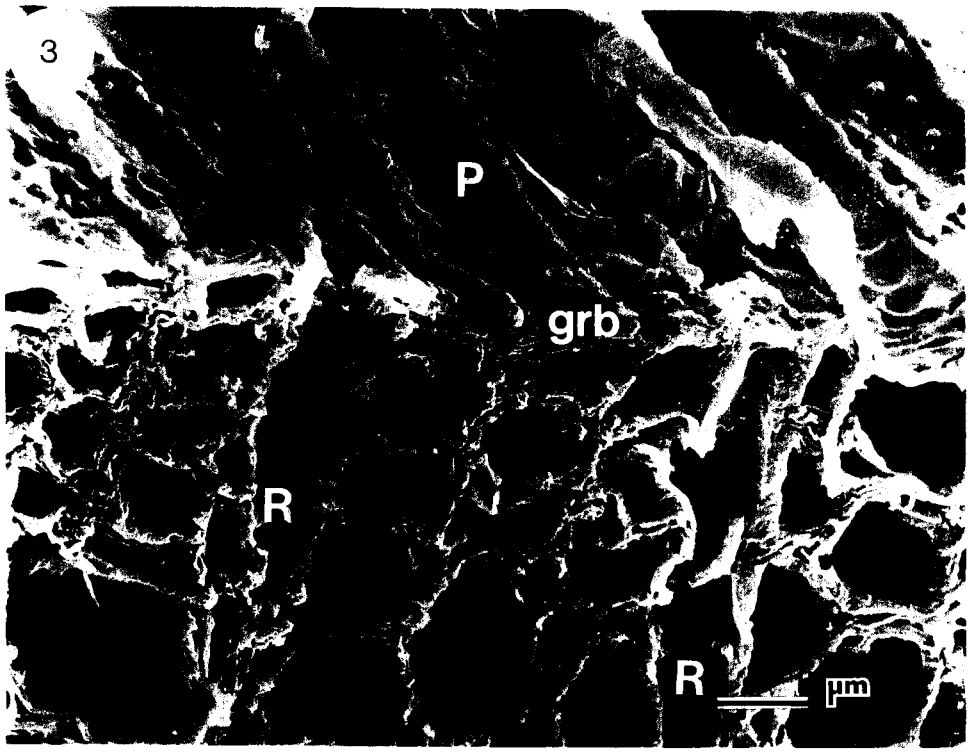

FIG. 3. Scanning electron micrograph of a smooth longitudinal split fracture plane (**P**) along the growth ring boundary of wood decayed by *Trametes versicolor*. Notice a gradual reduction in cell size from the bottom of the micrograph to the growth ring boundary. **R** indicates ray cells and **tw** indicates transwall failure.

controls were removed from the chambers as soon as the target weight loss was achieved.

Fracturing the specimens

After decay was completed, specimens were dried through a two-stage air-dry/oven-dry procedure to avoid warpage from excessive drying stresses. The final oven-dry weights were recorded for subsequent weight loss calculations. The specimens were wrapped in aluminum foil and stored in a desiccator to keep them in a dry state. All the specimens were loaded to failure on the tangential face by using a FPL toughness testing machine set at 60° pendulum angle. The broken specimens were subsequently examined for evidence of longitudinal split failures.

Scanning electron microscopy

Two or more fracture surfaces were obtained per specimen after failure. Portions containing the smooth longitudinal split fracture planes were carefully removed with sharp razor blades and fixed on double stick tape previously placed on SEM studs. In some cases, transverse sections of specimens were microtomed (with the split fracture plane left untouched) to reveal the exact location of the split plane within the wood anatomy. Conductivity between specimens and studs was facil-

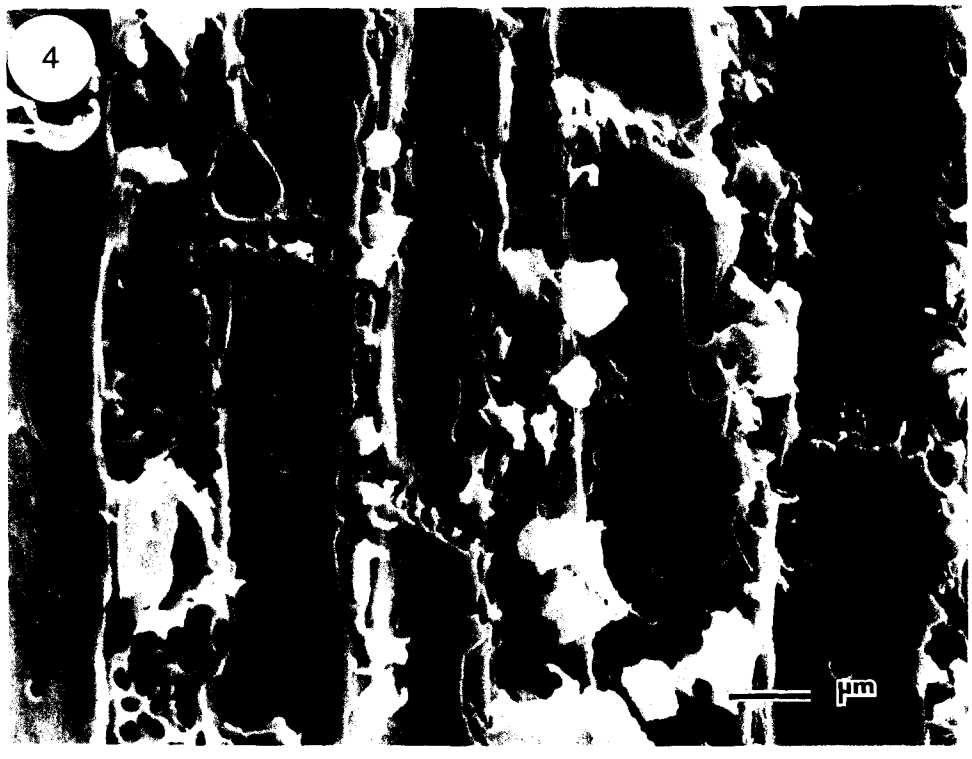

FIG. 4. Scanning electron micrograph of a smooth longitudinal (tangential) split fracture plane along a growth ring boundary. Wood was decayed by *Trametes versicolor* before failure. Notice severe degradation of the fracture plane as well as the ray parenchyma (**rp**) cells. **hy** are fungal hyphae while **hs** are hyphal sheaths around the cross walls (**cw**) of the marginal parenchyma cells. Large arrows indicate boreholes.

itated by carbon-coating the specimen edges with the studs. Specimens were finally coated with gold using a Technics sputter-coater before microscopic examination in the SEM.

Transmission electron microscopy

Epon embedding was done to study the fungal invasion patterns by using freshly decayed wood samples. Small specimens from freshly decayed aspen were fixed in glutaraldehyde-formaldehyde mixture (Karnovsky 1965) buffered with 0.1M Na cacodylate to pH = 7.2. Post fixation was done in 2% O_sO_4 in 0.1M Na cacodylate buffer at 4 C in the dark. The specimens were rinsed in distilled water, dehydrated, and embedded in Polybed 812 Epon. Thin sections (90–120 nm) were obtained by using a diamond knife on a Sorvall MT-2B ultramicrotome. Sections were stained with uranyl acetate and lead citrate.

RESULTS

It took 6 weeks to obtain the target weight loss with *T. versicolor*. The average weight loss (measured as a percentage of original oven-dry weight) at the end of this period was 10.4 ± 2.8%. Decay with *B. adusta* took a longer time, and the

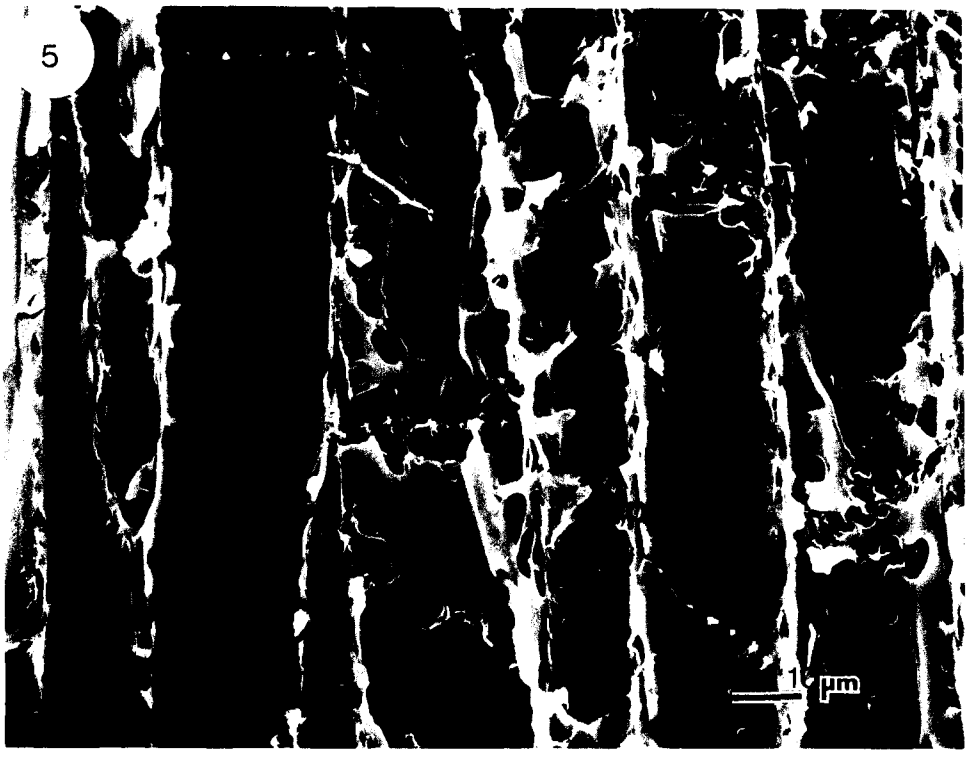

FIG. 5. Scanning electron micrograph of a smooth longitudinal split fracture plane along the growth ring boundary of wood decayed by *Bjerkandera adusta*. The lumen surface (**Lu**) was exposed during the fracture process indicating that failure was primarily transwall. Observe degradation of the ray parenchyma **rp**. Also notice hyphal sheath (**hs**) on the smooth fracture plane. **cw** is a marginal parenchyma cross wall.

specimens were harvested at the end of 10 weeks when the average weight loss was 9.0 ± 2.0%. The specimens were impact-loaded and all of them failed at a single blow of the pendulum. One-third of the specimens decayed by *T. versicolor* showed evidence of smooth longitudinal splits, while a greater fraction (about two-fifths of the population) of samples decayed by *B. adusta* produced this failure type. None of the control (undecayed) specimens produced smooth longitudinal split failures. Longitudinal shear planes (Fig. 1b) in sound wood did not adhere strictly to the grain direction. They can generally be described as rough, nonplanar and of a "corrugated" appearance.

Scanning electron microscopy of the fractured samples indicated that the longitudinal split plane occurred essentially along the growth ring boundaries in the tangential plane (Figs. 2 and 3). Closer examination of the fracture plane revealed fungal hyphae (Fig. 4), which appeared on the fracture plane because the cell lumens were exposed. Exposure of lumens further indicated that the line of fracture generally crossed all the cell-wall layers as opposed to occurring within or between cells. This type of failure is generally described as *transwall* (Côté and Hanna 1983). Prominent on Figs. 4 and 5 are marginal parenchyma cross walls (i.e., boundary between two adjoining parenchyma cells along the fiber axis) and hyphal

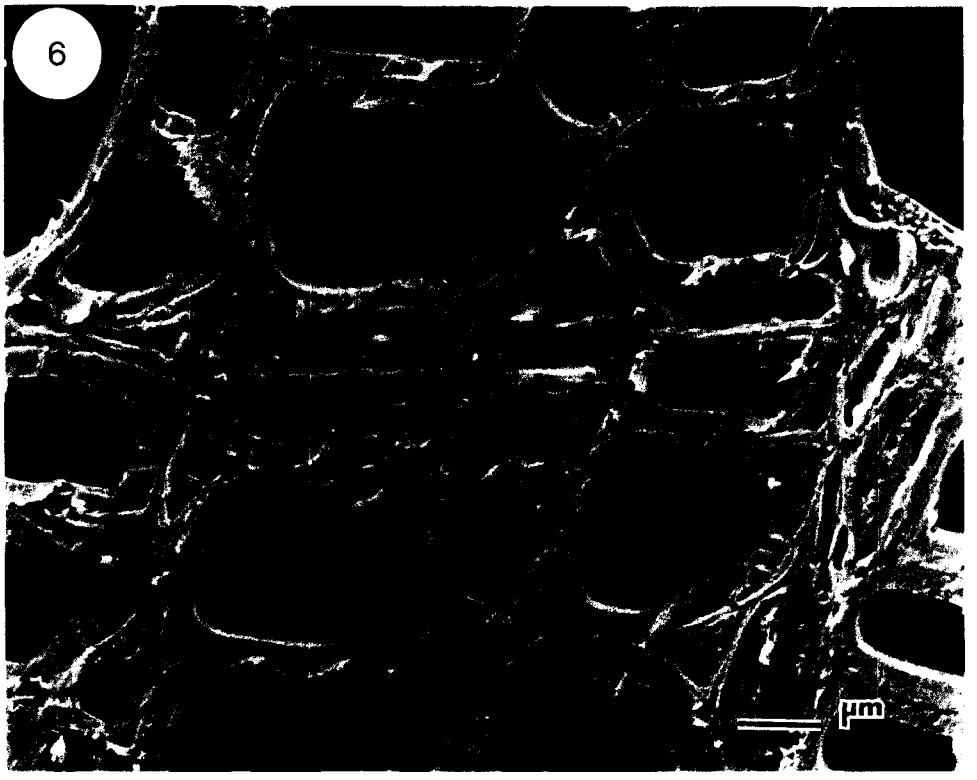

FIG. 6. Scanning electron micrograph of a cross-section of aspen showing marginal (**mp**) and ray (**rp**) parenchyma cells at the growth ring boundary and ray zone respectively.

sheaths. Except in a few cases, the orientation of the cross walls is perpendicular to the longitudinal axis.

Aspen contains marginal and ray parenchyma cells (Fig. 6). Results obtained from wood samples embedded in epon revealed that these regions were preferentially invaded at the early stages of decay (weight loss <10%) by *T. versicolor* and *B. adusta* (Figs. 7, 8, 9, and 10) and this could account for localized fracturing along the parenchyma cells. No morphological difference was found between longitudinal split planes formed under the influence of the two decay fungi.

DISCUSSION

P. tremuloides is a diffuse porous hardwood with a defined growth ring boundary. It has low specific gravity, 0.35 green to 0.38 at 12% M.C. (Wood Handbook 1974). For low specific gravity hardwoods, Côté and Hanna (1983) noticed that when tangential shear occurred, the fracture plane was generally through the vessels, and the fracture pattern was predominantly transwall. The resultant exposure of vessel lumens across the plane of fracture explains the somewhat corrugated appearance of the longitudinal shear fracture plane in sound aspen. To obtain a smoother fracture plane logically requires that the exposed surface area should be reduced. This is possible when the fracture line traverses, for the major part, cells of smaller circumferences than the vessels. When aspen was decayed

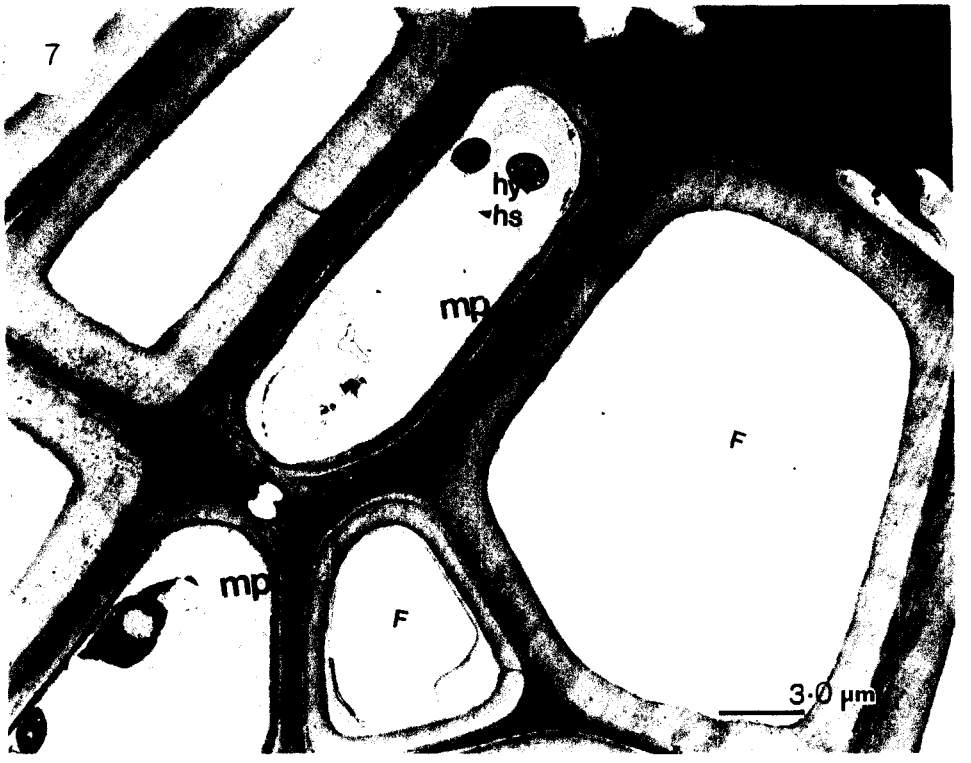

FIG. 7. Transmission electron micrograph of a cross-section of aspen decayed by *Bjerkandera adusta*. Notice fungal hyphae (**hy**) and hyphal sheath (**hs**) within lumen of the marginal parenchyma cells (**mp**). **rp** is the ray parenchyma region and **F** are fiber cells. The hyphae will only appear on the fracture plane when the lumen is opened (i.e., transwall failure).

by *T. versicolor* and *B. adusta,* the longitudinal split failure that resulted passed through the marginal (axial) parenchyma cells rather than through the vessels or fibers. Marginal parenchyma cells are located at the growth ring boundaries and examination of Figs. 2 and 3 shows that they are smaller than the neighboring cells. Parenchyma cells may be brick shaped or isodiametric (Harada and Côté 1985). For this reason, it is conceivable that their cross walls may appear perpendicular to the longitudinal axis of the fibers and vessels. That the identified cross walls on the smooth split fracture plane are generally perpendicular to the fiber axis supports the observation that the line of fracture went essentially through the parenchyma cells since vessels and fibers have their ends tapered at an angle to the longitudinal axis.

Figures 7 and 9 demonstrate preferential invasion of the marginal parenchyma cells by hyphae of *T. versicolor* and *B. adusta,* respectively. This invasion pattern is believed to have caused localized degradation of the growth ring boundary and this promoted fracture development. Carbohydrates in the parenchyma cells may be an initial food source, making these cells more readily decomposed than other types. Longitudinal split failure through the parenchyma cells probably occurred with white-rot fungal attack because the fungal cellulases were acting close to the

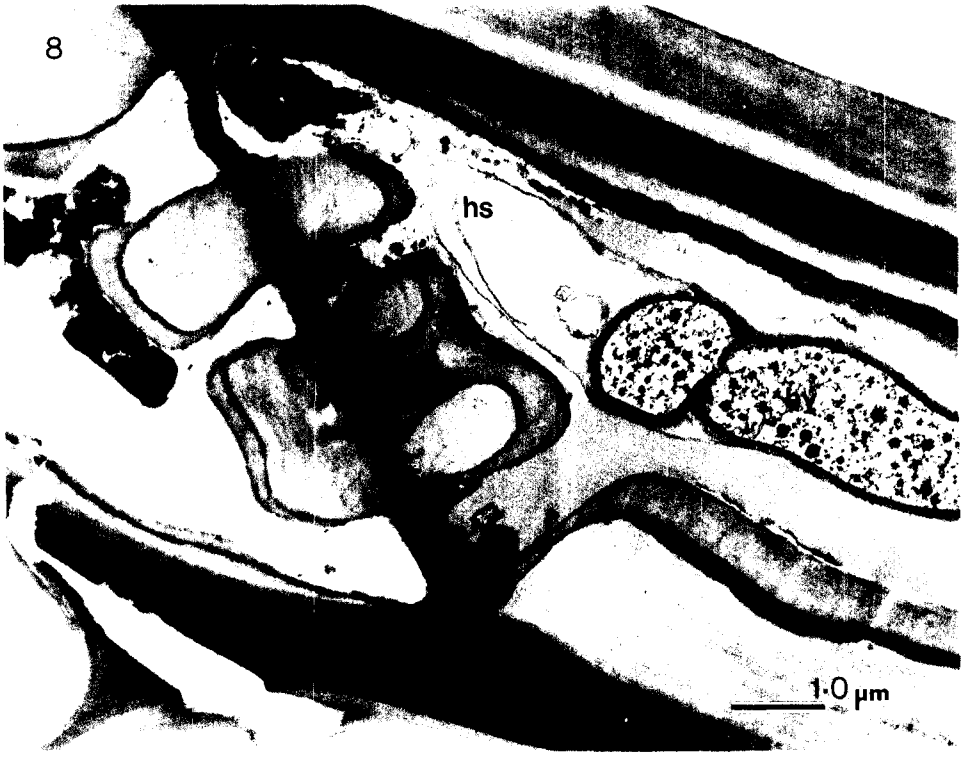

FIG. 8. Transmission electron micrograph showing a cross-sectional view of ray parenchyma cells invaded by *Bjerkandera adusta*. Hypha (**hy**) and hyphal sheath (**hs**) were pictured near a cross wall (**cw**) with few pit apertures inside the lumen of the ray cell.

fungi in this area instead of diffusing freely to weaken adjacent cell-wall areas, as already reported by Blanchette and Shaw (1978) and Highley and Kirk (1979) for brown-rot fungi. Preliminary experiments conducted with a brown-rot fungus, *Gloeophyllum trabeum,* in our laboratory showed no evidence of split failures with characteristic smooth fracture planes.

It is possible that hyphal sheaths, recognized on the split fracture plane (Figs. 4 and 5), also contributed to fungal degradation of the parenchyma cells. Murmanis et al. (1984) already depicted degradation of Western hemlock cell walls by hyphal sheaths of *Ganoderma applanatum* even in the absence of any visible hyphae. Similar observation can be made from Fig. 9. Longitudinal split fracture planes produced after *T. versicolor* and *B. adusta* attack were identical, and it would not have been possible to separate them had the specimens not been premarked. The original expectation was to find evidence of delamination (intercell failures through the middle lamella) on the fracture planes of specimens decayed by *B. adusta* since this fungus was already known to be capable of selective delignification (Blanchette et al. 1985). It is possible, however, that the strain of *B. adusta* utilized here is different from that of Blanchette and coworkers, and may not have acted as a selective delignifier under the present experimental conditions. The failure pattern that led to smooth split formation, as already indicated, cuts through the

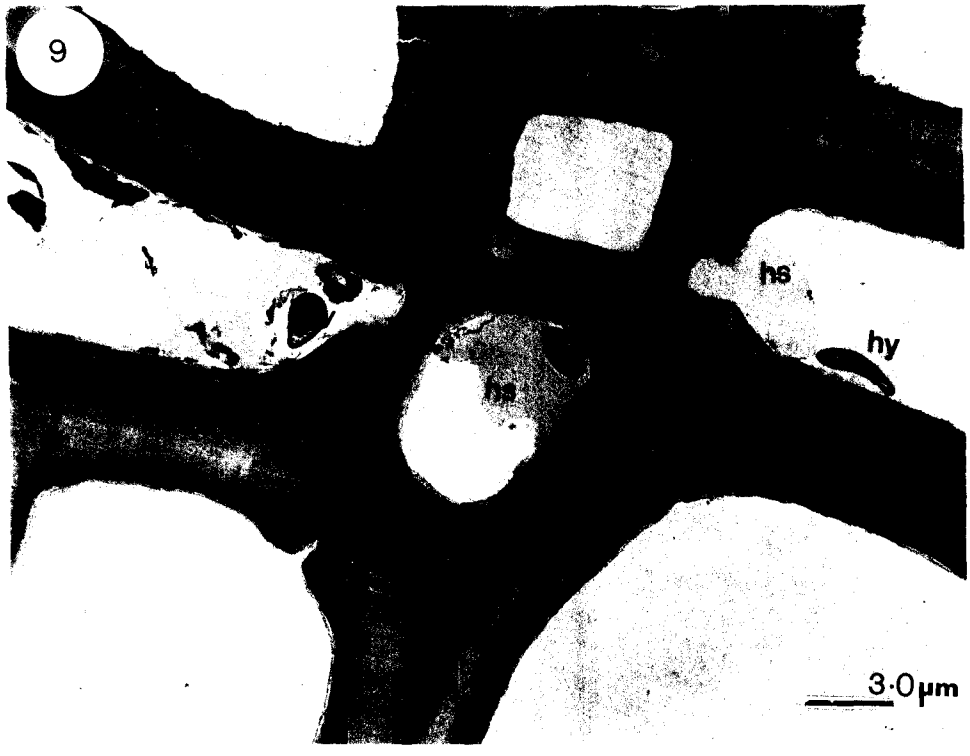

FIG. 9. Transmission electron micrograph of aspen decayed by *Trametes versicolor*. Notice invasion of the marginal parenchyma cells by fungal hyphae (**hy**) and hyphal sheaths (**hs**). Arrows indicate cell erosion initiated by hyphal sheaths.

cell walls, propagated lengthwise along the fiber axis, and opened the lumens in the process. It is therefore possible that middle lamella separation requires more delignification than could be achieved at 10% weight loss. An identical failure pattern by both *T. versicolor* and *B. adusta* may be explained by the fact that both fungi are capable of localized removal of the carbohydrate fraction of wood.

Longitudinal split failure generally occurred along the growth ring boundaries to expose the tangential fracture plane. Although both the marginal and ray parenchyma cells were degraded by the two fungi, it is possible that the consistent failure along growth ring boundaries, rather than rays, was a result of tangential loading employed during specimen failure. It is suggested that future experiments should consider both radial and tangential loading designs to clarify this point. Another issue of interest is whether this unique longitudinal split fracture will be associated with all wood having marginal parenchyma and decayed by white-rot fungi or if it is just a failure pattern associated with *P. tremuloides*. This question can not be answered with the present results, but observations in this study strongly suggest that the presence of parenchyma cells in *P. tremuloides* is what attracted fungal invasion, localized degradation and fracture development. Severe degradation of parenchyma cells has, however, been previously reported in Western hemlock, *Tsuga heterophylla* (Raf.) Sarc., decayed by *Ganoderma applanatum* (Murmanis et al. 1984), in birch wood decayed by *Coriolus versicolor* (Blanchette et al. 1985),

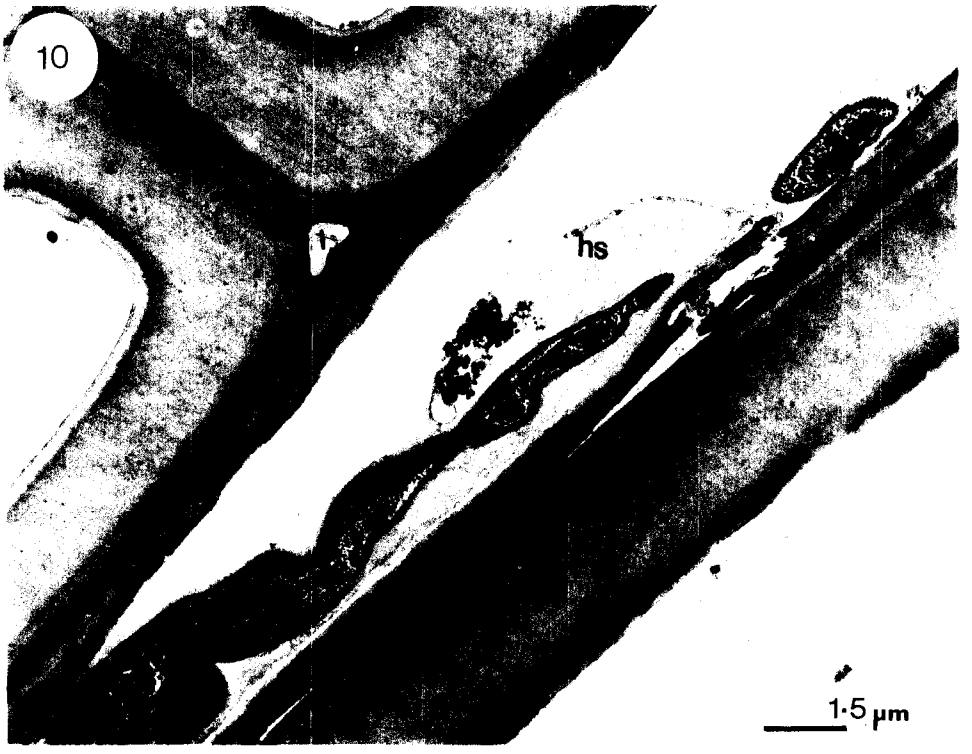

FIG. 10. Transmission electron micrograph showing invasion of a ray parenchyma cell by hypha (**hy**) and hyphal sheath (**hs**) of *Trametes versicolor*. **I** is an intercellular air space.

and in Oak *Quercus alba* L. delignified by *Innonotus dryophillus* (Berk.) Murr (Otjen and Blanchette 1986). During the decay of hemlock by *Ganoderma applanatum*, Murmanis et al. reported that the parenchyma cells were attacked much more severely than tracheids. In view of all these observations, it would appear that longitudinal split failures having smooth fracture planes may occur in many woods with marginal parenchyma attacked by white-rot fungi.

CONCLUSIONS

Longitudinal split failures were generated along the growth ring boundaries of aspen decayed by *T. versicolor* and *B. adusta* to 10% weight loss and impact-loaded on the tangential plane. The fracture planes were similarly smooth for both fungi and a microscopic examination of the fracture pathway revealed that the fracture lines propagated essentially in a transwall manner through the marginal parenchyma cells. The fungal invasion patterns were positively correlated with the site of fracture development, and hyphae and hyphal sheaths that were observed on the fracture planes constituted direct evidence of fungal invasion. Orientation of parenchyma cross walls on the fracture plane is another evidence that the fracture line went through the parenchyma cells. It is concluded that white-rot fungal invasion and degradation patterns influence the development and morphology of longitudinal split failure in wood.

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