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Ultrastructural Aspects of Cellulose Decomposition by White-Rot Fungi

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Ultrastructural aspects of cellulose decomposition by white-rot fungi

Summary

Five white-rot fungi were grown on cellulose fibers and the degradation was observed by scanning and transmission electron microscopy. The white-rot fungi produced hyphal sheaths when grown on cellulose fibers, but sheaths generally were not present between the hyphae and fibers. Autolyzing hyphae and diffusion of cytoplasmic material into sheath or fiber was seldom observed. Therefore, the sheath may not be involved in the degradative process but functions for attachment, nutrition, and protection. The fungi disrupted fiber surfaces. However, degradation was not localized around hyphae but occurred at a distance from them, Thinning of fibers did not occur. Thus, the white-rot fungi must produce a diffusible cellulose-degrading system.

Schlüsselwörter (Sachgebiete)
Holzzerstörung
Cellulosefasern
Weißfäule-Pilze
Hyphenscheiden
Ultrastruktur
Raster-Elektronenmikroskopie
Transmissions-Elektronenmikroskopie

Feinstrukturuntersuchungen über den Cellulose-Abbau durch Weißfäule-Pilze

Zusammenfassung

Fünf Weißfäule-Pilze wurden auf Cellulose-Fasern gezogen, und der Abbau wurde raster- und transmissions-elektronenmikroskopisch verfolgt. Die Weißfäule-Pilze erzeugten Hyphenscheiden während des Wachstums auf den Cellulose-Fasern, die Scheiden waren jedoch nicht überall zwischen den Hyphen und den Fasern. Autolyse der Hyphen und Diffusion von zytoplasmatischem Material in die Scheiden oder Fasern wurde selten beobachtet. Daher dürfte die Scheide nicht in den Abbauprozeß einbezogen sein, könnte jedoch eine Hilfsfunktion wie Ernährung und Schutz ausüben. Die Pilze reißen die Fasern an den Oberflächen auf. Der Abbau ist aber nicht rund um die Hyphen beschränkt, sondern kommt auch in einiger Entfernung von ihnen vor. Ein Dünnerwerden der Fasern tritt nicht ein. Jedoch müssen die Weißfäule-Pilze ein diffusionsfähiges Cellulose-Abbau-System erzeugen.

Introduction

White-rot attack in wood is characterized by progressive thinning of the secondary wall, beginning at the lumen and progressing outward toward the middle lamella (Cowling 1961; Liese 1970; Wilcox 1970). The action of the enzyme system apparently is restricted to the immediate vicinity of the hyphae, and mainly to the exposed surface of the cell wall. However, diffusion of certain enzymes into the secondary wall is possible. Circumstantial evidence for this was reported by Cowling (1961) who observed that thinning of cell walls without hyphae proceeded at about the same rate as cells containing hyphae. In addition, white-rot fungi produce cavity-shaped fissures in the S_2 layer of the secondary wall at some distance from hyphae (Liese 1970; Wilcox 1970). Thus, polysaccharide-degrading enzymes of white-rot fungi may diffuse into the cell wall to produce cavities, whereas the complete cell wall disintegration takes place from the surface of the cell wall.

The presence of lignin and other materials in the wood cell wall makes visualization of the sites of attack in cellulose difficult from microscopic observations. In a previous study with brown-rot fungi (Highley et al. 1983b), we employed electron microscopy to monitor the sites of attack and the morphological changes that occurred during the degradation of isolated cellulose fibers. In this study, we used the same approach to obtain information on the ultrastructural changes in cellulose and diffusibility characteristics of the cellulolytic degradative system during white-rot attack of cellulose fibers.

Experimental

Five white-rot fungi were examined: *Coriolus versicolor* (L.:Fr.) Quel., *Pycnoporus sanguineus* (L.:Fr.) Murr., *Phellinus pini*

Purchased by U.S. Department of Agric.,
Forest Service, Forest Products Laboratory,
for official use.

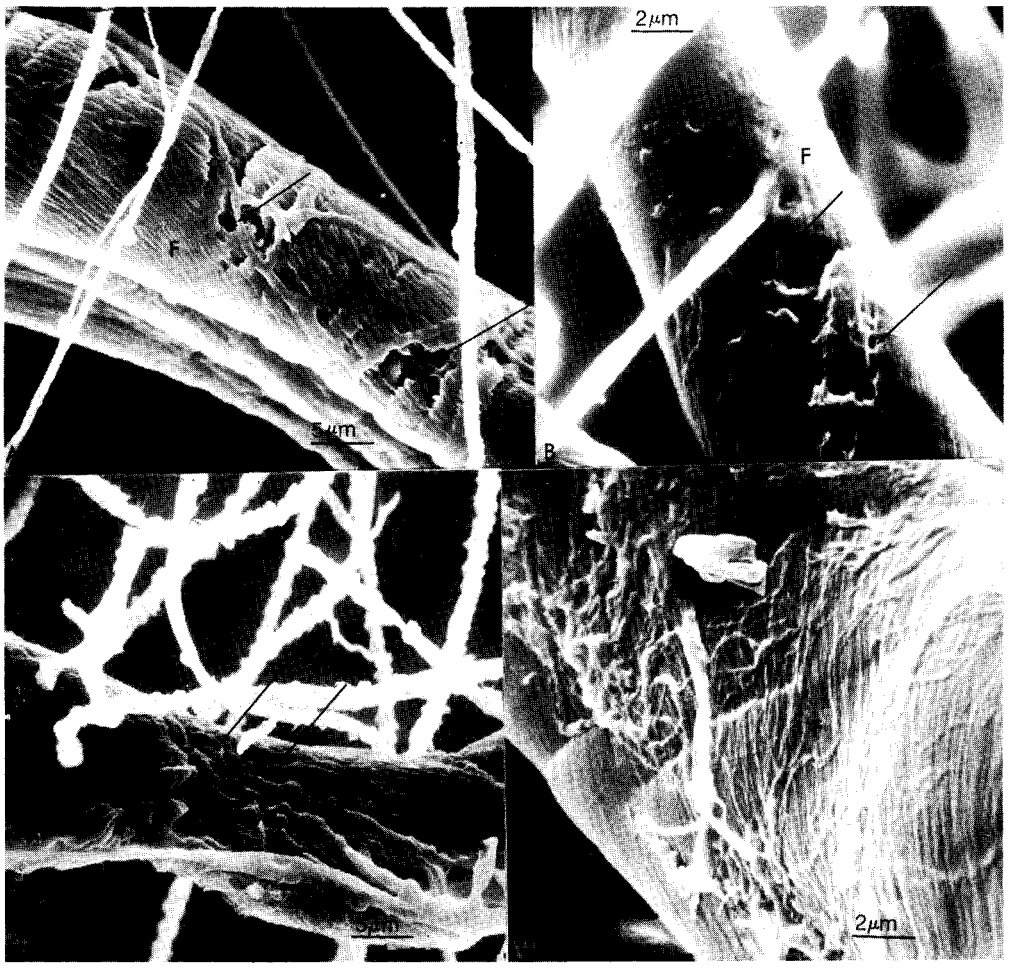

Fig. 1. Surface degradation of cellulose. (SEM)
A. *P. sanguineus:* bore holes in fiber (F). 1580X
B. *Ph. pini;* bore holes in fiber (F). 3900X
C. *X. frustulatus:* bore holes in fiber. 1580X
D. *Ph. chrysosporium:* fiber (F) surface fibril disruption. 3900X

(Thore:Fr.) A. Ames., *Phanerochaete chrysosporium* Burds., and *Xylobolus frustulatus* (Pers.:Fr.) Boid.

Cellulose fibers (Hercules cotton, Type 600–A) were degraded in chambers patterned after those in the ASTM soil-block method (ASTM 1971) as previously described (Highley et al. 1983a). Specimen preparation techniques for scanning (SEM) and transmission (TEM) electron microscopy are detailed in Highley et al. (1983a).

Results

Scanning electron microscopy showed that *P. sunguineus, Ph. pini,* and *X. frustulatus* penetrated the cellulose fibers by means of bore holes (Fig. 1A, B, C, respectively). Penetration of the cellulose fibers by the other two white-rot fungi was not obvious from SEM. *Ph. chrysosporium* (Fig. 1D) disrupted the fiber surface, but did not produce bore holes. However, observation by TEM showed hyphae of all the white-rot fungi, except *Ph. chrysosporium,* in the fiber cell walls as well as in the lumina (Figs. 2A, 3B, C, 4A). In *Ph. chrysosporium* growth appeared to be limited to the fiber surface.

Our SEM and TEM micrographs of the white-rot fungi in this study did not show fine, filament-like hyphae often called "microhyphae" (Liese 1970).

All of the white-rot fungi disrupted the fiber surface, as evidenced by fibril separation (Figs. 1A, C, D, 3A) or eroded areas (Figs. 3B, C, 4A). Degradation, however, was not localized immediately around hyphae but occurred at a distance from hyphae; the cellulose-degrading system evidently diffused throughout the fiber (Figs. 2B, 4A, B, C, D). Degraded fibers were split in the direction of the fiber axis as well as transversely to the axis (Fig. 4A, B, C, D). Thinning of fiber cell walls was not evident.

An extracellular matrix of hyphal sheath was not apparent in SEM, but was visible in TEM (Figs. 2A, 4C, D, 5A, B). *C. versicolor* produced the most distinguishable hyphal sheath (Fig. 2A); several hyphae were encased within the same sheath. With the other white-rot fungi, sheaths covered only a single hypha (Fig. 5A, B) or no hypha (Fig. 4C, D). The sheath may be fragile, for often only remnants or broken sheaths were observed (Figs. 4B, D, 5A, B (arrows)). Sheaths appearing as a dark line in cross section usually were closely appressed to the fiber surfaces and extended a considerable distance from hyphae (Figs. 4C, D, 5A, B). Sheaths generally were not present

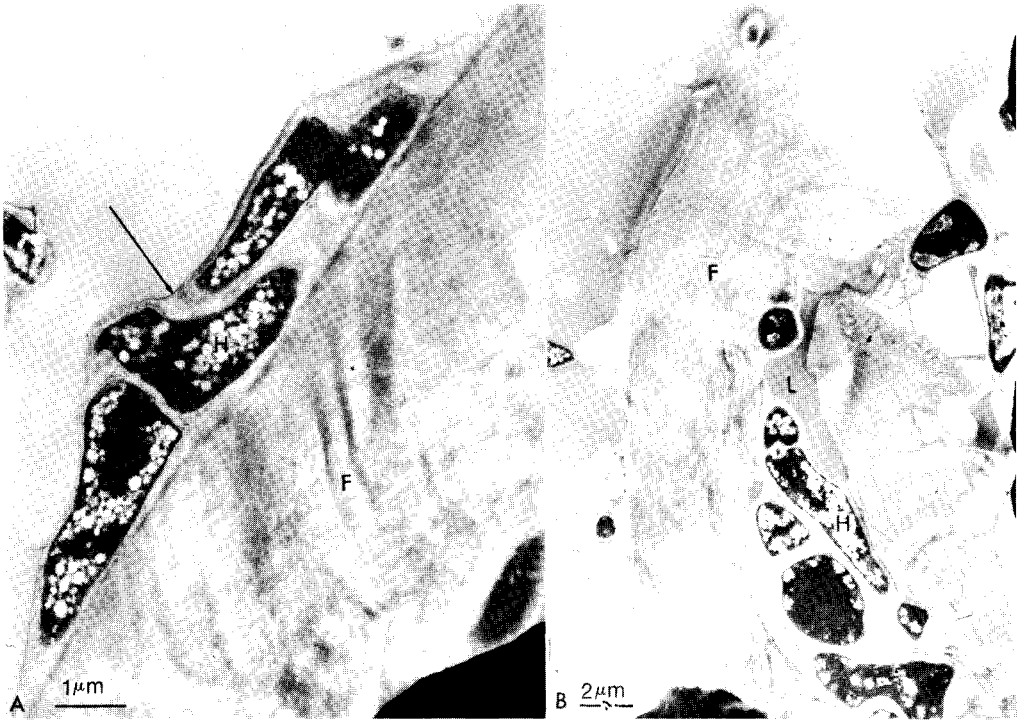

Fig. 2. Sheath enclosing hyphae and hyphae in fiber wall and lumen. (TEM)
A. *C. versicolor:* hyphae (H) appressed to fiber (F) surface, are encased by sheath (arrow). 9000X
B. *C. versicolor:* hyphae (H) in lumen (L) and fiber (F) wall. 3550X

between the hyphae and fibers; thus, hyphal cell walls were in contact with fibers (Figs. 4B, 5A, B). Autolysis of hyphae and extrusion of cytoplasmic material into sheaths or fibers were rarely seen.

Discussion

The results of this study demonstrate that the five white-rot fungi studied produce a diffusible cellulose-degrading system. Ultrastructural changes in degraded cellulose fibers were remarkably similar to those produced by brown-rot fungi (Highley et al. 1983b). Localized degradation of cellulose near hyphae was rarely observed by either white- or brown-rot fungi. This is in contrast to attack by isolated cellulases on cellulose where a slow, localized erosion of cellulose is observed near point of contact (Porter et al. 1960; Eriksson 1981). Microscopic analysis of cellulose degradation by nonwood-decaying cellulolytic fungi or bacteria also shows localized attack near cells of the invading organism (Porter et al. 1960; Basu and Ghose 1962; Berg, Hofsten, and Pettersson 1972; Berg and Hofsten 1976). Cellulolytic enzymes would be expected to produce a localized surface attack on cellulose because cellulases isolated thus far are too large to penetrate the microstructure of cellulose, and the affinity of cellulases to cellulose restricts their diffusion. Thus, our results suggest that white-rot fungi; like brown-rot fungi, produce a small, nonenzyme depolymerizing agent that is involved in cellulose degradation.

Eriksson (1981) suggests an additional mechanism for cellulose degradation in white-rot fungi, wherein hydrolysis by cellulases is combined with an oxidative step. Possibly radical-generating oxidative enzymes produce reactants such as superoxide anion ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), or hydroxyl radical ($OH^{\cdot}$) that are involved in the primary attack of crystalline cellulose. $^1O_2$ and $OH^{\cdot}$ are particularly reactive radicals (Pryor 1976). These reactants are small enough to penetrate the microstructure of cellulose and predispose cellulose to endo- and exoglucanases that white-rot fungi produce. Recently, white-rot fungi have been reported to produce radicals that could react with cellulose (Eriksson 1981; Nakatsubo et al. 1981). Nakatsubo et al. (1981) report $^1O_2$ formation by *Ph. chrysosporium* and provide evidence that it plays an integral role in lignin biodegradation. Because lignin and cellulose occur together in wood cell walls, this radical may also react with cellulose. Eriksson (1981) found that $O_2^{\cdot-}$ is produced extracellularly by the white-rot fungus *Sporotrichum pulverulentum* (synonymous: *Ph. chrysosporium)* as well as other wood-destroying fungi. The origin of radical-generating oxidases and their involvement in cellulose degradation by white-rot fungi warrant further study.

The white-rot fungi in our study did not produce thinning of cellulose fiber walls often reported to be produced in lignocellulose walls (Cowling 1961; Wilcox 1968). Similarly, Ruel et al. (1981) did not observe

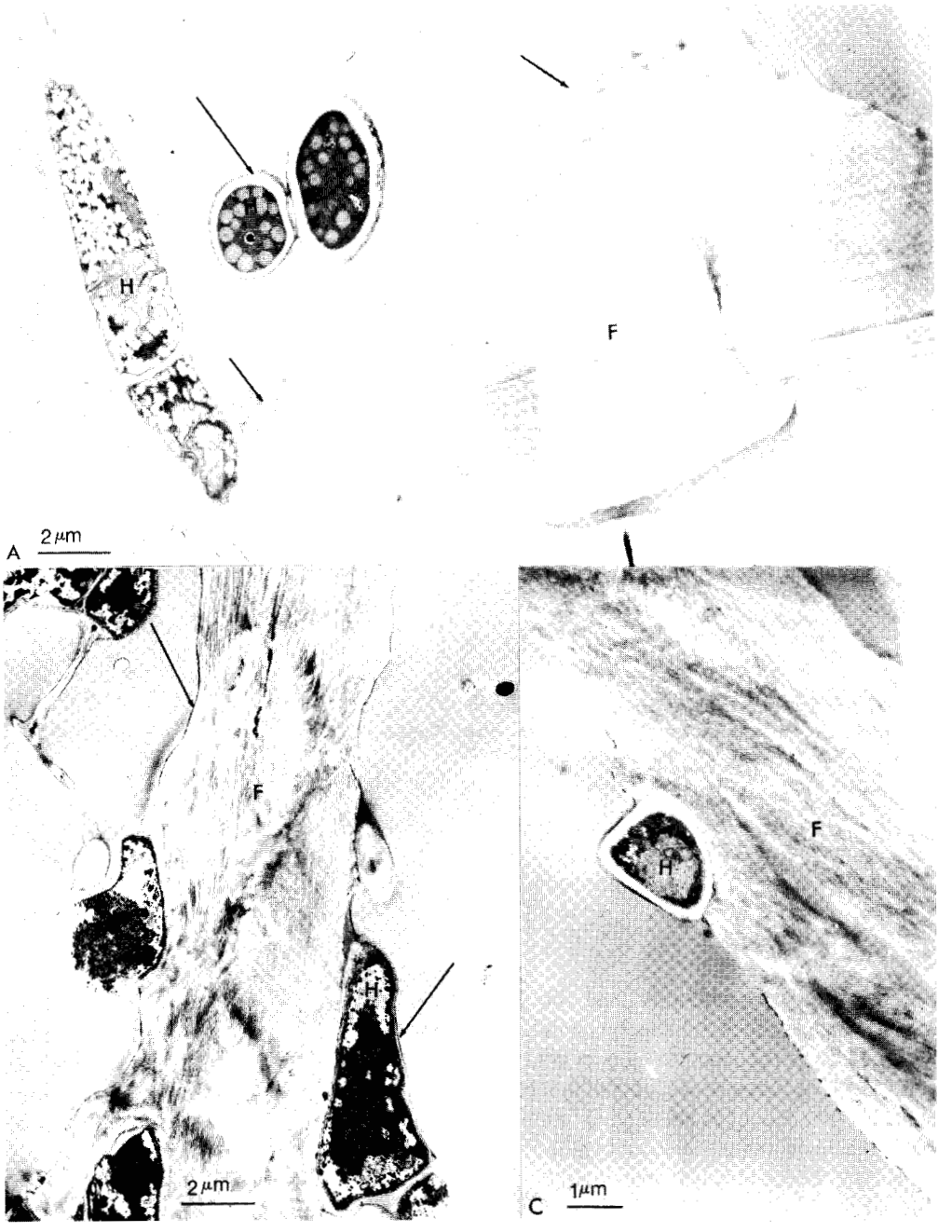

Fig. 3. Disruption of fiber surface. (TEM)
A. *Ph. chrysosporium:* surface digestion (short arrows); one hypha (H) in contact with fiber (F) surface; hyphae away from fiber encased by sheath (long arrow). 4850X
B. *C. versicolor:* erosion of fiber (F) surface by hyphae (H). Sheath (arrows) surrounds hyphae and fiber. 4950X
C. *Ph. pini:* erosion of fiber (F) surface by a hypha (H)

progressive thinning of the secondary wall from the lumen of spruce cells degraded by *S. pulverulentum.* They also provided evidence that lignin-degrading enzymes diffuse from the fungal hyphae. Accordingly, with a diffusible cell-wall-degrading system, thinning of cell walls by white-rot fungi would not be expected.

Both white-rot and brown-rot fungi form hyphal sheaths in contact with cellulose. However, the physiological function of the sheath may differ between the two types of fungi. With brown-rot fungi, autolysis and extrusion of cytoplasmic material into sheaths (Palmer et al. 1983) and into cellulose was commonly observed (Highley et al. 1983b). Autolyzing hyphae were seldom observed in the examined white-rot fungi, and cytoplasmic material was seldom observed in the sheath or fiber.

Generally, the hyphal cell wall of white-rot fungi in contact with fibers eroded the fiber surface. This erosion was not observed with brown-rot fungi; the

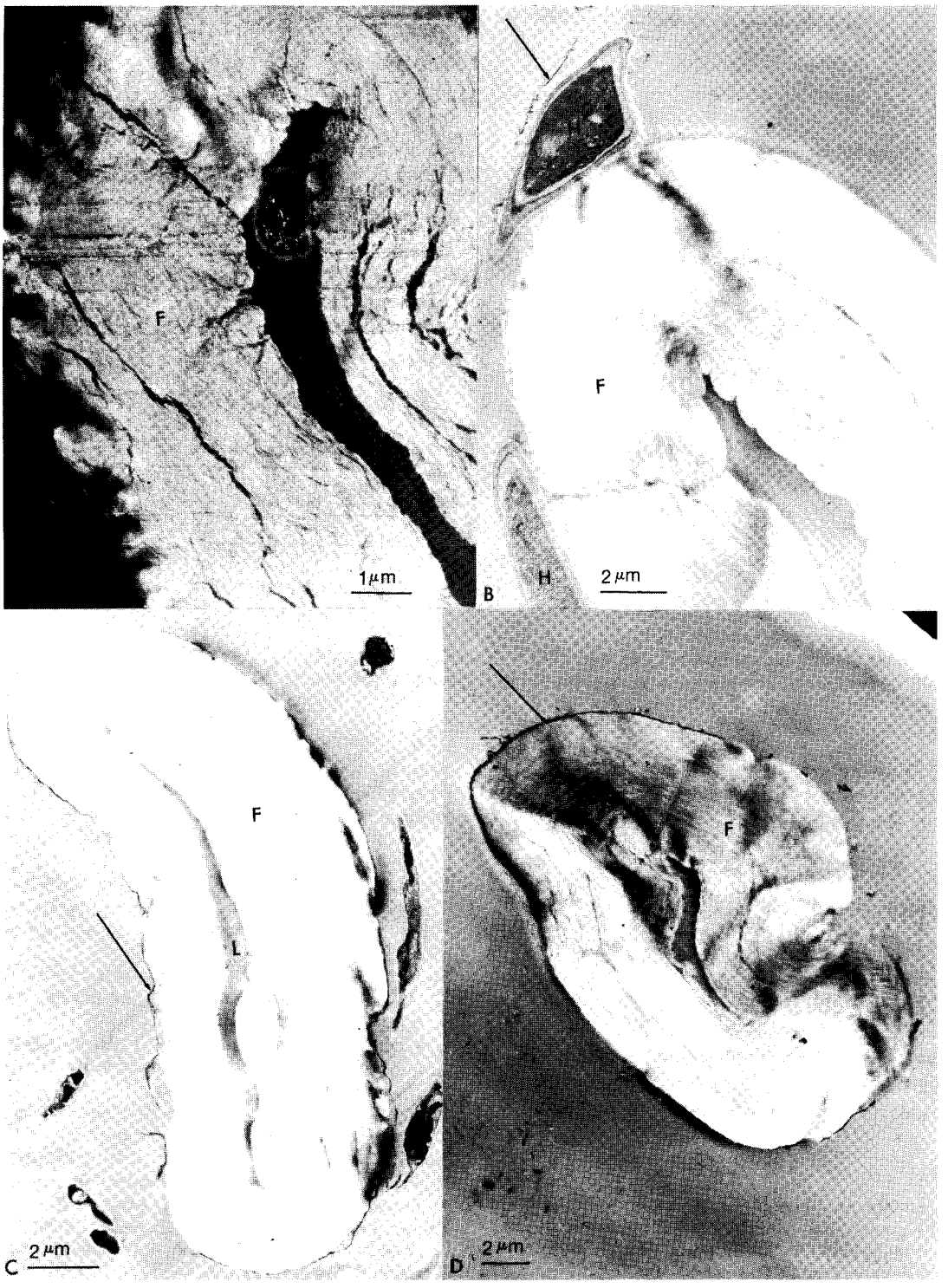

Fig. 4. Advanced fiber degradation. (TEM)
A. *P. sanguineus:* hypha (H) in fiber (F) lumen (L); eroded fiber surface at left. 9100X
B. *Ph. chrysosporium:* hyphae (H) appressed to fiber (F) surface; hyphal sheath (arrow) remnants are present. 4550X
C. *X. frustulatus:* sheath (arrow) encases fiber (F). The voids adjacent to lumen (L) are artifacts. 4850X
D. *Ph. pini:* sheath (arrow) partially broken, encases fiber (F). 3330X

hyphal sheath and its contents appeared to interact intimately with the cellulose fibers (Highley et al. 1983b). Thus, in white-rot fungi the sheath may function for attachment, nutrition, and protection, whereas in brown-rot fungi it may also serve as a medium for housing and transporting agents for degrading cell wall components. Green et al. (1980) suggest that another function of the sheath in white-rot fungi is to restrict movement of the degradative enzymes, and this accounts for the localized attack

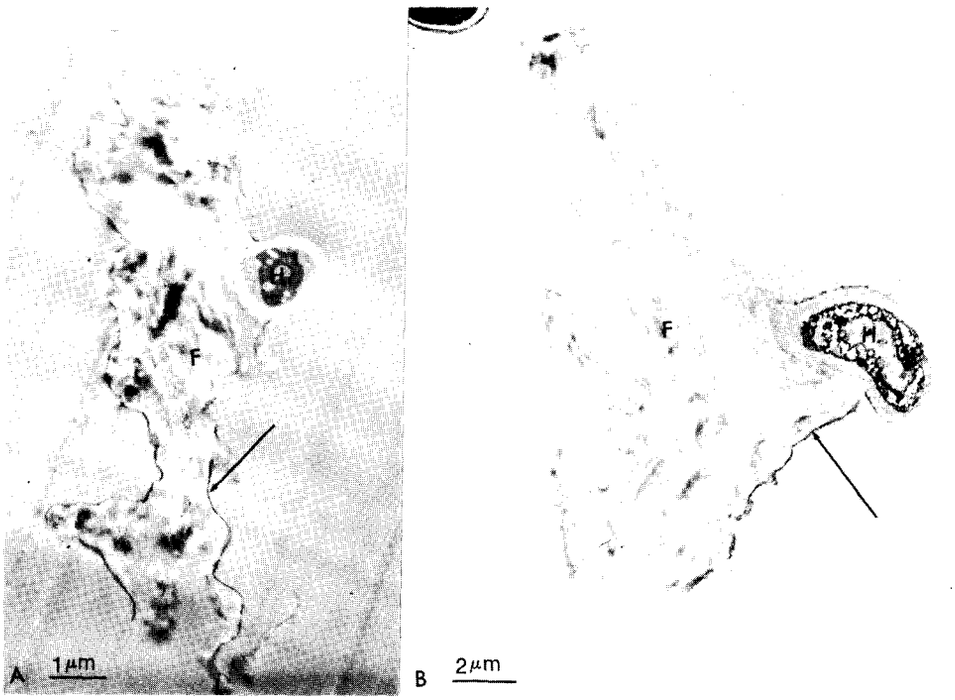

Fig. 5. Very advanced fiber degradation. (TEM)
A. *Ph. pini:* sheath (arrow) encases fiber (F), hypha partially embedded in fiber. 7300X
B. *Ph. chrysosporium:* sheath (arrow) partially present on hypha (H) and fiber (F). 3850X

observed by white-rot fungi in lignocellulose. However, our study shows that the sheath does not restrict movement of the cellulolytic system of white-rot fungi.

Observations

1. White-rot fungi grown on cellulose produced hyphal sheaths.
2. Cellulose was degraded at a distance from hyphae of the white-rot fungi.
3. Thinning of cellulose fibers did not occur.
4. The white-rot fungi apparently produce a diffusible cellulose-degrading system.

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