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VISUALIZATION OF HYPHAL SHEATH IN WOOD-DECAY HYMENOMYCETES. II. WHITE-ROTTERS

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ABSTRACT

Hyphae of each of five isolates representing five species of white-rot fungi had hyphal sheaths when grown in axenic culture. Sheaths were most evident when hyphae were appressed against a solid substrate such as steel, glass, cotton, or agar. The amount of sheath around hyphae vaned among isolates. Most sheaths were fine fibrillar in texture, but often inclusions were present which altered the histological appearance. Only two isolates had protoplasmic inclusions from autolyzing hyphae within the sheaths. Necrotic cells occurred but lacked sheaths.

Key Words: hyphal sheath, Hymenomycetes, wood-decay fungi, white-rotten, autolysis.

In a previous study (Palmer *et al.,* **1983),** we showed that twelve brown-rot fungi had hyphal sheaths. In another study with isolated cellulose (Highley *et al.,* **1983),** a brown-rot fungus produced hyphal sheath that extended a considerable distance from hyphae, encircling the cellulose fibers. Fibers were extensively degraded where touched by sheath even in absence of hyphae. This supports the views of others (Green *et al.,* **1981;** Jutte and Sachs, **1976),** that the hyphal sheath may be functional in wood decay. Whether white-rot fungi have sheaths as regularly as do brown-rot fungi and whether sheaths of white-rotters show distinctive differences from those of brown-rotters were investigated in this study. Differences, if they exist, may assist in explaining differences in attack by these fungi in wood.

MATERIALS AND METHODS

Cultures of five North American white-rot isolates were obtained from the culture collections of the Center for Forest Mycology Research at the Forest Products Laboratory. *Phanerochaete chrysosporium* Burds., ME-446, was isolated from beechwood chips. *Coriolus versicolor* (L. : Fr.) Quél., MAD-697, is used in soil-block decay testing and was isolated from a forest tree. *Phellinus pini* (Thore: Fr.) A. Ames, L-14602-Sp; *Pycnoporus sanguineus* (L. : Fr.) Murr., FP-103380-S; and *Xylobolus frustulatus* (Pers.: Fr.) Boid., FP-24059-S, were isolated from forest logs or slash.

Mycelia were grown on sterile cellulose from cotton (Highley *et al.,* **1983)** or on 2 per cent malt agar in some cases overlain with cellophane disks, glass cover slips, or stainless steel squares as described previously (Palmer *et al.,* **1983).** Procedures of sample preparation for reflectance light microscopy (RLM), trans-

[1] Maintained at Madison, Wisconsin, in cooperation with the University of Wisconsin.

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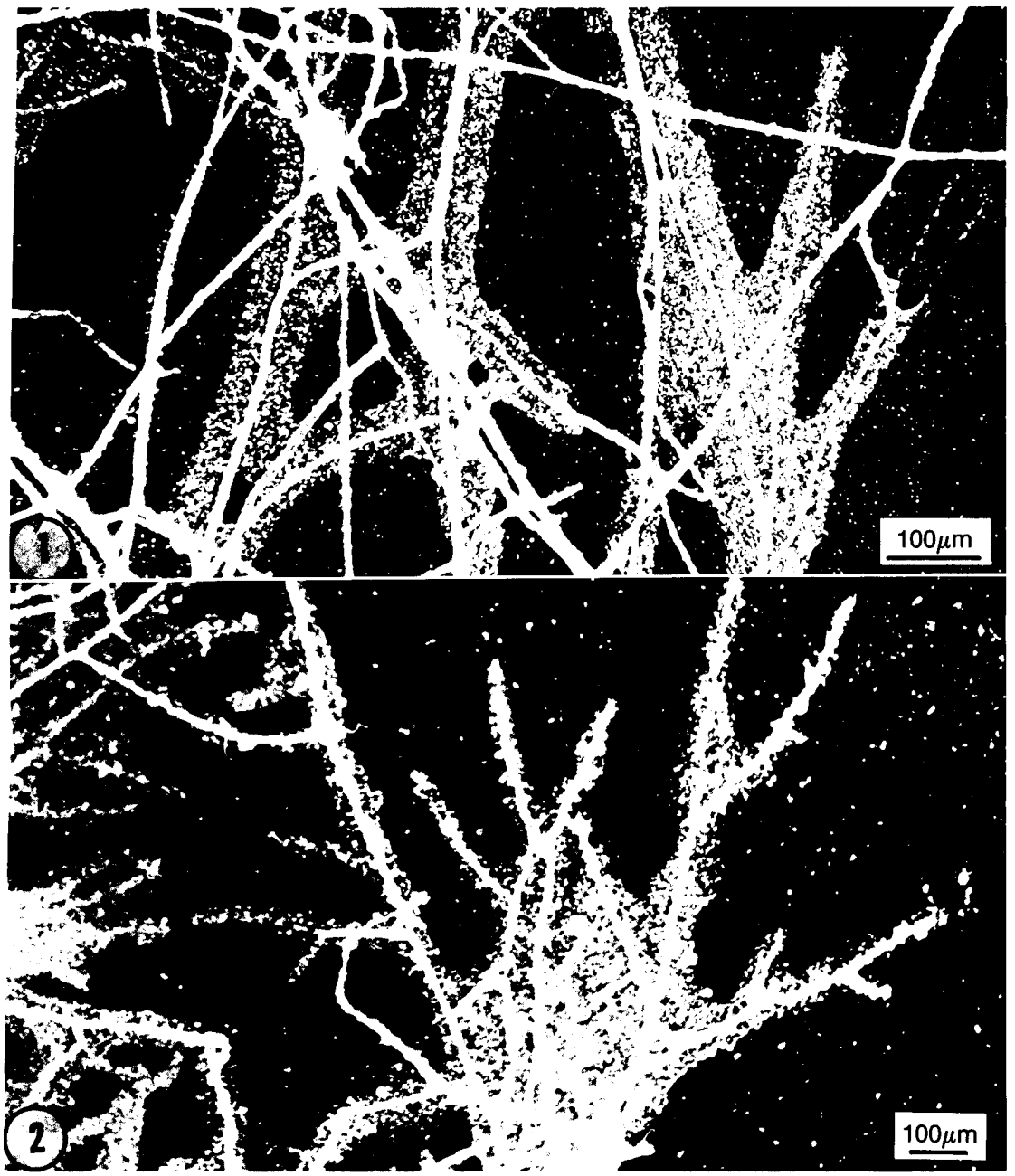
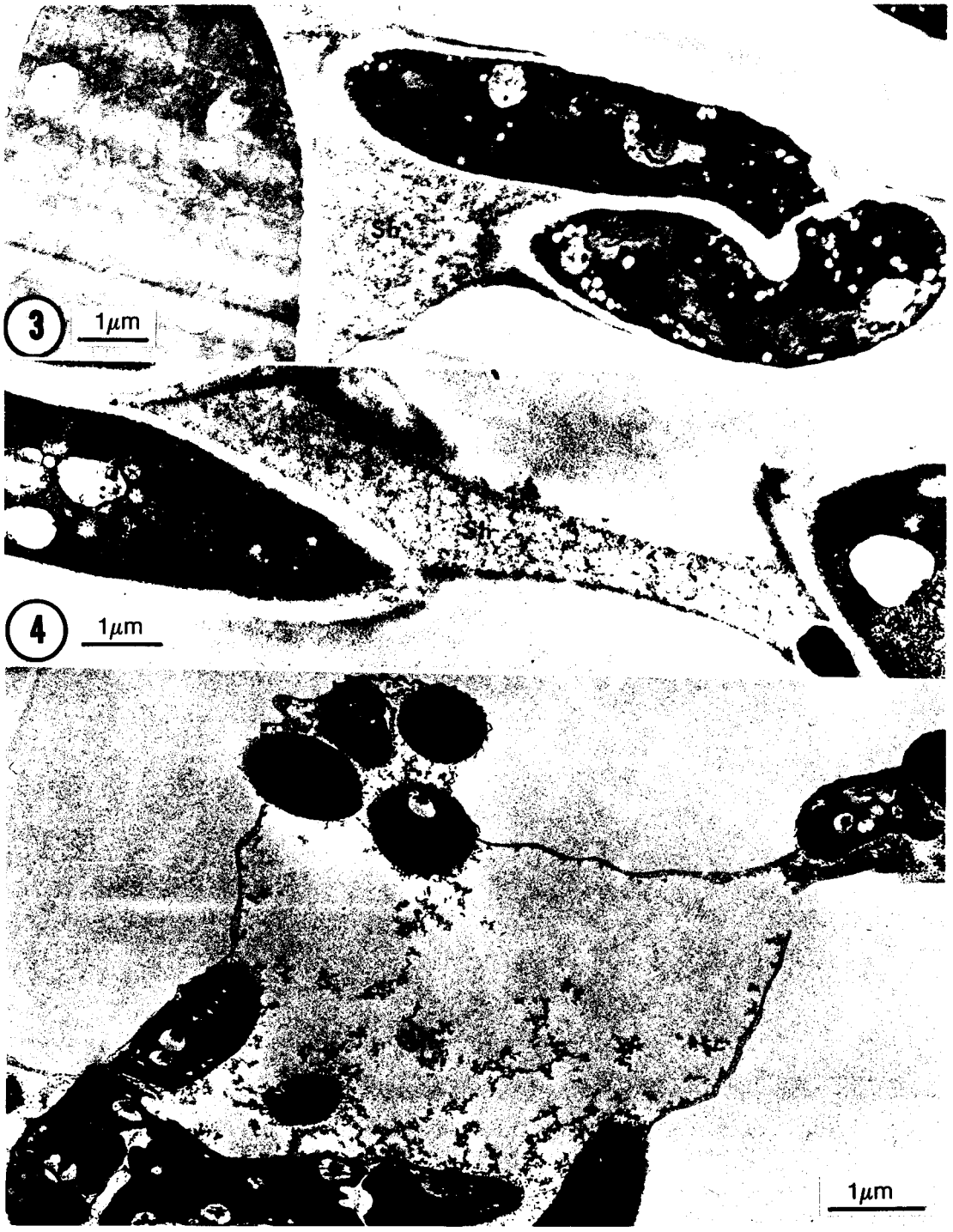
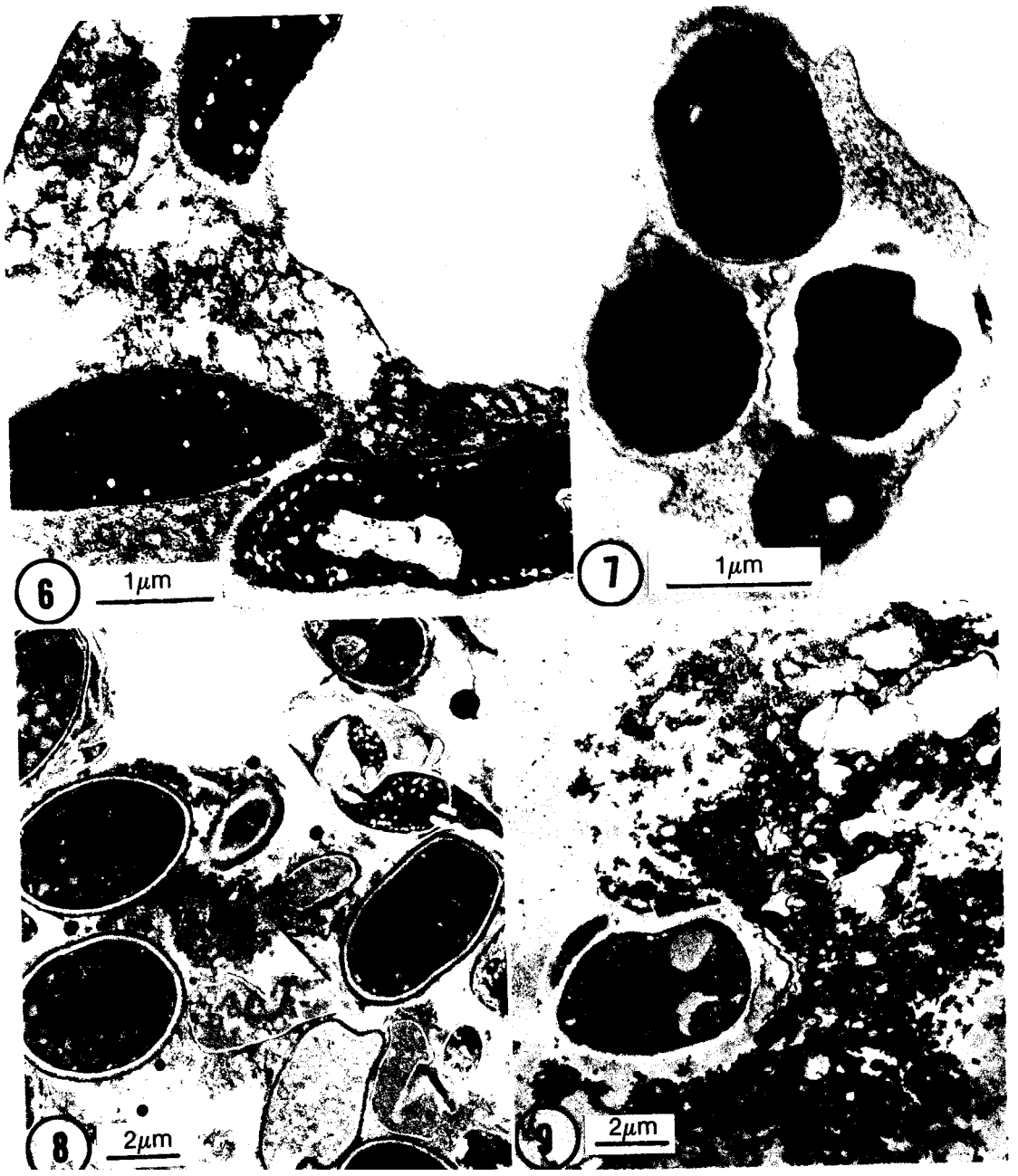


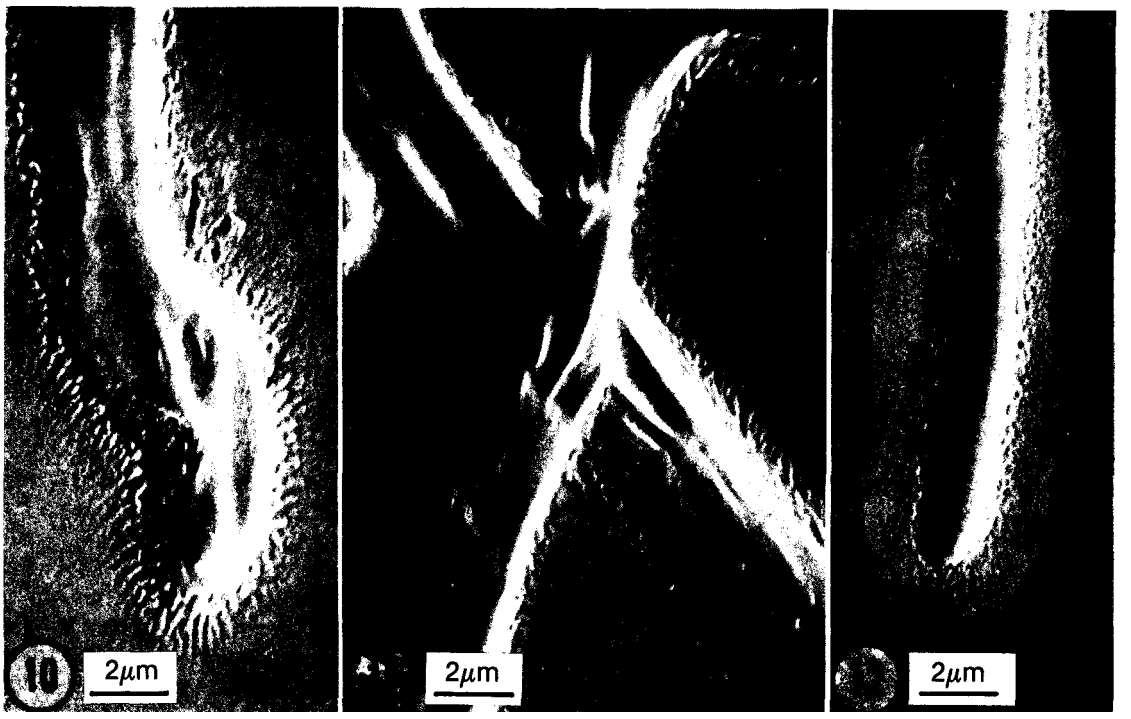
FIGS. 1, 2. Hyphal sheaths surrounding growing points and rest of hyphae. RLM. 1. *Xylobolus frustulatus,* × 130. 2. *Phanerochaete chrysosporium,* × 80.



FIGS. 3–5. Fine fibrillar sheath (Sh). TEM. 3. *Coriolus versicolor,* × 1 1,400. 4. *Xylobolus frustulatus.* × 12,300. 5. *Pycnoporus sanguineus,* × 14,140.



FIGS. 6, 7. Dense granular or finely fibrillar sheath (Sh). TEM. 6. *Coriolus versicolor*, ×16,800. 7. *Pycnoporus sanguineus*, ×20,700. FIGS. 8, 9. Released cellular contents. TEM. 8. *Phanerochaete chrysosporium*. Cellular contents coming from autolyzing hyphae (arrows), ×5100. 9. *Coriolus versicolor*. Released cellular contents surround an intact hypha (H); autolyzing cells not in sight, ×10,250.



FIGS. 10–12. Hyphal sheaths (arrows) surrounding growing points and rest of hyphae. SEM. 10. *Phanerochaete chrysosporium,* growing point, ×5000. 11. *P. chrysosporium,* rest of hyphae, ×5000. 12. *Pycnoporus sanguineus,* growing point, ×5000.

mission electron microscopy (TEM), and scanning electron microscopy (SEM) have been described previously (Highley *et al.,* **1983;** Palmer *et al.,* **1983).**

RESULTS

Each of the five white-rot fungi examined had a hyphal sheath when incubated on agar with or without cellophane disks, coverslips, or stainless steel squares, and on cotton fibers. When negatively stained with PTA for RLM observations, hyphae had sheaths surrounding their growing points and extending along their lengths (FIGS. 1, 2). The amount of sheath material varied; there was more sheath on hyphae of *X. frustulatus* (FIG. 1) than on *Phanerochaete chrysosporium* (FIG. 2). Hyphae of *Phellinus pini* and *Pycnoporus sanguineus* also had comparatively narrow sheaths. Texture and structural details of sheaths were apparent in TEM micrographs. All five fungi had fine, fibrillar sheaths limited by a denser bordering line as was pictured for *C. versicolor* (FIG. 3), *X. frustulatus* (FIG. 4), and *Pycnoporus sanguineus* (FIG. 5). In some cases, the sheath had partially broken down (FIG. 5). This probably occurred during some step in the preparation procedure and indicates the fragile nature of the sheath. The textural appearance of sheaths differed within the same isolate and among all isolates examined. In addition to fine, fibrillar texture, the sheath of C. *versicolor* could also be dense and contain granular and membranous materials (FIG. 6). FIGURE 7 shows a sheath in *Pycnoporus sanguineus* that was denser and more finely fibrillar than the sheath pictured in FIG. 5.

Phanerochaete chrysosporium (FIG. 8) and C. *versicolor* (FIG. 9) were the only species of those examined in which protoplasmic contents from autolyzing hyphae were observed in the sheath. FIGURE 8 showed two hyphae (arrows) undergoing autolysis. In FIG. 9, released cellular contents surrounded an intact hypha, but no hyphae undergoing autolysis were in sight.

SEM micrographs supported the RLM evidence that hyphal sheaths surrounded growing points and extended along the lengths of hyphae (FIGS. 10–12), especially when appressed against a solid such as glass or cotton. Hyphal sheaths were not visible around necrotic hyphal cells.

DISCUSSION

Structurally the hyphal sheath in white-rot fungi did not differ from that in brown-rot fungi. With both types of fungi, the sheath showed variability in texture and surrounded living but not necrotic hyphae. However, diffusion of protoplasmic contents into the sheath from autolyzing cells was much less common in the white-rot fungi. Only C. *versicolor* and *Phanerochaete chrysosporium,* two out of five white-rot fungi examined, showed hyphal autolysis and then much less frequently than did any of the 12 brown-rot fungi examined previously (Palmer *et al.,* **1983).**

Even if structurally the sheath had similar appearance in the brown- and white-rot fungi, its function may differ in these two types of fungi. Green *et al.* **(1980),** presented evidence that the sheath of wood-decaying white- and soft-rot fungi bound cellulases while that of brown-rot fungi did not. On the other hand, cotton fibers were degraded when only the sheath of a brown-rot fungus was attached to the fibers (Highley *et al.,* **1983),** suggesting that sheath in brown-rot fungi may also serve as a medium for housing and transporting lignocellulosic depolymerizing agents. Further research on determining the nature and the localization of wood-degrading enzyme systems would be of great interest.

In addition to housing or transporting depolymerizing agents, the sheath in both brown- and white-rot fungi may serve as a source of support and nutrition.

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