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MICROSCOPY OF PROGRESSIVE DECAY OF COTTONWOOD BY THE BROWN-ROT FUNGUS *GLOEOPHYLLUM TRABEUM*[1]

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ABSTRACT

Eastern cottonwood (*Populus deltoides* Bartr.) samples subjected to various degrees of brown-rot decay by *Gloeophyllum trabeum* (FPL 617) were studied by scanning electron (SEM) and polarizing microscopy. A technique was developed to prepare decayed wood specimens for SEM. Ray cells were heavily decomposed in early stages of decay. Bore holes were produced in early stages to facilitate hyphal penetration into fiber tracheids. Degradation of fiber tracheid walls began with the formation of radial checks or voids in the S2 layer, followed by the removal of the entire S2 layer, which often caused the separation of the S3 layer from the remaining cell wall. The S3 layer often was removed before the decomposition of the S1 layer. The compound middle lamella remained intact even after the complete removal of the secondary wall.

Keywords: *Populus deltoides*, hardwood, *Gloeophyllum trabeum*, brown rot, cell-wall structure, polarizing microscopy, scanning electron microscopy.

INTRODUCTION

In favorable conditions, microorganisms invade and degrade wood in use, causing great losses. Each particular microorganism has its unique pattern of attacking wood. Earlier studies on the subject of microscopical changes of wood attacked by microorganisms have been thoroughly reviewed by Wilcox (1970, 1973). In addition, because of the importance of estimating loss of wood strength

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due to biodegradation, Wilcox (1978) also has compiled such data from the available literature in a convenient reference.

Brown-rot fungi degrade cell walls by releasing cellulolytic enzymes capable of penetrating the cell-wall structure (Cowling 1961; Wilcox 1968; Highley et al. 1983). Microscopic studies have shown that brown-rot fungi typically initiate cell-wall degradation in the S2 layer of the secondary wall and that decomposition of the S1 and S3 layers does not occur before the complete removal of the S2 layer (Meier 1955; Wilcox 1968). In birch, Meier (1955) found that vessel walls were more resistant than fiber walls against brown-rot decay, whereas in sweetgum, both vessels and rays were resistant to the attack of a brown-rot fungus (Wilcox 1968). Relative decay resistance of different wood elements and cell-wall layers against brown-rot fungi has been attributed to differences in cell-wall density and chemical composition (Meier 1955) and to different degrees of lignification (Meier 1955; Wilcox 1968).

Most of the earlier microscopic studies of biodegradation of wood used mainly light microscopy. Since 1970, scanning electron microscopy (SEM), because of its high resolving power, depth of focus, and simplicity of specimen preparation, has been extensively used in the field of wood pathology (Bravery 1971; Jutte and Sachs 1976; Blanchette et al. 1985). Because of difficulties in preparing decomposed or partly decomposed wood samples, most SEM studies have observed longitudinal surfaces of wood samples. Therefore, the objectives of the present study were to develop a technique to prepare satisfactory transverse and longitudinal surfaces of decayed samples for SEM observation and to use this technique to demonstrate the progressive decay of eastern cottonwood by a brown-rot fungus.

MATERIALS AND METHODS

Test blocks, 1.9 by 1.9 by 0.95 mm with the 0.95-mm dimension in the grain direction, were sawn from eastern cottonwood (*Populus deltoides* Bartr.) heartwood. To determine weight loss due to decay, five test blocks were prepared for each of the 0, 2, 4, 6, 8, 10, and 12 weeks of exposure to the fungus. A set of matching test blocks also was prepared for microscopic studies. Test blocks were exposed to the brown-rot fungus *Gloeophyllum trabeum* (FPL 617) by means of the agar-block method described by McNabb (1958).

At each designated decay termination date, weight loss of each block was determined on an oven-dry basis. The matching sample for microscopic studies was cut into small pieces having a transverse surface area approximately 0.5 cm^2. Small specimens for microscopic studies were immediately fixed, without drying and weighing, with a 10:5:85 mixture of Formalin-glacial acetic acid-50% aqueous ethanol (FAA) for at least 2 days. After removal of the fixative with several changes of distilled water, specimens for polarizing microscopy were embedded in paraffin by a process described by Berlyn and Miksche (1976). Transverse (10 μm) and longitudinal (25 μm) sections were obtained by using a rotary microtome. Some sections were double-stained by a picro aniline blue method (Wilcox 1964), but most sections were examined unstained with a Zeiss polarizing microscope.

SEM specimens were prepared in the following manner. The FAA-fixed specimens were immersed overnight in a 50:50 mixture of polyethylene glycol (PEG-1540) and water, transferred into two changes of pure PEG-1540, and individually mounted on wooden blocks. The transverse, radial, and tangential surfaces of

embedded specimens were prepared by a razor-blade cutting technique (Exley et al. 1974). Cubic specimens containing razor-blade-cut surfaces were removed from the mounting blocks, washed with several changes of warm distilled water to remove the embedding matrix, and were subsequently dehydrated by a methanol-acetone-pentane solvent exchange sequence (Thomas and Nicholas 1966). Dehydrated specimens were mounted on SEM stubs, coated with approximately 300 A of gold-palladium (40:60) in a sputter coater, and examined with a JEOL-JSM-35 SEM at 25 Kv.

RESULTS

At the end of the 2-week exposure to the fungus, test blocks sustained a 2.7% weight loss. Fungal hyphae were distributed mainly in large vessels and adjacent ray parenchyma (Fig. 1). Some vessels were more heavily infected than others, and fungal hyphae extended from one cell to another mainly through pits. Polarizing microscopy indicated that no apparent reduction in cell-wall birefringence occurred at this early stage of decay.

Exposure of test blocks to the fungus for 4 weeks caused a 4.3% weight loss. The entire sample surface was covered with the mycelium, and fungal hyphae had extended from vessels and rays into fiber tracheids (Fig. 2). Fiber tracheids adjacent to more heavily infected vessels showed reduced cell-wall birefringence, forming isolated and elongated pockets of decaying tissues. In the decaying pockets, ray cell walls were degraded, and small bore holes having an angle oblique to the cell axis were produced in fiber tracheid walls (Fig. 3). Bore holes were found to be the main passageway by which hyphae penetrated from vessels and rays into fiber tracheids. Accompanying active cell-wall penetration and hyphal proliferation, deposition of patches of a granular substance on the fiber tracheid walls also was observed (Fig. 4).

At the end of 6 weeks, test blocks sustained an average weight loss of 11.1%. Elongated pockets of decay continued to expand, more rapidly along the grain than across the grain. This expansion of the decay allowed some pockets to merge. At this stage, the cell wall of fiber tracheids at the periphery of enlarged decay pockets sustained a mild degradation, resulting in a porous appearance of the cell wall (Fig. 5). Meanwhile, fiber tracheid walls in the decay pockets were more heavily degraded (Fig. 6). The corresponding transverse appearances of Figs. 5 and 6 in the 8-week samples (25.5% weight loss) are shown in Figs. 7 and 8, respectively. Most of the fiber tracheid walls show radial cracks and checks, representing the initial stage of cell-wall degradation (Fig. 7). Fiber tracheids adjoining heavily degraded rays have already lost that portion of the secondary wall adjacent to the ray except the S3 layer. Figure 8 shows a more advanced decay of fiber tracheid walls in that the S2 and S3 layers are mostly decomposed but the S1 layer, compound middle lamella, and cell corners are still relatively intact.

After 10 weeks of exposure to the fungus, test blocks lost 43.5% of their original weight. Even at this late stage of decay, some test blocks contained regions that displayed exceptionally high decay resistance. This localized decay resistance seems to be associated with a bacterial infection (Fig. 9). The secondary walls of some fiber tracheids in the center of original decay pockets were completely decomposed. Most fiber tracheids only had the S2 layer decomposed, causing the

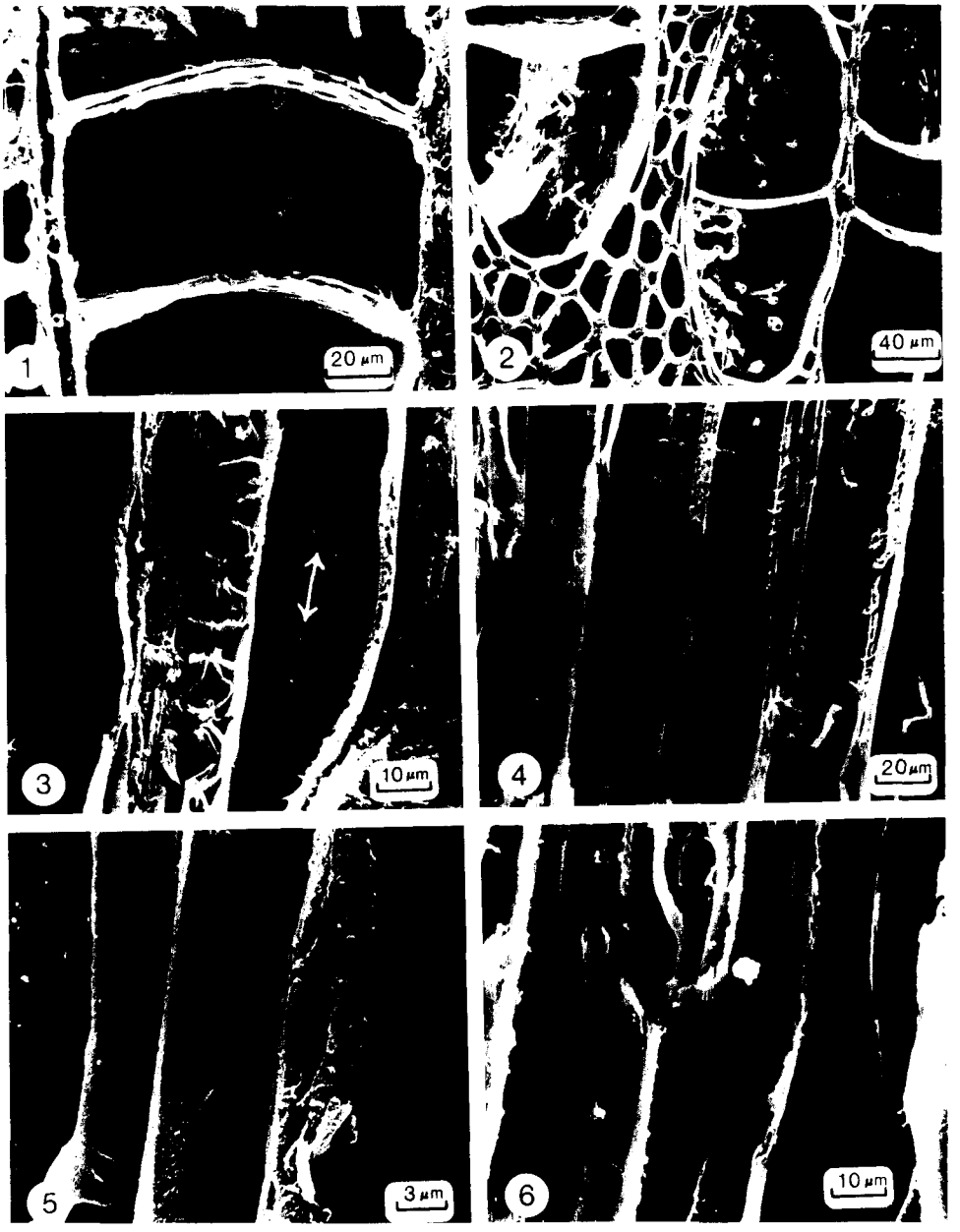

FIG. 1. Week 2 (2.7% weight loss), showing presence of fungal hyphae only in vessels and rays.

FIG. 2. Week 4 (4.3% weight loss), showing permeation of hyphae from vessels into adjacent fiber tracheids.

FIG. 3. Week 4 (4.3% weight loss). Ray cells are heavily degraded and bore holes (arrows) having an oblique angle to cell axis are produced.

FIG. 4. Week 4 (4.3% weight loss), showing hyphal proliferation, cell-wall penetration, and deposition of extracellular materials in fiber tracheids.

FIG. 5. Week 6 (11.1% weight loss). A longitudinal view of an early stage of cell-wall degradation, showing porous appearance of cell walls.

FIG. 6. Week 6 (11.1% weight loss). A longitudinal view of fiber tracheids adjacent to vessels, showing enlarged bore holes and heavily decayed cell walls.

S3 layer to separate from the remaining S1 layer and compound middle lamella (Fig. 10). At this stage, some residual S2 material was still attached to the S1 and S3 layers (Figs. 11 and 13). After the major portion of the S2 layer was decomposed, the detached S3 layer lost birefringence before any appreciable amount of S1 decomposition was observed (Fig. 12).

The 12-week test blocks sustained an average weight loss of 63.0%. At this late stage of decay, there was an increased number of fungal hyphae in fiber tracheids. The secondary walls of most fiber tracheids were completely decomposed, leaving behind the intact compound middle lamella (Fig. 14). The vessel walls, especially intervessel walls and perforation rims in vessels, were found more decay resistant than the cell wall of fiber tracheids and ray cells (Figs. 15, 16).

DISCUSSION

The method of embedding FAA-fixed samples in polyethylene glycol, followed by razor-blade cutting and the subsequent solvent-exchange drying has been shown an excellent technique to prepare decayed wood samples for SEM studies. The mechanical support provided by the polyethylene glycol matrix during razor-blade cutting and prevention of excessive shrinkage by the solvent-exchange drying method minimize artifacts during specimen preparation. By using this method, the turgid appearance and the natural position of fungal hyphae in wood samples can be well preserved (Figs. 1 and 2). In addition, this method also preserves microscopic integrity of decayed wood samples (Figs. 10, 11, and 14) and thus, facilitates the study of changes in cell-wall structures during progressive stages of decay.

In cottonwood, the mycelium of *Gloeophyllum trabeum* became quickly established, first in large vessels, and then from these large vessels into adjacent rays and fiber tracheids. Hyphal penetration between vessels and from vessels to rays was mainly through pits, whereas hyphae penetrated into fiber tracheids mainly through bore holes. Because bore holes were observed mainly in fiber tracheid walls and because the number of bore holes remained relatively constant even at advanced decay, most of the bore holes were produced during early stages of decay, apparently to facilitate hyphal penetration into fiber tracheids. Hyphae normally bored through fiber tracheid walls perpendicular to the cell axis, but bore holes having an angle oblique to the cell axis also were observed (Fig. 3). Longitudinal hyphal growth within the cell walls was not observed in cottonwood, probably because of insufficient cell-wall thickness to accommodate such growth.

Hyphal sheaths that often encase hyphae of many brown-rot fungi during degradation of cellulosic materials (Jutte and Sachs 1976; Highley et al. 1983) were not observed in this study. It is not clear whether the failure to observe hyphal sheaths in the present study is due to the insufficient fixation by the FAA used, to dissolution during processing, or to the fact that they were not produced. However, deposition of a granular substance at some distance away from hyphae was seen during the stage of bore hole production (Figs. 4 and 9).

Wilcox (1968) found that sweetgum ray parenchyma was more resistant to brown-rot decay than fiber tracheids, but cottonwood ray cells were found heavily decomposed in early stages of decay (Fig. 3). Similar to the results in studying sweetgum (Wilcox 1968) and birch (Meier 1955), vessel elements were found to be the most decay-resistant cell type against brown-rot in eastern cottonwood

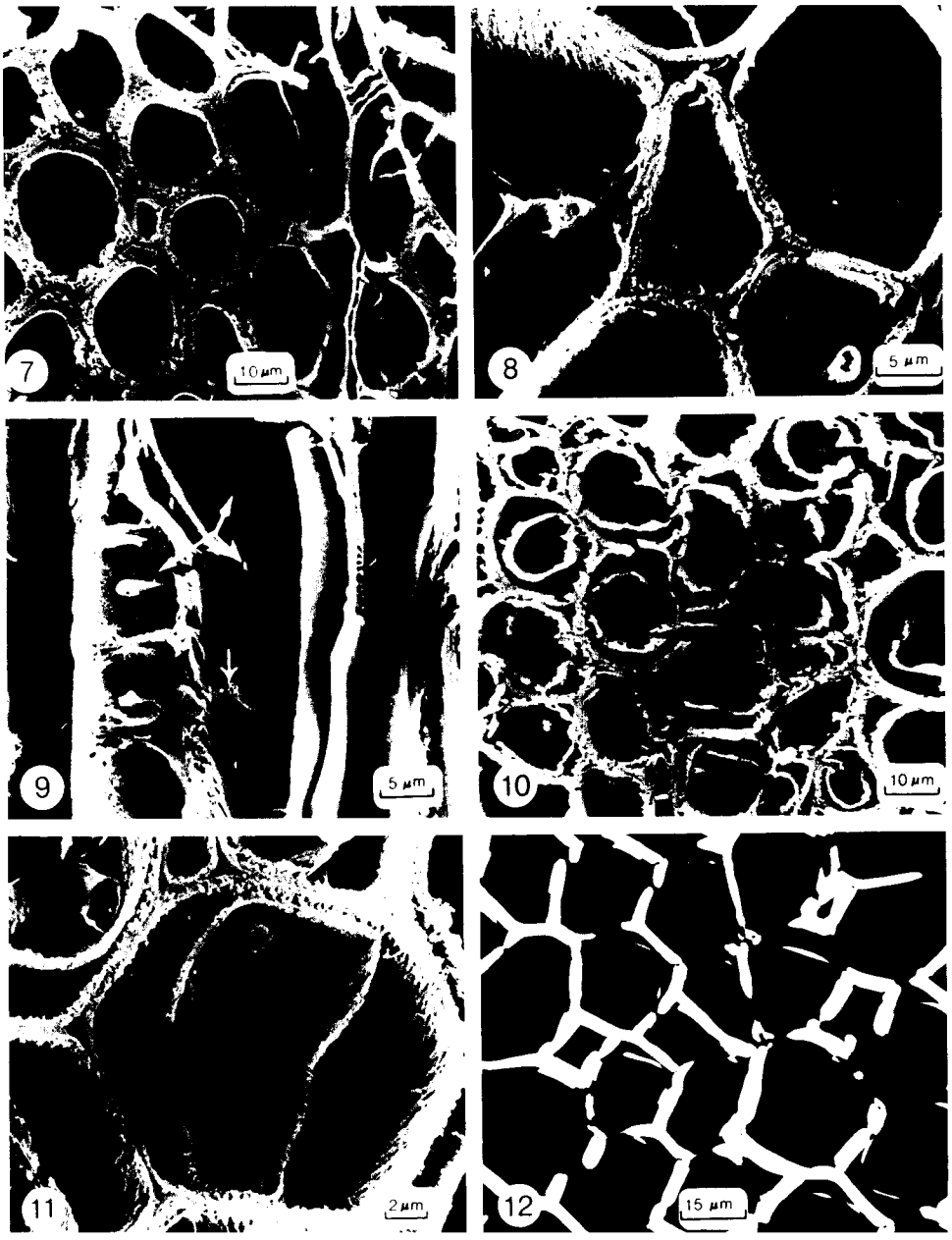

FIG. 7. Week 8 (25.5% weight loss). A transverse view of less decayed area, showing development of radial checks or voids in S2 layer. Portion of fiber walls bordering heavily decomposed ray already has lost secondary wall except S3 layer.

FIG. 8. Week 8 (25.5% weight loss), showing an area with advanced decay where S2 and S3 layers of fiber tracheids have been decomposed.

FIG. 9. Week 10 (43.5% weight loss), showing an area infected by bacteria (long arrows). Process of hyphal penetration into fiber tracheids has just started, and cell walls are still relatively intact. Also note extracellular material (short arrow) associated with hypha penetrating fiber wall.

(Figs. 15 and 16). Wilcox (1968) attributed the decay resistance of vessel walls to a high lignin content. Recently, Saka and Goring (1985) found that the secondary wall of white birch vessel elements had a higher lignin concentration (0.26 g/g) than that of fiber tracheids (0.14 g/g). By using an infrared spectroscopic method, Kuo et al. (1988) also found that cottonwood vessel elements contained about 20% more lignin than fiber tracheids. Fergus and Goring (1970) found that the lignin in the vessel wall of birch wood was composed predominantly of guaiacylpropane units and that the lignin deposited in the fiber and ray parenchyma secondary walls was composed mostly of syringylpropane units. Therefore, a higher degree of brown-rot decay resistance of vessel walls may be associated with both the quality and quantity of lignin in the vessel walls.

The S2 layer in the secondary wall of softwood tracheids has been found to be the first layer removed by brown-rot fungi (Meier 1955; Wilcox 1970; Ruel and Barnoud 1985). Because the lignin concentration in the secondary wall of softwoods is lower in the S2 layer than in the S1 and S3 layers (Saka and Thomas 1982), the greater brown-rot decay resistance of the S1 and S3 layers has been attributed to a greater degree of lignification in these layers (Wilcox 1968). However, Saka and Goring (1985) have reported that the lignin is uniformly distributed across the S1, S2, and S3 layers in white birch fiber walls. Because the pattern of preferential attack of cell-wall layers found in softwoods also has been observed in birch (Meier 1955), sweetgum (Wilcox 1968), and in cottonwood in the present study, the concept of relating brown-rot decay resistance to degree of lignification cannot be applied to hardwoods. Therefore, differences in density and chemical composition between different cell-wall layers, as proposed by Meier (1955), should be investigated. In addition, the possibility that the preferential attack of different cell-wall layers by brown-rot fungi is related to carbohydrate/lignin linkages also should be studied.

The present study suggests that the S3 layer of cottonwood fiber tracheid walls is decomposed before any appreciable amount of cellulosic material in the S1 layer is removed (Figs. 12 and 13). This order of cell-wall attack by the brown-rot fungus does not necessarily indicate that the S1 layer is more decay-resistant than the S3 layer because, after the removal of the S2 layer, the supply of cellulose-destroying enzymes from hyphae in the lumen to the S1 layer may be disrupted while hyphae often lie against the S3 layer. In fact, a reverse order of cell-wall attack was observed in the portion of the fiber tracheid walls adjoining highly decayed ray parenchyma in that the S2 and S1 layers were decomposed while the S3 layer remained intact (Fig. 7).

Wetwood formation caused by bacterial infections is common in several species

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FIG. 10. Week 10 (43.5% weight loss). S2 layer of majority of fiber tracheids has been removed, separating S3 layer from remainder of cell wall.

FIG. 11. Week 10 (43.5% weight loss). An enlarged view of Fig. 10, showing separated S3 layer and residual S2-microfibrils attached to S1 and S3 layers.

FIG. 12. Week 12 (63.0% weight loss). A polarized-light micrograph, showing degrading S3 layers and strong birefringence of S1 layers.

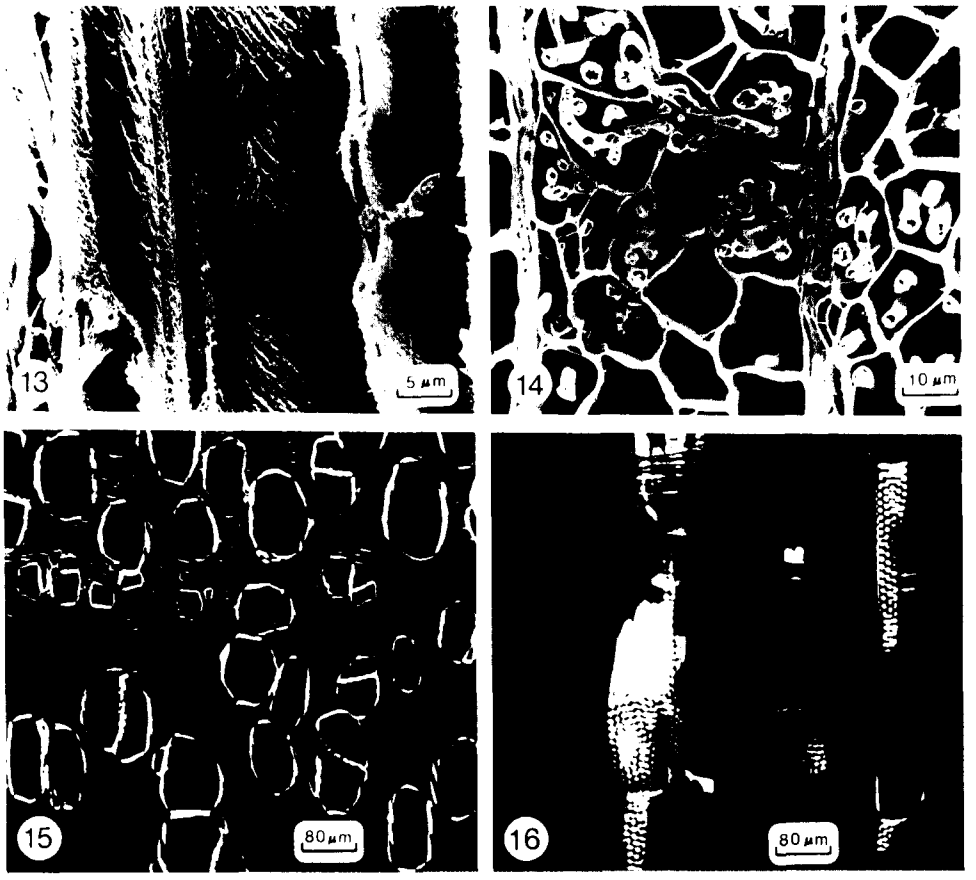

FIG. 13. Week 12 (63.0% weight loss). An SEM longitudinal view of a specimen corresponds to that of Fig. 12, showing removal of S3 layer and residual S2-microfibrils on S1 layer in center cell.

FIG. 14. Week 12 (63.0% weight loss), showing final stage of decay. Note that secondary walls have been completely removed, leaving intact compound middle lamella.

FIG. 15. Week 12 (63.0% weight loss). A polarized-light micrograph of a transverse section, showing relative decay resistance of vessels, rays, and fiber tracheids.

FIG. 16. Week 12 (63.0% weight loss). A polarized-light micrograph of a radial section, showing decay-resistant vessels.

of *Populus,* including eastern cottonwood (Clausen and Kaufert 1952; Hartley et al. 1961). Hossfeld et al. (1957) reported that aspen wetwood was more decay resistant than the surrounding sound wood. In this study, bacterium-infected regions in eastern cottonwood also were found to be more decay resistant than sound wood against the brown-rot fungus. At a late stage of decay, while the S2 layer of most fiber tracheids in sound wood was decomposed (Figs. 10 and 11), hyphae just started penetrating into fiber tracheids in regions with bacterial infection, and both ray and fiber tracheid cell walls showed only little degradation (Fig. 9). Hossfeld et al. (1957) found that fluorescent alcohol extracts present in aspen wetwood were responsible for its decay resistance. On the other hand,