

ULTRASTRUCTURAL CHARACTERISTICS OF RED MAPLE (*ACER RUBRUM* L.) WOOD¹

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ABSTRACT

The anatomy of red maple (*Acer rubrum* L.) was examined using the transmission electron microscope. Direct carbon replicas and ultrathin sections of inner and outer sapwood and inner and outer heartwood were prepared. In cross-sectional view sapwood intervessel pit membranes appear thin; in surface views of air-dried and extracted samples of the second sapwood ring and inner sapwood, openings in the intervessel pit membranes are visible. Intervessel pit membranes are permeated with extractives in the heartwood. Vessel-ray parenchyma pits have been described as similar to intervessel pits; but differences in shape, apertures, and pit membranes were detected in this study. The ray parenchyma cells appear different in ultrastructural details from those in species that have been studied with the transmission electron microscope as they do not have well-defined protective layers in the sapwood when they are adjacent to vessels, plasmodesmata channels are not apparent in the parenchyma-parenchyma pits, and there are no pits to the intercellular spaces in the rays. Red maple is an unusual hardwood as it has longitudinal intercellular spaces adjacent to the fibers; the appearance of these spaces is similar to that of the longitudinal intercellular spaces in members of the conifer family, Araucariaceae.

Keywords: Red maple, *Acer rubrum*, transmission electron microscopy, intervessel pitting, parenchyma pitting, pits.

INTRODUCTION

In the last few years, there has been increasing interest in the utilization of hardwoods from the eastern United States. Much remains to be learned about the fundamental properties of these woods; little is known of their fine structure. Red maple, *Acer rubrum* L., grows on a variety of sites in the southeast. It has been called the most abundant and widespread tree of eastern North America (Harlow and Harrar 1969) and so represents a large percentage of the hardwood resource. This paper describes anatomical details of this species as seen with the light and transmission electron microscopes. It represents a further contribution in a continuing study whose purpose is to characterize the ultrastructure of southeastern United States hardwoods and when possible to establish relationships between their anatomy and their physical properties. Details of the anatomy of southern red oak and white oak (Wheeler and Thomas 1981), blackgum, sweetgum, and mockernut hickory (Thomas 1976) have already been presented. Because information about hardwood ultrastructure is not abundant, examination of these southern hardwoods will add to knowledge of this topic.

Stark (1954), Panshin and deZeeuw (1980), and Metcalfe and Chalk (1950) described the light microscope anatomy of maple wood; Stark specifically described

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red maple as well as other individual species. Red maple wood is diffuse-porous and characterized by small vessel elements that are solitary and in radial multiples, and have spiral thickenings, simple perforation plates, and crowded alternate intervessel pitting; sparse longitudinal parenchyma; fibers classified as both libriform fibers and fiber-tracheids by Panshin and deZeeuw (1980), and as just fiber-tracheids by Stark (1954); homocellular rays, 1–5 cells wide; and ray-vessel pitting described as similar to the intervessel pitting.

In many ultrastructural studies of pit membranes, only one region of the tree is examined, or no distinction is made between the outer and inner areas of sapwood and heartwood. In this study, cell-wall organization and pit structure in all cell types of maple were examined, and the surface appearance of pit membranes in outer and inner sapwood, and outer and inner heartwood were compared.

One explanation given for why studies of hardwood ultrastructure are rare compared to studies of softwood ultrastructure is that the anatomy of hardwoods is more complex (Schmid 1965). Because of the greater number of hardwood cell types, it can be difficult to determine pit-pair type in direct carbon replicas prepared from longitudinal surfaces as the type of cell to which a pit leads generally cannot be directly determined, but is inferred. Maple is a species in which vessel-ray parenchyma pits are described as resembling the intervessel pits. To be certain that intervessel and vessel-ray parenchyma pits could be distinguished from one another in longitudinal surfaces viewed with the electron microscope, direct carbon replicas were prepared so that either vessel-vessel or vessel-ray parenchyma contacts were visible.

MATERIALS AND METHODS

Samples of inner and outer sapwood and inner and outer heartwood were obtained from a mature tree of red maple (*Acer rubrum* L.). The samples were either 1) air-dried, 2) solvent-dried by being placed for one week in methanol followed by one week in acetone in a soxhlet extracting apparatus prior to drying, or 3) extracted with a 1:1 acetone-benzene mixture in a soxhlet extracting apparatus before drying. Specimens for ultrathin sectioning were embedded in a mixture of n-butyl and methyl-methacrylate (75:25). Methacrylate was removed prior to shadowing the sections with platinum at an angle of approximately 20° in a high vacuum evaporator.

Direct carbon replicas of radial and tangential surfaces were prepared using the procedure described by Côté et al. (1964). Intervessel pitting in maple is more common in tangential surfaces because there are many radial pore multiples and vessel-ray parenchyma pitting is more common in radial surfaces, but there are occasional oblique pore multiples and rays curve around vessels so there are some intervessel pits on radial walls of vessels and some ray-vessel contact on tangential walls of vessels. Consequently, to be sure that intervessel and vessel-ray parenchyma pitting could be distinguished, the following procedure was used. For intervessel pitting, a pore multiple without ray contact was located in cross-section using a dissecting microscope and a tangential section with this multiple in its center cut. The surface of the wood surrounding the multiple was disrupted with a dissecting needle so that only the vessel would be smooth. For vessel-ray

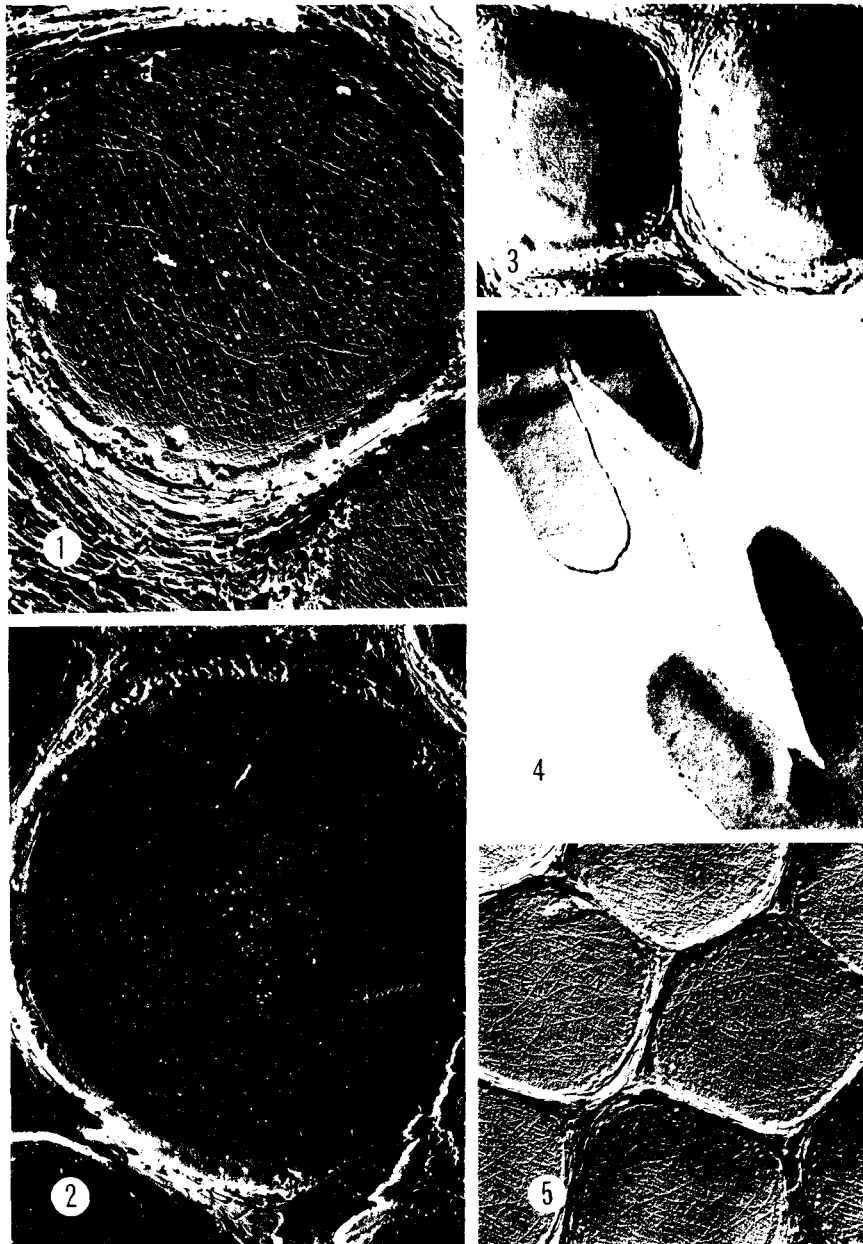


FIG. 1. Intervessel pit membrane from the outermost sapwood ring, solvent-dried, $\times 12,000$.

FIG. 2. Intervessel pit membrane from the second sapwood ring; note the small openings in the membrane, extracted, $\times 10,500$.

FIG. 3. Intervessel pit membranes from the second sapwood ring, air-dried; all membranes are displaced to one side of the pit chamber and small holes are visible where the membrane is next to the aperture, $\times 6,000$.

FIG. 4. Cross-sectional view of a sapwood intervesSEL pit membrane, second sapwood ring, $\times 9,000$.

FIG. 5. Heartwood intervesSEL pit membranes with a coarse texture imparted by the encrusting extractives, air-dried, $\times 4,500$.

parenchyma pitting, a solitary pore with ray contact was similarly isolated in radial surfaces.

RESULTS AND DISCUSSION

Intervessel pitting

The arrangement of the intervessel pits is alternate, and the outline of individual pits is generally hexagonal as the pits are crowded. Pit apertures are oval, with the aperture being approximately one-third the horizontal width of the pit.

The intervessel pit membranes of red maple are fine-textured and have randomly arranged microfibrils (Fig. 1). In the outermost growth rings, there were no openings in the membrane. In the second sapwood ring and in the inner sapwood, openings in the membrane were present in air-dried and extracted preparations (Fig. 2), but not in solvent-dried preparations. Pit membranes are not displaced during solvent-drying and not subjected to the same stresses that occur during air-drying (Thomas and Nicholas 1966; Hart and Thomas 1967). The imprint of the pit aperture on the pit membrane is often seen in air-dried wood, indicating that the pit membrane is adpressed to the pit border (Fig. 3). In air-dried wood, the stretching of pit membranes during displacement to one side of the pit chamber and the removal of water has produced separations between microfibrils. In wood that was treated with acetone-benzene, materials that in-filled between the microfibrils were apparently removed so holes are visible even though the membranes were not displaced.

Hardwood pit membranes are generally described as lacking visible openings, yet openings have been observed in yellow poplar (*Liriodendron tulipifera* L.; Bonner and Thomas 1972); sycamore (*Platanus occidentalis* L.; Husin 1977); and in pit membranes near the scalariform perforation plates of tupelo (*Nyssa sylvatica* Marsh.; Parham 1973). These three species are diffuse-porous, and have scalariform perforation plates and small-diameter vessels. As intervessel pit membranes have been examined in relatively few species, the degree of variability in their appearance and correlations of any such variability with other wood anatomical features have not been established.

Intervessel pit membranes allow liquid flow between contiguous vessels, but prevent passage of air embolisms. When air appears in a vessel and the pit membrane moves to one side of the pit chamber, it is vital that the membrane maintain its integrity, for if it tore the air embolism could easily pass into the adjacent conducting unit and render it nonfunctional. Zweyffennig (1978) presented a formula to express the force that would be exerted on a pit membrane during displacement. According to that formula, the force on the membrane during displacement would be affected by the distance of displacement and also by the size of the pit aperture and pit chamber radius. As intervessel pit size varies between woods, the texture and thickness of the pit membrane and its resistance to displacement might also be expected to vary between species. Phillips (1933) observed that in dried samples of conifer wood nearly all springwood pits were aspirated, while a "certain proportion" of the summerwood pits remained un-aspirated. Sapwood intervessel pit membranes in maple are thinner and more delicate-appearing than in the other southern hardwood species examined to date.

In cross-sections, intervessel pit membranes in all regions of sapwood exam-

ined appeared thin and loose-textured (Fig. 4). This contrasts with the vessel-vasicentric tracheid pit membranes of white oak (*Quercus alba* L.) and southern red oak (*Q. falcata* Michx.), as in these species the membranes were thicker and appeared less permeable in the second sapwood ring than in the outermost sapwood ring. This difference correlates with the different sizes of the water-conducting regions of the ring-porous oaks and the diffuse-porous maple. Only the outermost growth ring conducts water in ring-porous oaks; more growth rings conduct water in the diffuse-porous maple (Huber 1935; Zimmermann and Brown 1971).

The appearance of intervessel pit membranes in solvent-dried and air-dried preparations of inner and outer heartwood is similar. Extractives appear to have coated individual microfibrils so the heartwood pit membranes have a coarser texture than the sapwood pit membranes (Fig. 5). Treatment with acetone-benzene did not remove the deposits from the membranes. In the extracted samples, a central area of the membrane, comparable to the position of the pit aperture, with a texture smoother than the peripheral regions of the membrane was observed. Sections of heartwood show the membrane to be infilled with extractives (Fig. 6).

Vessel element wall organization

The vessel elements in maple are described as having spiral thickenings. During this study, it was noted that spiral thickenings and intervessel pits generally did not occur in the same area. The microfibrillar organization of the vessel wall is generally regarded as being complex in the region of pits; in maple it seems particularly complex where there are spiral thickenings.

Parham and Kaustinen (1973) examined spiral thickenings in 19 species of hardwoods and 3 species of softwoods with the scanning electron microscope and presented a scheme for classifying spiral thickenings. According to these authors, red maple has prominent branched spirals that are firmly attached to the wall. With the transmission electron microscope, not only the branches that are obvious with the light microscope and SEM are apparent, but less prominent branches and swirls (Fig. 7) are seen. Often the wall has a granular appearance in areas that appear bounded by the branches. Treatment with sodium chlorite removed the granules and made apparent the complex organization of the microfibrils underneath these granular areas (Fig. 8). In some of these regions bounded by the branches, the orientation of the microfibrils is near vertical and the near-vertical microfibrils seem to dip under the spirals.

Parham and Kaustinen (1973) briefly reviewed the discussions on the nature of spiral thickenings; opinion is divided on whether spirals are part of the S_3 or an additional elaboration of the cell wall. Sections show that in red maple the spirals are part of the innermost cell-wall layer (Fig. 9).

In replicas where the vessel wall was split, it is apparent that the pit apertures do not follow the orientation of the S_3 , but rather the S_2 layer (Fig. 10). Harada (1965) and Panshin and deZeeuw (1980) suggest that the orientation of the pit apertures in *Fagus* and *Tilia* vessel elements parallels the S_3 . In other cell types, the pit aperture is oriented parallel to the S_2 (Preston 1974). When vessel walls of different species are examined between crossed nichols, they show varying degrees of optical heterogeneity; some species show uniform birefringence (War-

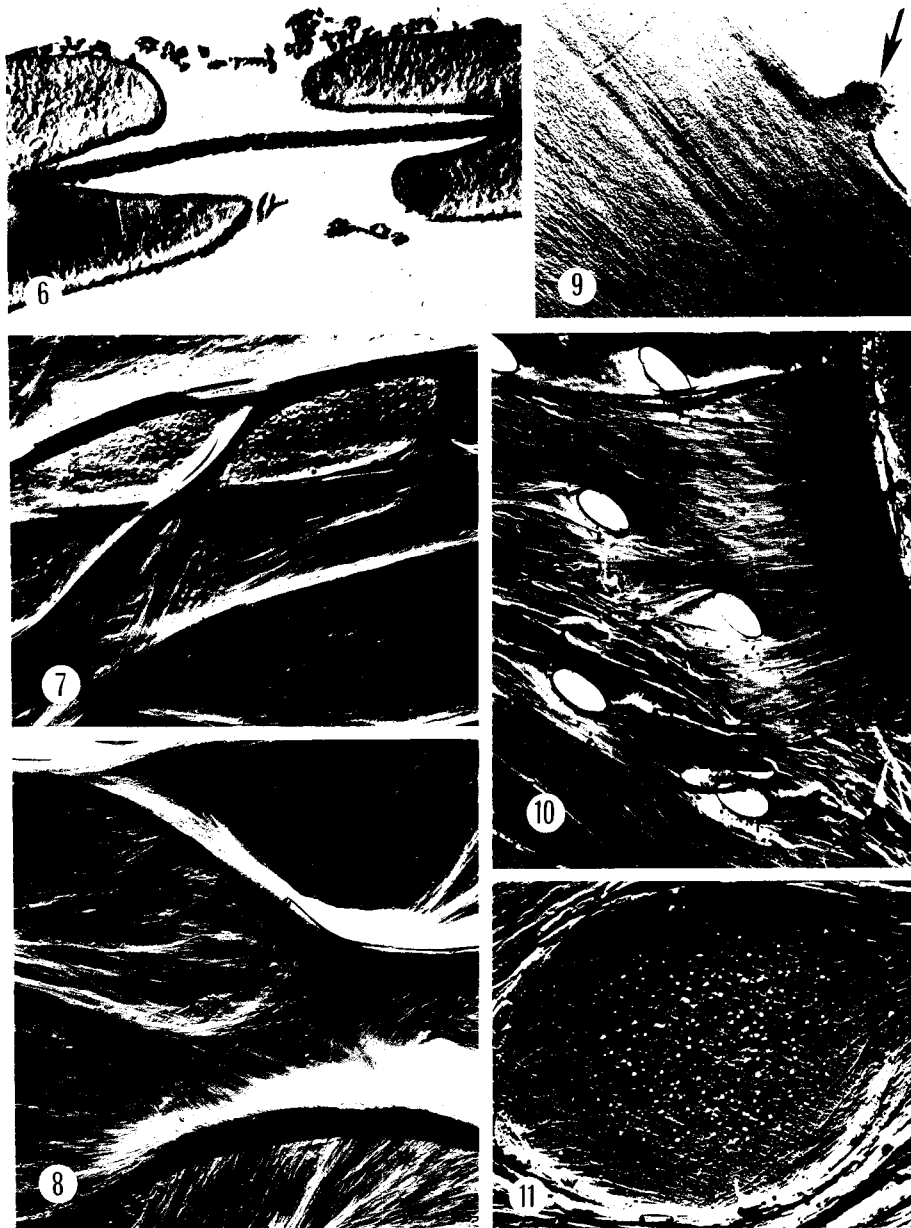


FIG. 6. Cross-sectional view of a heartwood intervessel pit membrane; extractives have infilled and coated the membrane, $\times 10,500$.

FIG. 7. Spiral thickenings in a vessel element, solvent-dried, $\times 3,000$.

FIG. 8. Spiral thickenings in a vessel element, air-dried then treated with sodium chlorite, $\times 5,250$.

FIG. 9. Cross-sectional view showing a vessel element adjacent to a fiber; the arrow points to the spiral thickening of the vessel element wall, $\times 10,500$.

FIG. 10. Layering in a vessel element wall; note the orientation of the pit apertures follows the microfibril orientation of the S_2 , not the S_3 , air-dried, $\times 3,000$.

FIG. 11. Surface view of a vessel-ray parenchyma pit membrane, extracted, $\times 18,000$.



FIG. 12. Vessel-ray parenchyma pit membranes in the heartwood, air-dried, $\times 9,000$.

FIG. 13. Sapwood vessel (V)-ray parenchyma (R) pit membrane in cross-sectional view, an "extra" layer on the parenchyma side of the pit, $\times 19,500$.

FIG. 14. Tangential section showing a vessel (V), longitudinal parenchyma (L), and ray parenchyma (R) in the sapwood, $\times 3,000$.

FIG. 15. Ray parenchyma; the intercellular spaces (i) associated with these cells are illustrated, air-dried, $\times 3,000$.

FIG. 16. Tangential section of a ray, $\times 3,500$.

FIG. 17. Two fibers and a longitudinal intercellular space; the lining of the space and the strands traversing it are shown, solvent-dried, $\times 9,000$.

drop 1964). Wardrop (1964) presented three explanations of the latter phenomenon: 1) layering is absent; 2) the cell-wall layers differ only slightly in helical microfibril orientation; 3) microfibril orientation is disturbed because of the presence of pits. It is possible that *Fagus* and *Tilia* are species in which there is little difference in the microfibril orientation of the cell-wall layers and there is a large S_2 angle. The layered structure of red maple vessel walls is apparent in sections; in this species the orientation of the cell-wall layers is different enough that it is relatively easy to determine that the vessel pit apertures have the orientation of the S_2 .

Vessel-parenchyma pits

Although the vessel-ray parenchyma pits of maple are described as similar to the intervessel pits (Metcalf and Chalk 1950; Panshin and deZeeuw 1980), differences between the two are apparent when they are viewed with the transmission electron microscope. Vessel-ray parenchyma pits are lens-shaped, not hexagonal; have more elongate apertures than intervessel pits; and the aperture extends nearly to the edge of the border. In the sapwood, these pit membranes are more encrusted than intervessel pit membranes; the microfibrils at the periphery are tightly packed and tend to a parallel alignment. There is not as sharp a demarcation between the pit membrane and the adjacent wall as in intervessel pits (Fig. 11).

In the heartwood, extractives have accumulated over the entire membrane and in all three types of preparations thicker oblong-shaped deposits were apparent within the area that would have been beneath the aperture (Fig. 12).

In cross-section, it is obvious that the vessel-ray parenchyma pit membranes are thicker than the intervessel pit membranes. Protective layers were not always observed; in some preparations "extra" layers with attachment to the pit membrane were seen (Fig. 13).

Longitudinal parenchyma is rare in maple. A tangential section of a region where longitudinal parenchyma cell was adjacent to a vessel showed two well-developed extra layers in the parenchyma cell (Fig. 14). These layers were thickest along the vessel-parenchyma pit and indistinguishable along a wall common to two longitudinal parenchyma cells. It is not clear why there should be better-defined protective layers in the longitudinal parenchyma cells than in the ray parenchyma cells.

Parenchyma

In direct carbon replicas, the lamellation of ray parenchyma cell walls is apparent, as is the association of intercellular spaces with the ray parenchyma cells (Fig. 15). The lining of these spaces has a smooth texture. Intercellular spaces are also apparent in tangential sections (Fig. 16). Pits between ray cells and intercellular spaces were not observed in sections.

Simple pits are formed between contiguous parenchyma cells (Fig. 14), but the membrane did not show any indication of the presence of plasmodesmata.

Fibers

Intercellular spaces adjacent to red maple fibers are common. In replicas of longitudinal surfaces, they appear similar to the longitudinal interstitial spaces

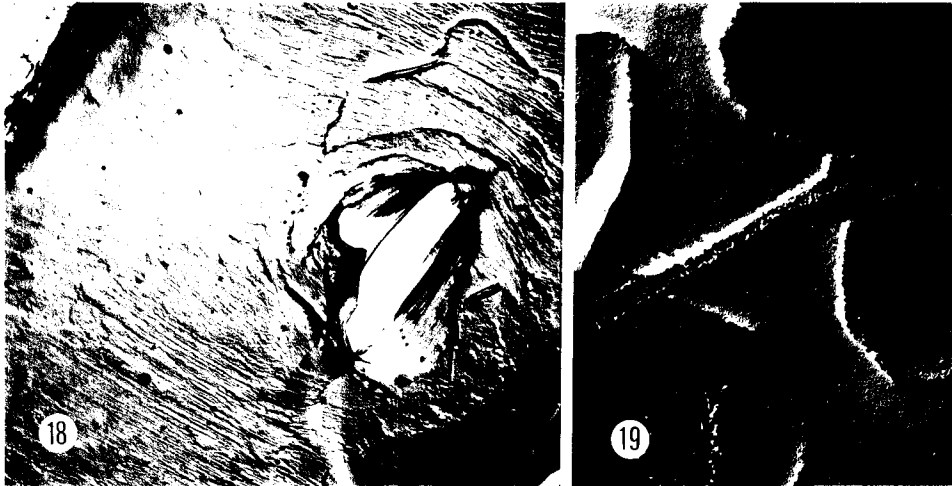


FIG. 18. Pit in a fiber wall, air-dried, $\times 7,500$.

FIG. 19. Cross-sectional view of a fiber-fiber pit, sapwood, $\times 23,000$.

Bolton et al. (1975) described in wood of the conifer family *Araucariaceae*; they have a smooth lining, and fibrils extend into the intercellular spaces or completely bridge them (Fig. 17). The shape of the fibrils in *Araucariaceae* wood and red maple wood is similar to intercellular pectic strands in leaf palisade parenchyma (Carr et al., 1980). Fibrils may be a general feature of intercellular spaces that are created by cells separating from one another. Pits between fibers and intercellular spaces were not observed.

Bolton et al. (1975) in their review of the literature on interstitial spaces indicate that longitudinal intercellular spaces have received little attention in the literature and refer to only two publications which they say show longitudinal channels in hardwoods (Preusser et al. 1961, illustrations of *Fagus* wood; and Isebrands and Parham 1974, illustrations of *Populus* tension wood). However, the micrographs in the article by Preusser are cross-sections of rays, i.e. tangential sections, and so these are radial intercellular spaces, not longitudinal spaces. Examination of the illustrations in the Isebrands and Parham article shows there are spaces between the longitudinal cells in the kraft-pulped tension wood, not in the untreated tension wood. Reference to intercellular spaces associated with maple fibers has been made in reports of light microscope observations (Metcalf and Chalk 1950; and Braun 1970).

Pits were more common on the radial walls of fibers than on tangential walls and generally were not centrally located (Fig. 18). The pit aperture was only slightly inclined from the longitudinal axis of the fiber, indicating a very low angle for the S_2 microfibrils. No surface views of fiber-fiber pit membranes were obtained. In cross-section, fiber-fiber pit membranes appear thicker than intervessel pit membranes (Fig. 19).

The distinction between libriform fibers and fiber-tracheids is the apparent lack of pit borders in the libriform fibers and their presence in the fiber-tracheids. This is, of course, based on the cells' appearance as seen with the light microscope. These two cell types intergrade with one another and both types are said to occur

in red maple (Panshin and deZeeuw 1980). All maple fibers examined in this study did have bordered pits, as seen with the electron microscope.

Relationship of anatomy to treatability

MacLean (1952) classified the relative resistance of the heartwood of various species to penetration by wood preservatives. Silver maple (*Acer saccharinum* L.), not red maple, was classified. Red maple and silver maple are both termed soft maples, have similar anatomy, and their properties and uses are often described together (e.g. Panshin and deZeeuw 1980). Consequently, remarks about the penetrability of silver maple should also apply to red maple. The heartwood of silver maple is classified as moderately difficult to penetrate; the categories MacLean (1952) used are: easily penetrated, moderately difficult to penetrate, difficult to penetrate, very difficult to penetrate. In an earlier study, Teesdale and MacLean (1918) noted that penetration took place mainly in the vessels and fibers of maple with only very slight penetration of the rays. Vessels and fibers are both considerably longer than ray parenchyma cells and so would offer better pathways for preservatives than the ray cells. The parenchyma-parenchyma pit membranes are thicker than both the intervessel pit membranes and the fiber-fiber pit membranes and so not as good pathways for liquid flow. The interparenchymatous pit membranes of maple appear unusual as they lack plasmodesmata channels and this could also contribute to the rays' greater resistance to penetration.

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