OCCURRENCE AND LIGNIFICATION OF LIBRIFORM FIBERS IN NORMAL AND TENSION WOOD OF RED AND SUGAR MAPLE[1]

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ABSTRACT

Morphology and histochemistry can be used to distinguish between libriform fibers and fiber tracheids in tension and normal wood of red (*Acer rubrum* L.) and sugar (*Acer saccharum* Marsh.) maple. Utilization of a new differential staining method using safranin and astra blue dissolved in ethyl alcohol and an ultraviolet illumination technique (355–375 nm excitation) provides an effective method to distinguish between these fibers in microtome sections. Observations of the morphology of these tissues and their histochemical analyses were made using light and scanning electron microscopy. The Mäule reaction indicated less syringyl lignin in secondary walls of tension wood (outside of the G-layer) than in normal wood. Libriform fibers do not fluoresce in UV light in the wavelengths used, although fluorescence was observed in some of the cell corners, probably due to the presence of guaiacyl lignin in the cell corners. In normal and tension wood of both species, libriform fibers occur in interrupted wavy bands, have larger lumens than fiber tracheids, and intercellular spaces are commonly present.

Keywords: *Acer,* astra blue, histochemistry, libriform fibers, lignin, normal wood, red maple, safranin, sugar maple, tension wood.

INTRODUCTION

This paper is part of a larger study on properties of fibers in sugar (*Acer saccharum* Marsh.) and red (*A. rubrum* L.) maple. Our previous work determined that the combination of safranin-O and astra blue stains in ethyl alcohol solution differentiate libriform fibers from fiber tracheids in microtome sections. A notable characteristic of these two species was a positive cellulose staining reaction in groups of fibers in both normal and tension wood. These fibers appeared to be not well lignified as confirmed by the absence of fluorescence using UV light, and occurred in interrupted wavy bands or in groups; they were identified as libriform fibers (Vazquez-Cooz and Meyer 2002). These interesting results motivated us to study the libriform fibers of these two species in more detail.

Libriform fibers may remain alive for several years (Esau 1965), which demonstrates their close relationship to longitudinal parenchyma (Magendans 1999). The name libriforrn fiber was given by early botanists because of their similarity to phloem fibers, which were known as ''liber'' (Eames and MacDaniels 1947). Chalk (1983) stated that in *Acer* a paratracheal sheath of living fibers separates the ground tissue of fibers from vessels. The primary function of fiber tracheids and

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libriform fibers is to provide mechanical support to the tree, although fiber tracheids can also participate in conduction (Ilvessalo-Pfäffli 1995). Given that there is a difference in form and function between fiber tracheids and libriform fibers, it is important to be able to distinguish between them when conducting anatomical investigations.

Bailey (1936) realized the difficulty of separating tracheids, fiber tracheids, and libriform fibers. Bailey also established the concept of imperforate tracheary elements. Currently, it is accepted that a tracheary element is a cell with a secondary wall derived from a fusiform cambial initial (in secondary xylem; derived from procambium in primary xylem) that has neither perforations (or a single perforation) nor is subdivided into a strand of cells each surrounded by a secondary wall (Carlquist 2001).

In 1964, the IAWA Committee on Nomenclature recognized Bailey's categories of hardwood fibers and wrote definitions. A libriform fiber was defined as ''an elongated, commonly thick-walled cell with simple pits; usually distinctly longer than the cambial initial as inferred from the 1ength of vessel members and parenchyma strands'' (IAWA 1964). Fiber tracheids, on the other hand, were defined as being ''commonly thick-walled with a small lumen, pointed ends, and bordered pit-pairs having lenticular to slit-like apertures'' (IAWA 1964). The primary distinction between libriform fibers and fiber tracheids was therefore based primarily on the type of pitting—simple in the case of libriform fibers and bordered in the case of fiber tracheids. The terminology for fiber tracheid could be applied to the latewood tracheids in gymnosperms, but this term is restricted to angiosperms by most authors (Panshin and de Zeeuw 1980).

In 1989 the International Association of Wood Anatomists published a numerical listing for coding wood features when using computer programs for wood identification (Wheeler et al. 1989). This coding system does not list *type* of fiber; instead, it separates fibers with simple to minutely bordered pits from fibers with distinctly bordered pits. IAWA relaxed the 1964 distinction between fiber tracheids and libriform fibers by stating that fibers with simple to minutely bordered pits (chambers < 3 μm dia.) were ''libriform fibers,'' while fibers with distinctly bordered pits (pit chambers > 3 μm dia.) were ''fibers (or fiber tracheids or ground tissue tracheids).'' A critical statement by the IAWA Committee (Wheeler et al. 1989) is: ''The terms libriform fibres, fibre-tracheids, and ''true tracheids'' have been deliberately avoided as descriptors in this list because there is no consensus on their definitions.'' We offer this paper as an assist in helping to identify types of hardwood fibers.

Carlquist (1986a, b) discussed occurrence and characteristics of imperforate cells in hardwoods and recommended retaining the order IAWA definition for libriform fibers in having simple pits. Baas (1986), on the other hand, argues that libriform fibers may have simple or minutely bordered pits (borders less than 3 μm dia.), while fiber-tracheids have distinct borders (greater than 3 μm dia.). Intermediate forms of fibers between libriform fibers and fiber tracheids might occur, but they are rare (Magendans 1999). In addition, Magendans and van Veenendaal described funnel pits that occur in libriform fibers as having pit canals that extend from the pit membrane to the fiber lumen, where they form elongated apertures (1999). Furthermore, complementary pits in neighboring walls of adjacent libriform fibers are not in perfect alignment, while the complementary pits of fiber tracheid pit-pairs are aligned as mirror images of each other (Magendans and van Veenendaal 1999).

Libriform fibers are present in the secondary xylem of many arboreous families (Carlquist 2001) as well as in herbaceous plants such as cactus (Mauseth and Landrum 1997). Several studies have examined aspects of libriform fibers ranging from morphological modifications (Czaninski 1964) including the presence of thin transverse walls (Parameswaran and Liese 1969), to chemical composition (they contain syringyl lignin but not

guaiacyl lignin), and UV light absorbance (Schwarze et al. 2000). Yoshinaga et al. (1997) studied cellular distribution of guaiacyl and syringyl lignins within an annual ring in oak (*Quercus mongolica* Fischer var. *grosseserrata*), and found that the tips of cells were rich in syringyl lignin compared with the bodies (middle part) of the cells. It was suggested that guaiacyl and syringyl lignins were distributed heterogeneously in the axial direction of individual long fusiform cells. They also found that the proportion of fusiform cells rich in syringyl lignin tended to increase at locations most distant from vessel elements. Using SEM to observe pit structure, their examinations suggested that the cell types tended to change gradually from vasicentric tracheids to libriform fibers via fiber tracheids as distances from vessel elements increased. Yoshinaga et al. (1997) suggested that the proportions of syringyl lignins tended to increase as cell functions changed from water conduction to mechanical support. However, there is controversy about whether transitional fibers exist (Baas 1986; Carlquist 1986a, b, 2001) or not (Magendans 1999).

This paper is part of a larger study on wood machining. It was necessary for us to describe accurately the anatomy and histochemistry of wood used in a study of the cutting forces when machining green, dry, and frozen maple (Vazquez-Cooz, 2003). Over 300 microtome sections were prepared to characterize the wood anatomy of sugar maple and red maple. Observations revealed new information on libriform fibers: the purpose of the present paper is to report on the occurrence, lignification, and some morphological characteristics of libriform fibers present in tension and normal wood of red and sugar maple. The use of a differential staining method and an UV illumination technique made it possible to distinguish between libriform fibers and other elements. In addition, the distribution of libriform fibers within microtome sections was determined.

MATERIALS AND METHODS

Selection of trees

Three trees of red maple (*Acer rubrum* L.) containing tension wood and one tree containing normal wood were selected from ESF's Heiberg Memorial Forest in Tully, NY. One sugar maple (*Acer saccharum* Marsh.) containing tension wood and another sugar maple composed of normal wood were also obtained from Heiberg Forest. Two sugar maple trees containing tension wood were kindly provided by Native American Landscape Company of Syracuse, NY.

Red maple tension wood stem sections used to obtain sample material had from 44 to 70 growth rings; sugar maple tension wood material had from 50 to 76 rings. Normal wood stem sections had 46 (red maple) and 66 (sugar maple) growth rings. Tension wood samples were taken from the longest radius of decidedly elliptical stems, which had been harvested from leaning trees. Presence of tension wood was verified by observation of fibers with gelatinous layers. Normal wood was taken from round stem sections harvested from vertical trees. For each species, the tree containing normal wood was used as a control. Wood was stored in the never-dried (green) condition in a freezer.

Specimen preparation for anatomical and histochemical analysis

Small wood blocks for anatomical and histochemical analyses were cut to a size of 10 mm (tangential width) by 70 mm (radial length) by 10 mm (longitudinal depth). All blocks were taken from the outer part of the stem, within about 10 mm of the bark. Three tension wood and one normal wood sectioning blocks were taken from the individual trees of each species. Blocks were placed in containers with distilled water and then put into a glass bell jar to be evacuated for 2 h until they were water soaked. Sections 10 mm wide and 37 mm long were required for anatomical analysis of wood used for the machining study (Vazquez-Cooz 2003). After soaking, in order

to cut high quality, long sections of sugar maple, these blocks were softened with a 4% ETD (ethylenediamine) solution for 1.5 h at 70°C (Kukachka 1977). Red maple did not require softening. Transverse, radial, and tangential sections (20 μm thick) were cut with a sliding microtome. A small number of sugar maple samples were embedded in Epon 812 epoxy for sectioning with an ultramicrotome. These sections, ~1.9 nm thick, were used to verify presence of intercellular spaces.

Permanent slides of unstained and stained sections were mounted using Histoclad media (No. 3920). A Spot-RT digital camera was used to photograph the sections with a Nikon Optiphot light microscope equipped with DIC and fluorescence optics. Unstained sections were observed using fluorescence. A Nikon UV-1A filter block was used, with 355-375 nm excitation (max. at 365 nm), and barrier filter at 400 nm.

A new differential staining formulation using Safranin-O and astra blue was developed, using ethanol rather than water as the solvent (Vazquez-Cooz and Meyer 2002). It was critical, for this study, to ensure that all sections used for histochemical analysis received identical treatment. Therefore, care was taken to apply the stains for the same number of seconds for each staining session by using a stopwatch. All sections from each sectioning block were stained simultaneously in the same watch glass with ethanol solutions of safranin-O (Matheson Coleman & Bell, C.I. No. 50240) and subsequently with astra blue (6GLL, Aldrich 36, 340-5). After the sections were stained with safranin for 120 min, they were rinsed quickly three times in 85% ethyl alcohol over a period of 5 to 10 s, which leached some of the dye. Then the samples were stained with astra blue for 120 s and rinsed quickly (three times) in 95% and absolute ethyl alcohol. The sections remained in absolute ethyl alcohol for a few minutes until the sections could be placed on slides and cleared with xylene (3 times), and then mounted in Histoclad.

In addition, some sections were stained with safranin-O in aqueous solution for 180 s, and counter-stained with astra blue (aqueous solution) also for 180 s, according to the procedure described by Srebotnik and Messner (1994). For these sections, staining times again were controlled to the nearest second, using a stopwatch.

When it became necessary to compare stain intensity of tension wood and normal wood sections in an effort to determine differences in degree of lignification, new slides were prepared using sections stained in the same watch glass so they received identical treatment. Red maple and sugar maple sections were prepared separately, but the tension wood and normal wood sections of each species were processed in the stains simultaneously. A small notch was cut into the tension-wood sectioning block so there could be no confusion about the origin of the two types of sections.

Over 300 sections were examined. All photographs were obtained digitally and the images processed using Adobe Photoshop Version 6.

The Mäule reaction was used to stain a set of tension wood and normal wood sections of red and sugar maple utilizing white pine as a control. Sections were stained with 1% $KMnO_4$, for 15 min, washed in distilled water 5 min, immersed in 14% HCl 5 min, washed in distilled water 10 min, and mounted in 5% $NaHCO_3$ (Rawlings and Takahashi 1952). To ensure identical treatment conditions, tension wood sections were identified with small notches so they could be stained simultaneously in the same watch glass. Examinations were completed within 40 min of mounting in the $NaHCO_3$ solution.

Some sections of sugar and red maple (unstained) were dried for 2 h in an oven at 100°C (±3°C), mounted on aluminum stubs, and sputter-coated with gold-palladium. A Jeol-5800 scanning electron microscope (SEM) was used to observe the samples.

RESULTS AND DISCUSSION

Anatomy

Double staining red and sugar maple wood with safranin and astra blue in aqueous solu-

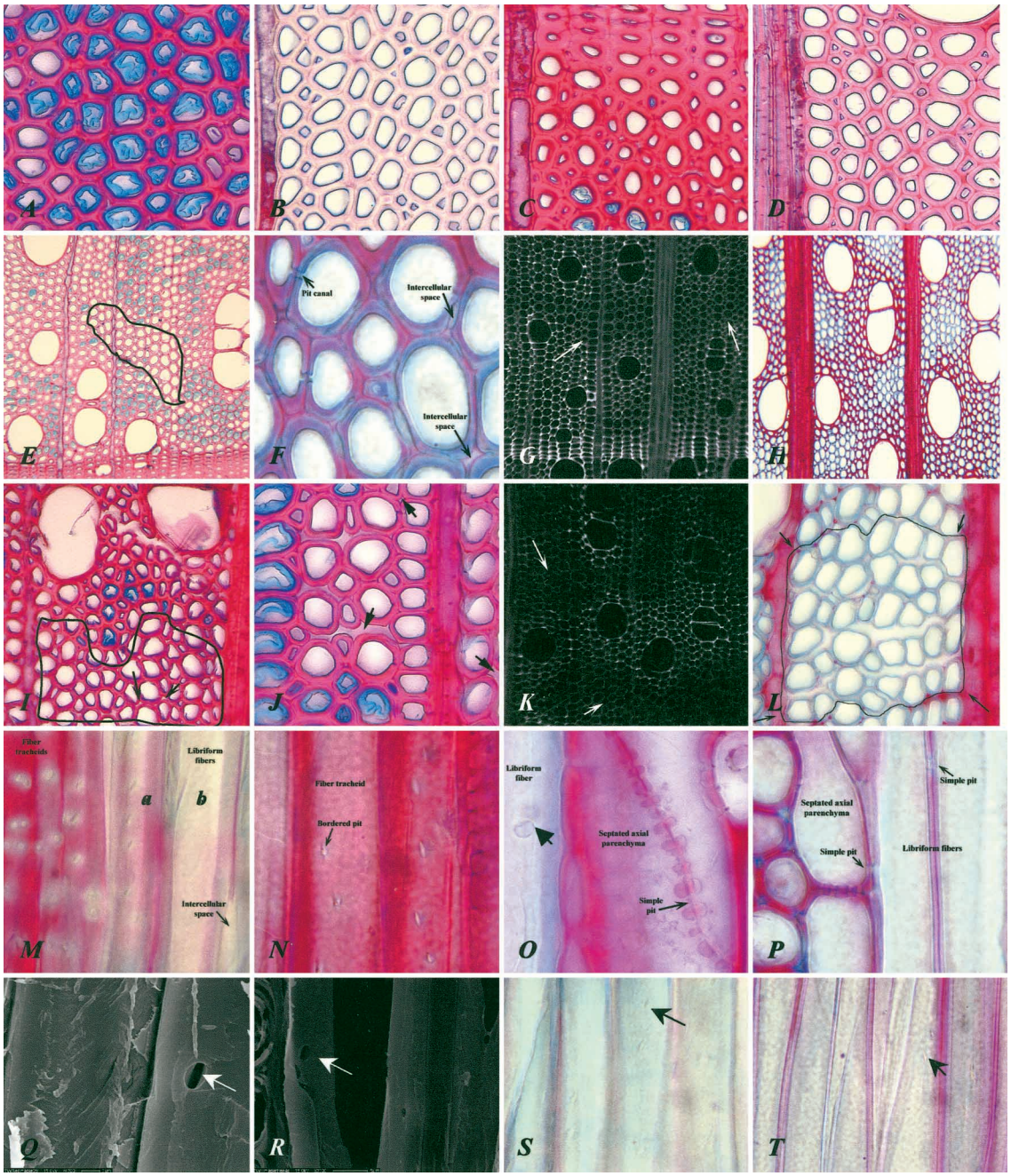

FIG. 1. Micrographs A, I–L, M, O, Q, and S are tension wood sections and B is normal wood of red maple. Micrographs C, E–H, N, P, R, and T are tension wood sections and D is normal wood of sugar maple. A–L show that the histochemical reactions of tension and normal wood of red and sugar maple are different for each type of wood, with tension wood (A and C) staining red, normal wood (B and D) staining pink. Notice in E and I that the areas composed of libriform fibers have large lumens (circled areas). Observe in F and J that lignin is present in the cell corners (red points), and notice intercellular spaces among the libriform fibers. In G and K (unstained sections) dark areas are composed of libriform fibers (see arrows) in which fluorescence is not observed using UV light. H and L show libriform fibers with a positive reation (blue) for cellulose; the areas in red (lignin positive reaction) are composed

tion produces a different histochemical reaction than if the same stains are dissolved in ethyl alcohol solutions (Vazquez-Cooz and Meyer 2002). When the stains are dissolved in aqueous solution, the tension wood fibers of red and sugar maple become red, while the G-layer stains blue (see Fig. 1A, C). Conversely, normal wood stains homogeneously pink (see Fig. 1B and D). Srebotnik and Messner (1994) have shown that safranin-O and astra blue in aqueous solution stain highly lignified wood fibers pink, whereas less lignified fibers stain red.

The stain reactions shown in Fig. 1A and C demonstrate the accepted view of lignin distribution—high levels in normal cell walls and none in G-layers. However, when tension wood and normal wood sections were stained simultaneously to ensure that they were treated identically, the intensity of the stain reactions demonstrated that tension wood does not contain as much lignin in its secondary walls as does normal wood (Fig. 1A vs. B for red maple and Fig. 1C vs. D for sugar maple). When comparing Figs. 1A, B, C, and D, it is important to note that a pink shade denotes more lignin than does a red shade (von Ausfess 1973; Srebotnik and Messner 1994). G-layers were commonly observed in fiber tracheids of both species (see blue walls above the circled libriform fibers in Fig. 1I for red maple). In many cases, when G layers were present in fiber tracheids, they were not present in libriform fibers that were located immediately adjacent to the fiber tracheids (see Fig. 2E in Vazquez-Cooz and Meyer 2002).

Safranin and astra blue in aqueous solution demonstrate lignified walls (red) and G layers (blue) nicely (Fig. 1E). However, the secondary walls of some groups of fibers stain blue when using safranin-O and astra blue in ethyl alcohol solution, while the surrounding fibers stain red; the contrast is so remarkable and the colors so vivid that it allows us to localize the groups of cells very easily (see Fig. 1H, L). These groups of fibers have been identified as libriform fibers (stained blue), while the red-stained cells have been identified as fiber tracheids or parenchyma based on type of pitting, cell-wall thickness, lumen diameter, etc., observed on both cross sections and radial sections (Vazquez-Cooz and Meyer 2002).

Libriform fibers have simple pits with elliptical shape (see Fig. 1O, P, S and T). According to Carlquist (2001), libriform fibers are notable for the sparseness and small size of pits as compared to other tracheary elements such as fiber tracheids—typically, pit apertures in libriform fibers are narrow or slit-like (lenticular); however, an elliptical shape may be present, which represents an opening along the predominantly parallel microfibrils. In our maple samples, pit apertures of both types of fibers are elongated, but the ends of the libriform fiber pit apertures are more round than the more acute ends of the pit apertures of fiber tracheids, (Fig. 2C). It was also observed that the fiber tracheids of red and sugar maple have small bordered pits (2.5 μm diameter approximately; see Fig. 1M, N, Q, and R). Pit diameter less than 3.0 μm is used in the new IAWA list of characters for wood identification as a feature of libriform fibers (Wheeler et al. 1989), but at least for these two maple

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of fiber tracheids and parenchyma. M, N, Q, and R show fiber tracheids with bordered pits (arrows in Q and R). Notice in M two libriform fibers (a and b) with an intercellular space that is a common characteristic in both species of maple. O shows a libriform fiber with a simple pit (arrowhead) and a strand parenchyma cell with simple pits. Note the clear demarcation in the staining reaction of the two cells. Observe in P strand parenchyma with a simple pit-pair and two libriform fibers with a simple pit-pair between them. S and T show libriform fibers with simple pits (arrows) with elliptical shape. A, B, C, D, E, I and J are sections stained with safranin-O and astra blue in aqueous solution. F, H, L, M, N, O, P, S and T are sections stained with safranin-O and astra blue in ethyl alcohol solution. Q and R are SEM micgoraphs. (A 30×, B 60×, C 15×, D 70×, E 16×, F 210×, G 15×, H 18×, I–L 65×, M, N 195×, O, P, S, and T 280×, Q 395×, and R 305×).

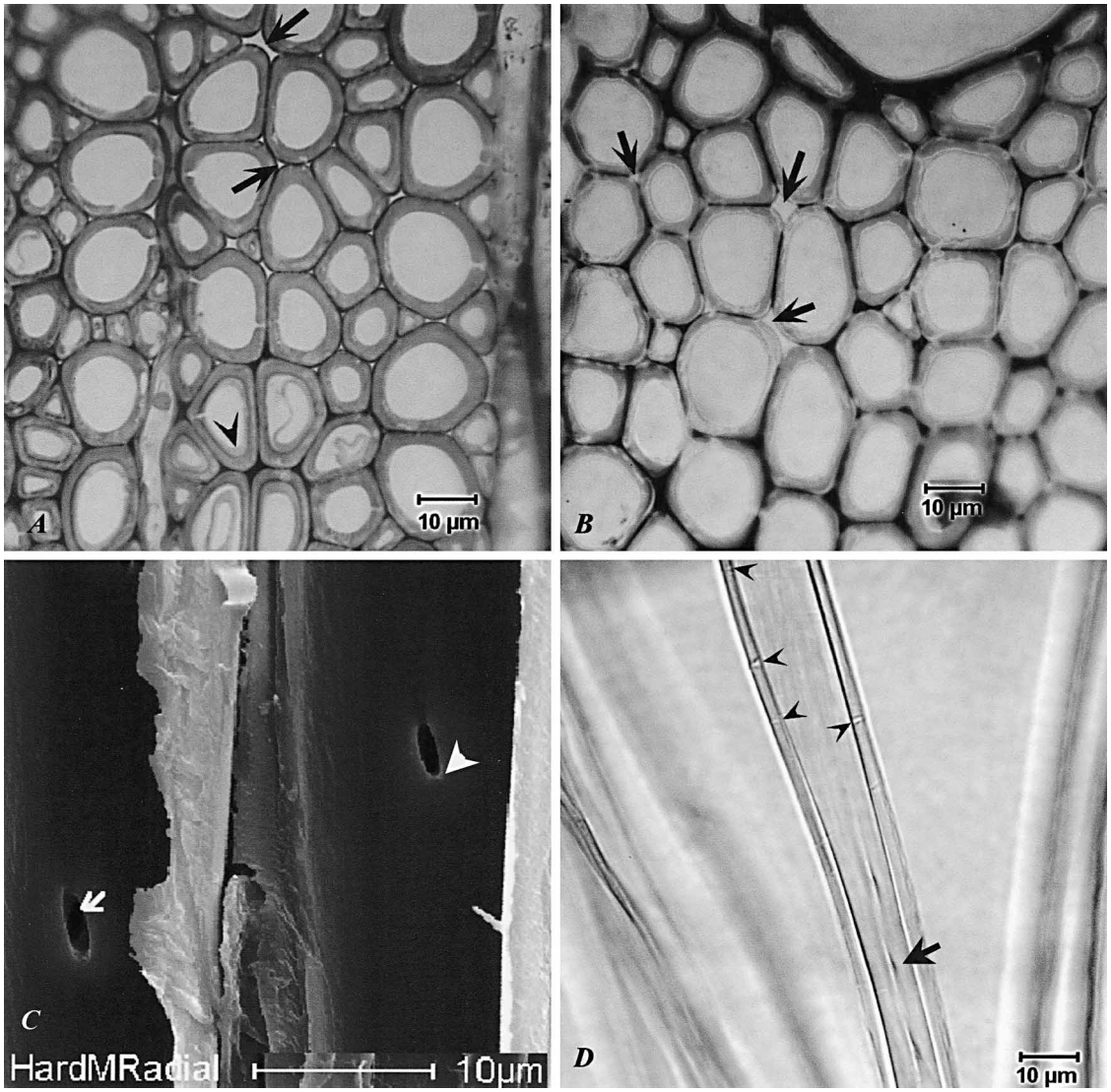

FIG. 2. Micrographs of tension (A, C, and D) and normal wood of sugar maple. A and B are thin transverse sections of embedded wood that demonstrate intercellular spaces (arrows) commonly present among libriform fibers. The arrowhead in A shows an intact (undisturbed) G-layer. C shows a fiber tracheid (left cell) and libriform fiber (right cell); the ends of the ellipitical pit aperture (arrow) of the fiber tracheid are more acute than the more rounded ends of the libriform fiber pit aperture (arrowhead). D shows a libriform fiber with simple pits (arrowhead) with apertures V-shaped toward the lumen and pits with ellipitical apertures (arrow).

species, since fiber tracheids have 2.5-µm diameter pits, the distinction does not apply. Pits in libriform fibers certainly were not rare (Fig. 2D) and, in fact, occurred on both radial and tangential walls (arrowheads vs. arrow in Fig. 2D) The arrowheads in Fig. 2D show libriform fiber simple pits that are V-shaped towards the lumen. Although both libriform fibers and axial parenchyma have simple pits, libriform fibers can be differentiated from strands of axial parenchyma because the parenchyma has cross walls, thinner longitudinal cell walls, and pa-

renchyma strands stain red while libriform fibers stain blue (Fig. 1O).

Libriform fiber lumen diameters were found to average 9.8 (C.I. ± 0.86) μm tangentially and 13.5 (C.I. ± 0.89) μm radially, while fiber tracheid lumen diameters were 8.6 (C.I. ± 0.69) μm tangentially by 11.3 (C.I. ± 0.88) μm radially. Over 100 fibers were measured on each cross section of normal wood and tension wood. Libriform fiber lumen diameters are significantly larger at the 95% confidence level, but wall thicknesses are similar for both types of fibers (Fig. 1, E and I, where the libriform fibers are encircled). The larger diameter of libriform fibers can be seen along the white line superimposed on Fig. 3A. In Fig. 3B, seven related cells (derived from the ''same'' cambial initial) are numbered. Fibers 1-4 are fiber tracheids and fibers 5-7 are libriform fibers. Note that since these fibers arose from the ''same'' cambial initial, they were sectioned at the same location along their length, so the difference in diameter is not due to some of the fibers being cut near their centers and others being cut near their tapering ends. Similar observations can be made in Fig. 1G, where areas that did not fluoresce as much are composed of larger-diameter libriform fibers. We have observed rows of libriform fibers several cells wide running radially for several growth rings. These wide rows seem to occupy the entire space between rays (see the pair of arrows in Fig. 4B).

Libriform fibers occur in groups in which there are intercellular spaces (Fig. 1F and J). These groups of cells range from scattered groups (Fig. 1H) to tangentially oriented bands (Fig. 1G, K).

Standard texts, such as Panshin and de Zeeuw (1980), report that sugar maple and red maple have both fiber tracheids and libriform fibers. However, they did not mention that the libriform fibers occur in distinctive groupings. When we examined old photomicrographs of maple cross sections in the files at ESF (some of which dated to the 1920s) and those in various publications, we were able to observe distinctive groupings of fibers based on lumen diameters and intercellular spaces (Table 1 and Fig. 4). We used the following terms to describe these patterns: interrupted wavy bands (Fig. 4A, B), wavy bands (Fig. 4C), large

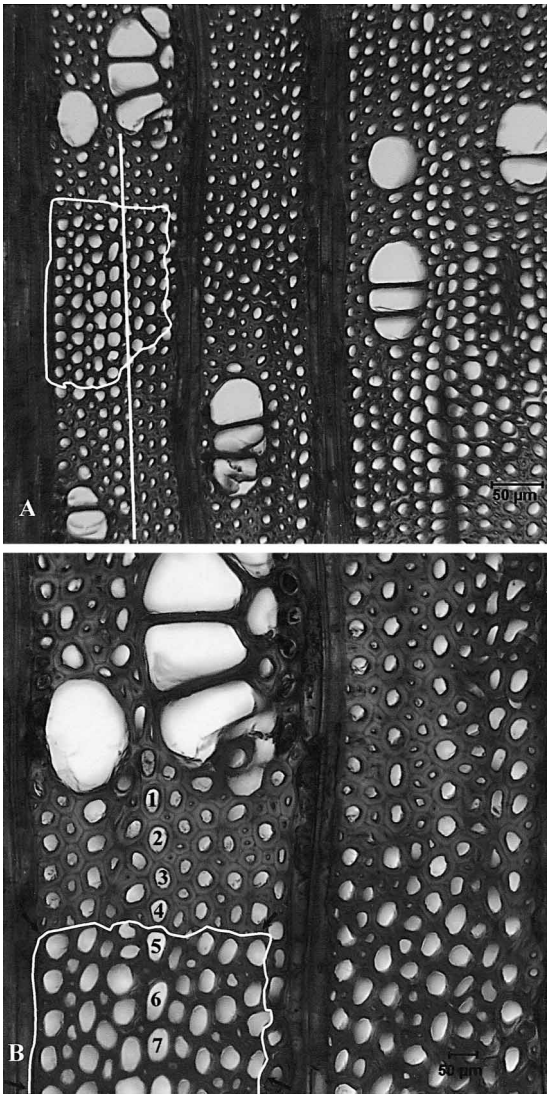

FIG. 3. Micrographs of tension wood of sugar maple stained with safranin and astra blue in aqueous solution. A shows a row of cells (white line) formed by the ''same'' initial. B shows an enlarged part of A pointing out part of the libriform fiber region (encircled area with arrows). Observe the difference in cell diameter and the differences in staining reaction (darker) in the libriform fiber regions (encircled area of B). Cells 1–4 are fiber tracheids; cells 5 to 7 are libriform fibers.

TABLE 1. *Distribution patterns for libriform fibers as observed in various Acer species.*

Species	Pattern	Source[a]
A. spp.	large groups	Miles 1978
A. caesium	large groups	File photo
A. campbellii	wide bands	File photo
A. campestris	large areas	Brown et al. 1932
A. carpinifolium	definite interrupted wavy bands	File photo
A. cultratum (A. pictum)	large groups	File photo
A. cultratum (A. pictum)	large groups	Pearson and Brown 1932
A. integrifolioxylon	large groups	Wheeler and Manchester 2002
A. kawakamii	interrupted wavy bands	Kanehira 1921
A. macrophyllum	large groups	Brown, 1928
A. morrisonense	large groups	Kanehira 1921
A. negundo	very large groups	1928 file photo by E.S. Harrar
A. nigrum	very large groups	1928 file photo by E.S. Harrar
A. oblongum	large groups	File photo (H.P. Brown n.d.) (see Fig. 2D)
A. oblongum	large groups	Pearson and Brown 1932
A. platanoides	interrupted wavy bands	File photo (H.P. Brown n.d.) (see Fig. 2C)
A. platanoides	large groups	Brown et al. 1932
A. pseudoplatanus	large groups	Kribs 1959
A. pseudoplatanus	large interrupted bands	Brown et al. 1932
A. pseudoplatanus	large interrupted bands	Brown and Berry 1941
A. rubrum	wavy bands	Brown 1928
A. saccharinum	large groups	File photo
A. saccharum	interrupted wavy bands	Brown 1928 (see Fig. 2B)
A. saccharum	interrupted wavy bands	File photo (Berry n.d.)
A. saccharum	interrupted wavy bands	E. Choong 1959
A. saccharum	large groups	Core et al. 1976, Fig 16c
A. saccharum	interrupted wavy bands	Harlow 1970
A. saccharum	large groups	Hernández and Rojas 2002
A. saccharum	interrupted wavy bands	Marra 1942
A. saccharum	interrupted wavy bands	Marra 1942
A. saccharum	interrupted wavy bands	Meyer 1963
A. tenuifolium	tangential and wavy bands	File photo (see Fig. 2F)
A. thomsoni	large groups	File photo

[a] Source of ESF file photo is given where known (name and date), see References for other publications.

groups (Fig. 4D), prominent wavy bands (Fig. 4E, F *arrow*), to nearly tangentially oriented bands (Fig. 4F) *arrowhead*). We have only been able to find one rather oblique reference to a grouping of fibers (Pearson and Brown 1932), in which, for the description of *Acer campbellii*, the authors stated that some fibers were ''. . . in the neighbourhood of the vessels and in the outer part of the wider rings thicker-walled and giving a patchwork appearance in cross section . . .'' It is interesting that groupings of variable lumen diameters is also evident in *A. integrifolioxylon* (Table 1), which is an extinct wood collected in Oregon (Wheeler and Manchester (2002).

Some of the photographic records at ESF that we examined included SEM micrographs. Cubes of wood that had been examined with the SEM again showed definite intercellular spaces around some of the fibers (e.g., see Fig. 16C in Core et al. 1976). We verified that intercellular spaces occur naturally in wood by examining thin sections (~1.9 nm thick) cut from Epon-embedded blocks (Fig. 2A and B). Intercellular spaces were common. These spaces vary in size from minute openings at the cell corners to larger openings essentially separating two cells (arrows in Fig. 2A and B). The spaces occur within the groups of libriform fibers. The occurrence of intercellular

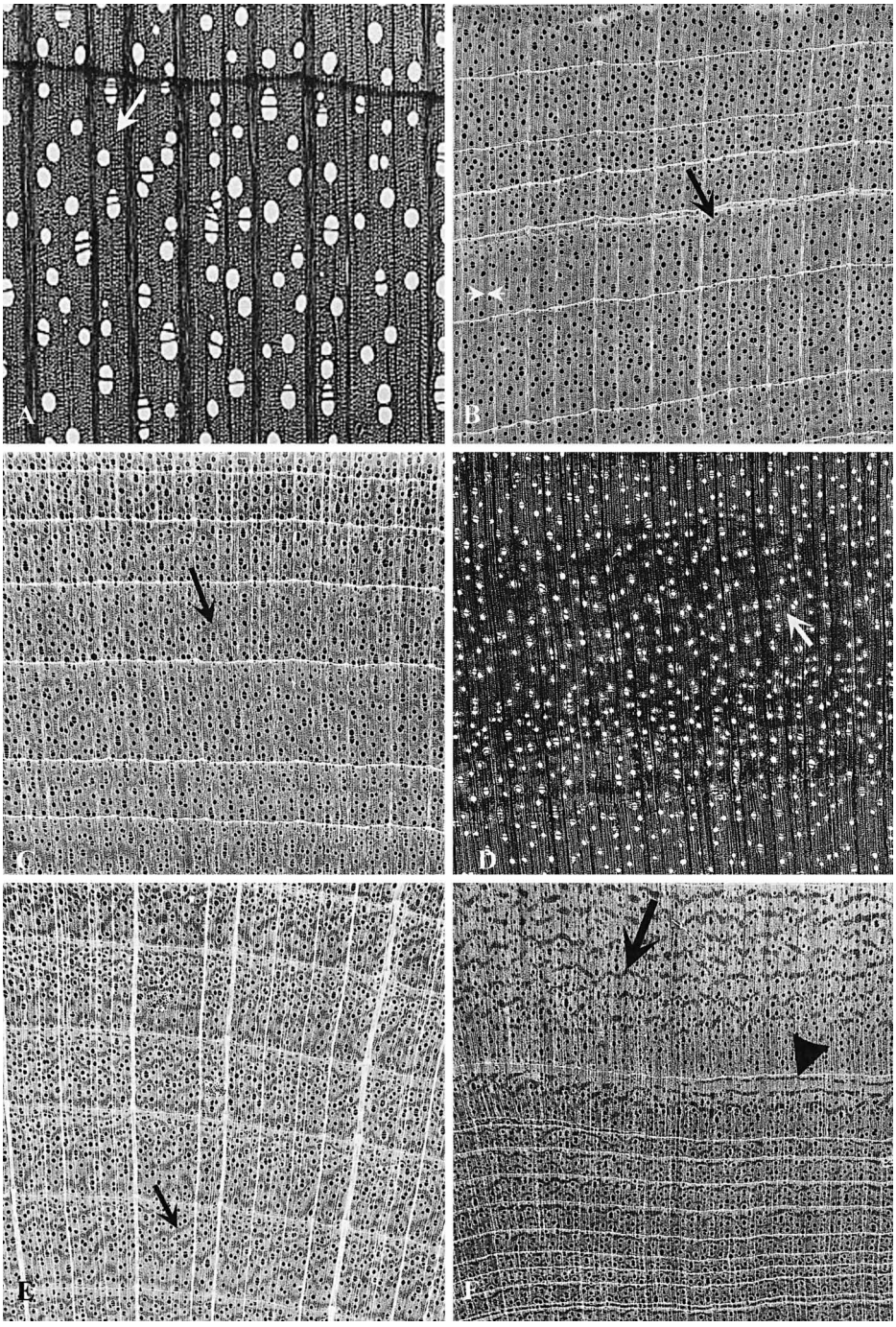

FIG. 4. Cross sections of various *Acer* species illustrating distribution and arrangement of libriform fibers. Arrows point out groupings of libriform fibers in each of the photomicrographs. A and B, *Acer saccharum*: libriform fibers arranged in interrupted wavy bands. B, the pair of white arrows locates a group of libriform fibers that runs in a radial line between a pair of rays. C, *A. pseudoplatanus*: libriform fibers in more definite wavy bands. D, *A. oblongun*: high concentration of libriform fibers through the central portion of the photomicrograph. E, *A. carpinifolium*: wavy bands are more prominent. F, *A. tenuifolium*: wavy bands are nearly horizontal and extend for long distances in the tangential direction, as shown by the large arrowhead (magnifications: A: 35×, B: 10×, C: unknown, D: 15×, E: 10×, F: 15×).

spaces adjacent to cell corners of libriform fibers, which are more round than are fiber tracheids, resembles the occurrence of intercellular spaces in cell corners of round coniferous compression wood tracheids or among the round tracheids of *Juniperus virginiana*. These intercellular spaces (Figs. 1F, J, L, 2A and B) are typically found in maple, although their existence has been nearly completely overlooked. Metcalfe and Chalk (1950) reported that fibers in *Acer* often have ''conspicuous intercellular spaces,'' but we have not been able to find any other published references to these conspicuous spaces. The minute spaces we observed in both embedded and unembedded sections have not been reported previously.

Some authors (Akachuku and Burley 1979; French 1923) have reported on the proportion of wood that is composed of fibers. French stated that red maple wood is composed of 68.1% fibers and sugar maple of 61.1%. However, the percentage of fibers varies not only between different species but also between different individuals of the same species, and even at different places in a tree (Taylor 1968). We did not estimate the proportion of libriform fibers in our samples of sugar and red maple. Examination of the photomicrographs shown in Fig. 4 and reported in Table 1 suggests that the proportion of libriform fibers varies within and between individual wood specimens as well as between rings of the same individual.

Hernández and Rojas (2002) reported that sugar maple is increasingly susceptible to crushing damage when planed by knives with increasingly larger jointed lands. When examining their photomicrographs, it appears that the crushed fibers are larger in diameter than surrounding fibers. These larger-diameter fibers appear to be libriform fibers. The larger lumens of libriform fibers would reduce specific gravity of wood in those areas where libriform fibers are located, which could lead to localized areas where wood properties may be affected, such as the failures that Hernández and Rojas (2002) observed.

Histochemistry

It is generally well accepted that wood in its natural state presents a solid heterogeneous lignin structure and a heterogeneous lignin distribution (Hori and Meshitsuka 2000). Schwarze et al. (2000) studied libriform fibers and fiber tracheids in *Quercus robur*. Libriform fiber lignins had a relatively high syringyl monomer content, as assessed by a strong reddish Mäule staining reaction and relatively short wavelength UV absorbance (maximum between 270 and 278 nm). Conversely, fiber tracheids had less syringyl lignin, as shown by their lighter red Mäule staining reaction, and a high guaiacyl monomer content, as shown by UV absorbance at a longer wavelength (maximum between 274 and 284 nm). Similar results were obtained by Yoshinaga et al. (1997) for *Q. mongolica* var. *grosseserrata*.

The presence of an unlignified, highly crystalline, cellulosic G layer in tension wood fibers goes without saying. Where the following discussion of lignin quantity and quality describes tension wood, consideration is restricted to lignins present in primary and secondary walls, not the G layer.

We conducted three experiments to demonstrate different types and amounts of lignin in libriform fibers compared to fiber tracheids and between normal wood compared to tension wood. First, sections stained with safranin and astra blue in ethyl alcohol solution produce a positive cellulose reaction in libriform fibers (blue color), but a lignin positive reaction in fiber tracheids and parenchyma cells (red color), but when the stains are applied in aqueous solution, a positive lignin reaction (red stain with safranin-O and no destaining with astra blue) is obtained in all types of cells. The aqueous-based stain results show that all cells have at least some lignin present and the ethanol-based differential stain distinguishes between different *types* of lignin (syringyl vs. guaiacyl).

Second, the Mäule reaction (Rawlings and Takahashi 1952; Nakano and Meshitsuka 1992), was used to distinguish between syr-

ingyl and guaiacyl *types* of lignin. Tension wood and normal wood stained with the Mäule reaction produced a definite contrast between normal wood and tension wood. We interpreted stain results according to Bland (1966) and Wardrop (1981). The G-layer, as expected, remained as transparent as in an unstained section. The secondary cell walls of tension wood of sugar and red maple contain less syringyl lignin than does normal wood because they stained light pink rather than red as did normal wood with the Mäule reaction.

Third, examination of unstained sections using UV illumination at 355-375 nm revealed that libriform fibers do not fluoresce, but fiber tracheids, longitudinal parenchyma and vessel elements do fluoresce (Fig. 1G, K).

Lignins contain subtle structural differences that may have an impact on fluorescence properties (Olmstead and Gray 1997). Lignin fluoresces when excited by UV light. Some studies have confirmed that a large number of potential fluorophores exist within the lignin structure (Lundquist et al. 1978). The observation that libriform fibers were not fluorescent in ultraviolet light at the wavelengths we used is of particular significance (Fig. 1G, K). Schwarze et al. (2000) found that the cell types in oak differ in their lignin composition—libriform fibers have a relatively high syringyl content, but in contrast, the fiber tracheids have a high guaiacyl content. In our experiments, we used an ultraviolet excitation filter (Nikon UV-lA) with an excitation wavelength at 355-375 and a maximum at 365 nm. Since fiber tracheids, which are high in guaiacyl lignin, fluoresce with this filter, but libriform fibers, which are low in guaiacyl lignin but high in syringyl lignin, do not fluoresce when examined with this filter, then this filter must excite some of the functional groups in guaiacyl lignin but not those in syringyl lignin. Bublitz (1981) demonstrated that fluorescence intensity was proportional to the quantity of lignin present in pulping liquors up to a concentration of 15 ppm. We observed fluorescence only in cell corners of some libriform fibers, so it appears that cell corners contain guaiacyl lignin; but the rest of the libriform fiber cell walls do not contain enough guaiacyl lignin to cause detectable fluorescence at 355-375 nm. Since parenchyma and fiber tracheid cell walls fluoresced uniformly, their cell walls contain a large amounts of guaiacyl lignin. The fluorescence results were confirmed by the histochemical reaction with safranin-O and astra blue in alcohol solution, in which the presence of a different type of lignin was identified in the cell corners, as shown by areas of red color (see Fig. 1F, L).

Bland (1966) studied sections of wood stained with the phloroglucinol reaction and lignin removed from various parts of trees. He found that while most of the lignin present in *Eucalyptus botryoides* is guaiacyl-syringyl, the type of lignin varied in different parts of the trees, and guaiacyl lignin was present in those parts where lignification is low. Srivastava (1966) found that the type of lignin and intensity of Maule and Wiesner color reactions varied between wood and bark, primary and secondary xylem, various types of cells, and even between cell-wall layer (middle lamella vs. secondary wall). Other authors have reported a sequential deposition of guaiacyl lignin in cell walls followed by syringyl lignin (Terashima et al. 1986; Saka and Goring 1988; Laigeng et al. 2001). Safranin/astra blue staining in aqueous solution indicates that tension wood is less well lignified than normal wood (Figs. 1A vs. B and 1C vs. D, where darker reds indicate less lignin). This implies that the less-well lignified tension wood may have a greater proportion of guaiacyl lignin, causing the ratio of guaiacyl to syringyl lignin in tension wood to be different than in normal wood.

There is also a gradation in lignin concentration across cell walls. Subtle differences in shade from pink to red indicate that lignin concentration is usually least next to the lumen. In Fig. 5, red shades photographed darker gray, indicative of less lignin closer to the cell lumens (note arrow in Fig. 5). Yoshizawa et al. (1993) found less absorbance in the inner

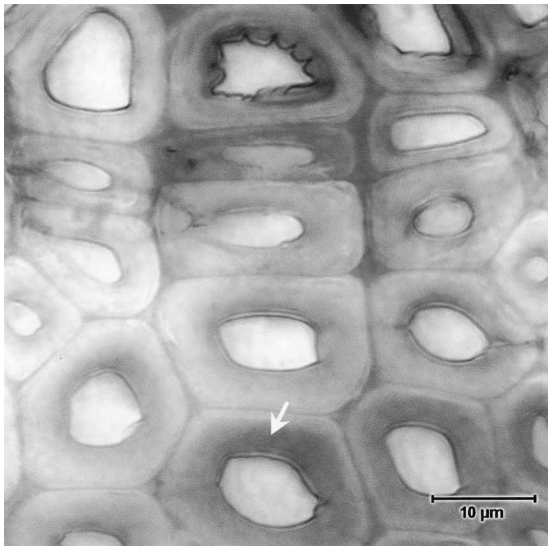

FIG. 5. Tension wood of red maple stained with safranin and astra blue in aqueous solution. The gradation in staining reaction across the cell walls demonstrates a lower concentration of lignin adjacent to cell lumens, where the cells stained darker red (e.g., arrow).

parts of cell walls next to lumens of fibers in *Betula*, substantiating our results.

CONCLUSIONS

Close examination of normal wood and tension wood of red and sugar maple revealed greater morphological and histochemical differences between libriform fibers and fiber tracheids than have been suggested by IAWA, which bases the distinction on pit characteristics. The differences we observed are based on differences in histochemistry (quantity and quality of lignin), cell morphology (cell shape and diameter), presence of intercellular spaces, and groupings of libriform fibers.

- The libriform fibers in general are larger in diameter than fiber tracheids and have a more round shape in contrast to the more rectangular fiber tracheids.
- As deduced from published and unpublished photomicrographs, libriform fibers occur in various patterns, ranging from small groups to interrupted wavy bands. In some cases, continuous, tangentially oriented bands occur.
- The wood of red and sugar maple contains areas of libriform fibers with intercellular spaces. These spaces are common and vary in size from minute openings at cell corners to separations between neighboring cells.
- The ends of the elliptical pit apertures of libriform fibers are more rounded than are the ends of the elliptical pit apertures of fiber tracheids, which are more acute. The libriform fiber pit apertures are V-shaped towards the lumen.
- Safranin-O and astra blue dissolved in ethyl alcohol is an effective differential staining method to identify and localize libriform fibers in red and sugar maple due to the blue stain that develops from astra blue reactions.
- Differences in fluorescence and safranin-O/astra blue differential staining indicate that libriform fibers do not contain as much lignin as fiber tracheids.
- The ratio of guaiacyl to syringyl lignin differs between libriform fibers and fiber tracheids; libriform fibers are lignified, but do not appear to contain guaiacyl lignin, except in their cell corners.
- Although libriform fibers and fiber tracheids are both considered to function as mechanical tissue, they are lignified differently, intercellular spaces are common among libriform fibers, and libriform fibers possess larger cell diameters than fiber tracheids.
- The lignified portions of secondary cell walls of tension wood (not considering the G-layer) do not contain as much lignin as the secondary cell walls of normal wood.

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