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DISTRIBUTION OF LIBRIFORM FIBERS AND PRESENCE OF SPIRAL THICKENINGS IN FIFTEEN SPECIES OF *ACER*

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SUMMARY

Fifteen *Acer* species were examined to study distribution and percentage by area of their libriform fibers. Safranin-O and astra blue dissolved in alcohol solution is an effective differential staining method to identify and localize libriform fibers. They have larger lumens than fiber tracheids, intercellular spaces, and occur in various patterns, ranging from large groups to wavy bands. In some cases they occur in radial files. The percentage by area of libriform fibers ranges from 14 to 40%. Inconspicuous to moderately visible spiral thickenings are part of the *Acer* libriform fibers and fiber tracheids. Differences in stain reactions and fluorescence indicate that libriform fibers differ in lignin concentration or composition from fiber tracheids – the concentration of syringyl lignin is greater in libriform fibers.

Key words*: Acer,* differential staining, fiber distribution, libriform fibers, fiber tracheids, maple, spiral thickening, lignification.

INTRODUCTION

Great efforts have been made to classify libriform fibers. In 1936, I.W. Bailey established the concept of imperforate tracheary elements in recognition of the difficulty of separating libriform fibers from tracheids and fiber tracheids. The occurrence and distribution of longitudinal parenchyma in wood are well documented (Panshin & De Zeeuw 1980); however, descriptive terms for distribution of libriform fibers have not been found, such as those for longitudinal parenchyma. Some authors (Carlquist 1986a and b; Baas 1986; Magendans 1999; Magendans & Van Veenendaal 1999) have studied occurrence and characteristics of the pits of the libriform fibers and fiber tracheids, but there are still controversies about the definition of libriform fibers. Magendans and Van Veenendaal (1999) stated that pits of adjacent libriform fibers are not in perfect alignment as are the pits of fiber tracheid pit-pairs.

Different techniques can be used to study and to identify libriform fibers in microtome sections. Double staining and the use of fluorescence are two methods. These methodologies have been proven and have been explained in our previous work (Vazquez-Cooz & Meyer 2004). Wood fluorescence is well known but is not widely used as a technique for wood identification. Miller (1981) selected fluorescence as an important

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criterion in the computer-assisted identification of timbers and Quirk (1983) also used the fluorescence of aqueous and ethanol extracts for computer-assisted identification of Leguminosae.

The purpose of this study is to report on the distribution patterns and the percentage of libriform fibers in 15 species of *Acer* and to contribute to the classification of libriform fibers based on differences other than pit characteristics. This work is a continuation of previous studies (Vazquez-Cooz & Meyer 2002 and 2004). The uses of the safranin-O-astra blue differential staining method, UV illumination technique, and polarized light all made it possible to distinguish between libriform fibers and other types of cells. We observed differences in histochemistry (lignin content), cell morphology (diameter and wall thickness), presence of intercellular spaces, and distribution of libriform fibers with respect to fiber tracheids within microtome sections.

MATERIALS AND METHODS

Blocks from several trees of *Acer rubrum* and *Acer saccharum* were observed in our previous research (Vazquez-Cooz & Meyer 2004). For this study, we extended the previous work by examining additional species. Fifteen *Acer* species (*A. barbatum*, *A. circinatum, A. floridanum, A. glabrum, A. grandidentatum, A. macrophyllum, A. negundo, A. nigrum, A. oblongum, A. pennsylvanicum, A. platanoides, A. pseudoplatanus, A. rubrum, A. saccharinum,* and *A. saccharum,*) were selected from the H.P. Brown Memorial Wood Collection to study the distribution of libriform fibers (Table 1).

Table 1. Distribution patterns and percentage of libriform fibers as observed in various *Acer* species.

Species	S-number[a]	Pattern	% Libriform fibers by area
A. barbatum	8420	interrupted wavy bands	37
A. circinatum	8620	wavy bands and radial files	40
A. floridanum	8333	interrupted wavy bands	26
A. glabrum	8246	interrupted wavy bands	24
A. grandidentatum	8533	large groups	37
A. macrophyllum	8521	large groups	26
A. negundo	8461	very large groups	14
A. nigrum	8157	very large groups	20
A. oblongum	15176	large groups	23
A. pennsylvanicum	8563	large groups and radial files	21
A. platanoides	10971	large groups	26
A. pseudoplatanus	31064	interrupted wavy bands	33
A. rubrum	8277	wavy bands	17
A. saccharinum	8596	interrupted wavy bands	23
A. saccharum	8003	interrupted wavy bands	17

[a] H.P. Brown Memorial Wood Collection accession number.
All samples from the USA except *A. oblongum* from Taiwan, *A. platanoides* from England, and *A. pseudoplatanus* from Belgium.

Small, 15-mm cube, wood blocks for anatomical and histochemical analyses were cut. The blocks were placed in vials with distilled water and then put into a bell jar to be evacuated for 3 hours until they were water soaked. Transverse sections (20 μm thick) were cut with a sliding microtome, and stained by the safranin-O and astra blue technique (Vazquez-Cooz & Meyer 2002). After ethyl alcohol dehydration, permanent slides 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 also observed using polarized light and fluorescence. For fluorescence, a Nikon UV-1A filter block was used, with 355–375 nm excitation (max. 365 nm), and barrier filter at 400 nm.

The differential staining formula that separates libriform fibers from the surrounding tissues (parenchyma and fiber tracheids) was used to stain the above *Acer* species to prove its efficacy in identifying libriform fibers and to study their distribution. All sections were stained with 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 1% safranin for 120 minutes they were rinsed quickly three times in 85% ethyl alcohol over a period of 5–10 seconds, which leached some of the dye. Then the samples were stained with 1% astra blue for 180 seconds 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 media.

Small unstained blocks were submerged in 2-methylbutane for 24 hours and air dried for 48 hours to prevent cells from collapsing. Then the blocks were mounted on aluminum stubs, and sputter-coated with gold-palladium. A Jeol-5800 scanning electron microscope (SEM) was used to observe the samples. The blocks were photographed digitally and the images processed using Adobe Photoshop Version 6.0 and Image-Pro Plus Version 5.0. Some areas of libriform fibers were macerated for 2 hours at 50 °C with a 50–50 solution of 30 percent hydrogen peroxide and glacial acetic acid. The fibers were then washed with distilled water for 24 hours, and a few dried fibers were stained for approximately one minute using chlorazol black E at 1 percent. The fibers were rinsed with distilled water and then with absolute ethyl alcohol (three times). They remained in absolute ethyl alcohol for a few minutes until they were cleared with xylene (three times), and permanent slides were mounted using Histoclad media No 3920.

RESULTS AND DISCUSSION

Double staining of maple sections with safranin-O and astra blue in aqueous solution produces a different histochemical reaction than if the same stains are dissolved in ethyl alcohol solutions (Vazquez-Cooz & Meyer 2004). 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). Double staining of maple with safranin and astra blue in ethyl alcohol solution can be used to visualize the distribution of libriform fibers and subsequently to estimate the percentage area

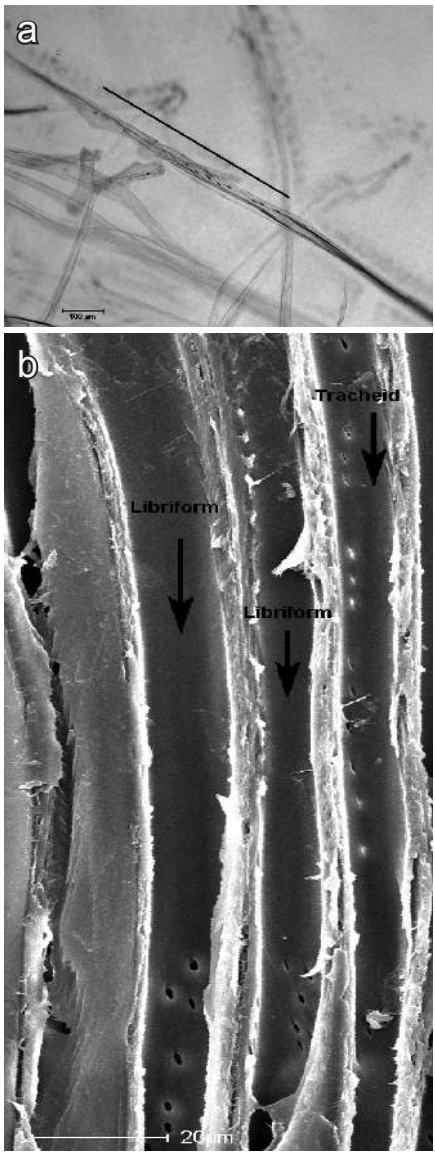

Fig. 1. Micrograph **a** shows a libriform fiber of *Acer platanoides* and **b** shows two libriform fibers and a tracheid of *Acer saccharum*. Micrograph **a** shows the concentration of pits toward the center of the libriform fiber (see line), while **b** shows the concentration of pits of the fiber tracheid approximately along the fiber length.

of libriform fibers on transverse sections. In previous studies, it was determined that in red maple and in sugar maple the groups of fibers that stained blue with this method were libriform fibers while the red-stained cells were identified as fiber tracheids and/or parenchyma (Vazquez-Cooz & Meyer 2002). These findings motivated the authors to study the distribution of libriform fibers in other *Acer* species.

Libriform fibers in *Acer* are non-septate and are differentiated from strands of axial parenchyma which are septate and are surrounded by their own secondary walls.

Reichenbach (2004) reported that the order *Myrtales* possesses septate libriform fibers but in *Acer*, which is included in the *Sapindales* order (Thorne 1992), we did not observe septate libriform fibers. In the species of *Acer* studied here, libriform fibers have simple pits with elliptical shapes. The pits are predominantly concentrated towards the centers of the libriform fibers, while in the fiber tracheids the pits are distributed approximately along the fiber length (Fig. 1). This pit distribution contrasts with the prevalent opinion that the pits in the conductive elements (tracheids) of conifers are concentrated toward the ends of the elements. According to Carlquist (2001), libriform fibers are notable for sparsity and small pit sizes as compared to other tracheary elements such as fiber tracheids; this agrees with our findings, since we counted only 14 pits in some libriform fibers. Usually, pit apertures of libriform fibers observed in this study were narrow with round ends (Fig. 2).

Controversy about how to differentiate libriform fibers from fiber tracheids based on pit morphology probably will continue for years. The trouble rests on the fact that in some species (for instance, *Ormosia paramensis*) the pits of libriform fibers are

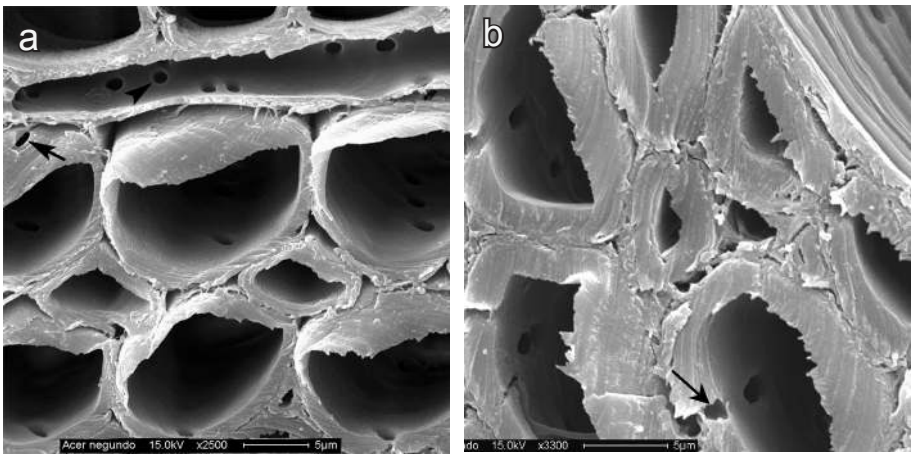

Fig. 2. Micrographs of *Acer negundo* (a & b) revealing narrow pit apertures of libriform fibers with round ends (arrow) in comparison with round-shaped simple pits in a ray parenchyma cell (arrowhead). Fig. 2b shows a section through a simple pit-pair (arrow).

minutely bordered (Baas 1986). Now, as a result of our study on *Acer*, the solution to this problem has been eased with the observance of the lack of fluorescence of the libriform fibers, the blue staining reaction that occurs with safranin-O and astra blue in alcohol solution, and the presence of intercellular spaces among libriform fibers. These characteristics were observed consistently in the 15 species studied. However, for the purpose of this study, we followed the definition of libriform fibers given by IAWA (IAWA Committee 1989). This definition points out that libriform fibers have simple pits or bordered pits with chambers less than 3 µm in diameter.

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 and have spiral thickenings. We used rather general terms to describe these patterns, ranging from large groups through interrupted wavy bands (see Table 1). Horáček *et al*. (2003) found that in *Quercus robur* libriform fibers formed radial strips. Files of large-diameter libriform fibers may be seen in Figure 3. Pearson and Brown (1932), in their description of *Acer campbellii*, stated that some fibers with thick walls occur in

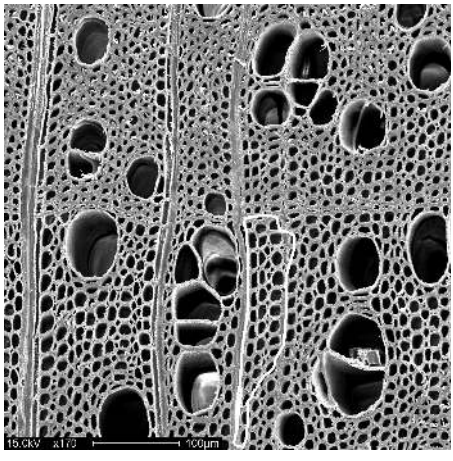

Fig. 3. SEM micrograph of *Acer pennsylvanicum* showing files of large-diameter libriform fibers (see encircled area).

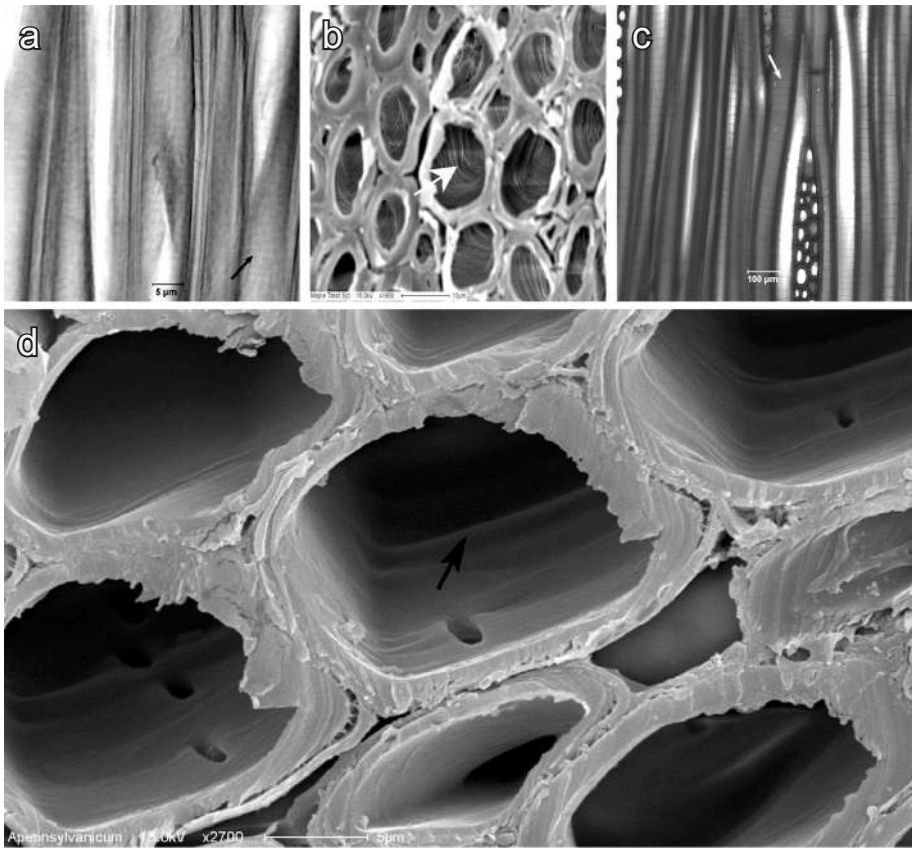

Fig. 4. Micrographs of *Acer saccharum* (a & b), *Ilex opaca* (c) and *Acer pennsylvanicum* (d) showing spiral thickenings in the fibers. Notice how prominent the spiral thickenings are in *I. opaca* (c, arrow) in comparison with *A. saccharum* where they are inconspicuous (a, arrow); however, using SEM (b & d), the spiral thickenings are more evident (arrows).

patchwork-appearing groups in transverse section. We believe that this patchwork appearance was due to groups of libriform fibers.

Part of the libriform fibers and fiber tracheids were found to have spiral thickenings that are not easily visible using a light microscope if we compare them with those spiral thickenings in *Ilex opaca* (Fig. 4a & c), but the spiral thickenings are visible using SEM (Fig. 4b & d). They are much less visible than in vessel elements. In addition, all of the species studied have libriform fibers with intercellular spaces (Fig. 5), and were always in groups, ranging from scattered groups to wavy bands (Table 1). The larger lumens of the libriform fibers form a pronounced pattern on photomicrographs (Fig. 6). The larger lumens also would reduce specific gravity, which should have a negative impact on wood strength. Damaged libriform fibers were commonly observed as an artefact of microtomy.

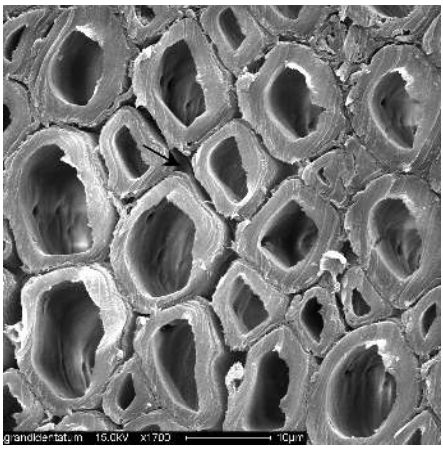

Fig. 5. SEM micrograph of *Acer grandidentatum* showing prominent intercellular spaces (arrow).

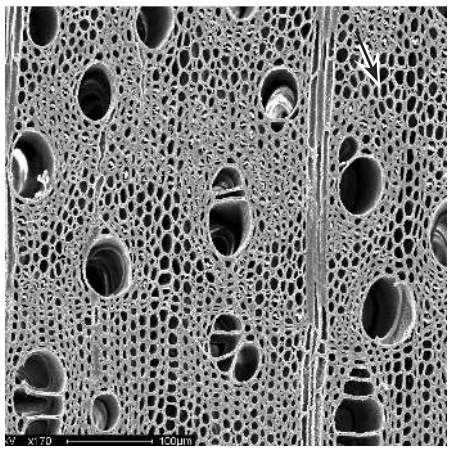

Fig. 6. SEM micrograph of *Acer circinatum* showing libriform fibers with larger lumens that make them stand out (arrow).

Kreitzberg *et al*. (1976) found interesting results regarding lignin skeletons of libriform fibers of birch. Their study indicated that the lignin of the S_2 layer in libriform fibers is more friable and the fibers are less lignified than the vessels. This fact could help explain our observations that libriform fibers of maple in the microtomed sections are damaged easily. Meier (1955) also found that in birch the secondary cell walls of the libriform fibers had less lignin than other wood elements.

In the present study we found that, when all species studied were stained with safranin and astra blue in ethyl alcohol solution, a positive cellulose reaction (lignin negative) occurred in libriform fibers, although some cell corners were lignin positive, and a lignin positive reaction occurred in fiber tracheids and parenchyma cells, confirming our past results (Vazquez-Cooz & Meyer 2004). Baas (1986), in his proposal for descriptive conventions of fiber terminology, pointed out that pits in libriform fibers are more common on the radial walls, as was observed in the species of maple studied here (Fig. 7).

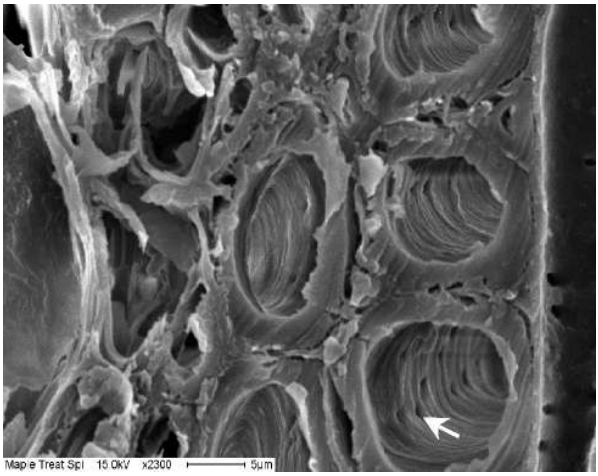

Fig. 7. SEM micrograph of *Acer saccharum* showing libriform fibers with simple pits which are more common on the radial walls (arrow).

Also, it was observed that in these fibers the pits are concentrated toward the center of the fiber (Fig. 1).

Lignin emits photon energy in the form of autofluorescence when excited by UV light, showing a maximum emission at 358 nm (Lundquist *et al*. 1978). Since cellulose does not fluoresce, the emission observed is caused by lignin and possibly by extractives (Tylli, pers. comm. 2000). For example, extractives such as flavonoids produce a bright fluorescence under UV light. Due to lignin's fluorescence, UV absorbance analysis is an important tool to find possible differences in wood chemical composition (Yoshinaga *et al*. 1997). The utilization of ultraviolet light in the wavelengths we used is a useful tool to identify those areas in wood sections that differ in lignin concentration.

Lignins contain subtle structural differences that may have an impact on fluorescence properties (Olmstead & Gray 1997), and some studies have confirmed a large number of potential fluorophores that exist within the lignin structure, such as carboxyl groups (Lundquist *et al*. 1978). The observation that libriform fibers are not fluorescent in ultraviolet light is of particular importance (Fig. 8). We found in the literature that

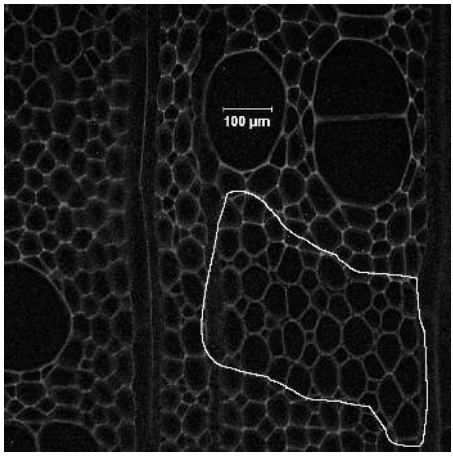

Fig. 8. Micrograph of *Acer circinatum* (unstained section) showing areas of libriform fibers (encircled area) in which fluorescence is only observed in some cell corners.

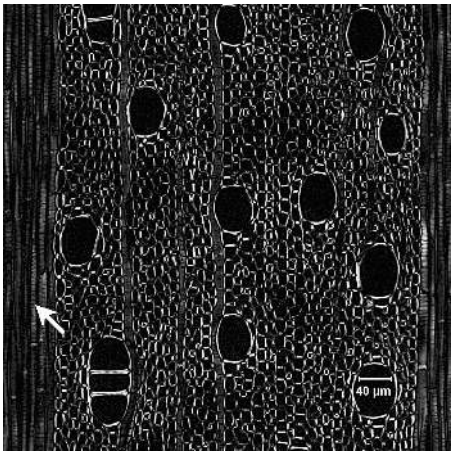

Fig. 9. *Acer barbatum* photographed with polarized light showing darker areas of libriform fibers and rays with prominent pit pattern (arrow).

in oak (*Quercus mongolica*) the proportion of syringyl lignins tends to increase in cells that are farther away from the vessel elements (Yoshinaga *et al*. 1997). Schwarze *et al*. (2000) indicated that the cell types in oak differ in their lignin composition – the libriform fibers have a relatively high syringyl content; in contrast, the fiber tracheids have a high guaiacyl content.

In general, a fluorescence microscope has a filter that only lets through radiation with a desired wavelength. In our case it was 355–375 nm, so this wavelength happened to match the fluorescing of vessels, fiber tracheids, and parenchyma cells but not the fluorescing of libriform fibers. As was mentioned above, some authors have found that syringyl lignin content is high in libriform fibers or increases in cells farther away from the vessel elements. Based on those results, the fluorescing areas observed in our experiments coincide with the cells that have a higher content of guaiacyl lignin. It appears that excitation at 355–375 nm excites some of the functional groups in guaiacyl lignin but not those in syringyl lignin. The histochemical reaction with safranin-O and astra blue confirms the presence of a different chromophore, possibly indicating a different type of lignin in the cell corners or the results indicate that the cell corners contain more lignin, as shown by areas of red color. Note that in Figure 8 the cell corners of libriform fibers fluoresce more intensely than the remainder of the fibers.

Another interesting observation was that areas of libriform fibers are darker in unstained sections observed using polarized light (Fig. 9, arrow), which suggests that microfibril angles are different between libriform fibers and fiber tracheids.

CONCLUSIONS

- Safranin-O and astra blue dissolved in ethyl alcohol is an effective differential staining method to identify and localize libriform fibers in the 15 *Acer* species studied here due to the blue stain that develops. This technique enables the distribution of the libriform fibers to be observed rapidly.
- The percentage of libriform fibers by area ranges from 14 to 40%.
- Libriform fibers in the species studied occur in various patterns, ranging from large groups to wavy bands and in some cases are oriented in radial files.
- All species of *Acer* studied contain areas of libriform fibers with intercellular spaces and pits concentrated toward the centers of the fibers.
- In *Acer*, not only vessel elements, but also libriform fibers and fiber tracheids have spiral thickenings, even though they are not readily visible with the light microscope.
- Differences in fluorescence suggest that libriform fibers have a greater proportion of syringyl lignin in comparison with fiber tracheids.
- Even polarized light confirmed that in *Acer* libriform fibers appear to be completely different from fiber tracheids, probably due to differences in microfibril angle. Polarized light also makes it possible to observe prominent pit patterns in ray parenchyma cells.
- Libriform fibers are easily damaged, due to their large lumens and different lignin composition.

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