FUNDAMENTAL DIFFERENCES BETWEEN TWO FIBER TYPES IN ACER

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SUMMARY

Our earlier studies on Acer have shown morphological and chemical differences between two fiber types. Our new research applies the soft-rot cavity method using the soft-rot fungus Chaetomium globosum to measure the microfibril angle (MFA) in these two fiber types in fifteen species of Acer (maple). Microfibril angles in fiber type 1 were significantly larger than those in fiber type 2. The greatest difference (11.6°) was noted in radial sections of Acer floridanum, in which the average MFA (n =100) obtained from soft-rot cavities for fiber type 1 was 23.3 degrees (95 % C.I. ±1.4°) and for fiber type 2 11.7 degrees (95 % C.I. ±1.2°). The second greatest difference (10.0°) was in Acer nigrum, where the average MFA for type 1 was 16.4 degrees (95 % C.I. ±1.07°) and for type 2 6.4 degrees (95 % C.I. ± 0.64°). When transverse sections were examined with polarized light, areas of fiber type 1 were darker (less birefringent) than those of type 2, which indicates that the MFA of the two cell types differs. After four weeks of exposure to C. globosum the fibers of type 1 were more intensely attacked by the fungus than were the fibers of type 2, suggesting a difference in cell wall chemistry. Fluorescence spectra of the two types of fibers support that observation. Differences in MFA supplement the differences in morphology and chemistry and demonstrate that for Acer species the two fiber types are fundamentally different. These two types of fiber differ in the distribution of their pits. In type 1 the pits are concentrated mainly toward the center of the fiber while in type 2 the pits are distributed along the fiber length.

Key words: Acer, wood anatomy, wood chemistry, Chaetomium globosum, fiber dimorphism, maple, microfibril angle, soft-rot cavity.

INTRODUCTION

Cellulose in the cell wall is arranged in strands known as microfibrils. Orientation of the microfibrils, especially in the S2 layer (greatest proportion of the wall thickness), affects physical and mechanical properties of wood. The term microfibril angle (MFA) refers to the angle between the direction of the helical windings of cellulose microfibrils in the secondary cell wall (S2) and the cell’s longitudinal axis (Barnett & Bonham 2004). Orientation of microfibrils is critical. Usually, the smaller the MFA, the
better the mechanical properties of wood and wood products. For instance, MFA is a determinant for paper strength in *Pinus taeda*, where it affects tensile strength, stretch, modulus of elasticity, stiffness, and hygroexpansivity in unrefined and refined pulps (Courchene et al. 2006). Microfibril angle is directly related to longitudinal shrinkage of softwoods (Harris & Meylan 1965; Preston 1974; Megraw 1985).

The formation of cavities by soft-rot fungi aligned along the microfibrils is an accurate method to measure microfibril angle [Anagnost et al. 2000, who reported that results using the method are closely correlated (0.97) to the X-ray diffraction method and to the iodine method (0.94)]. The soft-rot method provides the facility to measure microfibril angles of any magnitude and to distinguish the $S_2$ layer in thin-walled cells. Because fibers are spindle-shaped, measurement of the orientation of soft-rot cavities in longitudinal sections must be made in the portion of the wall lying over the lumen midway between the two ends (Anagnost et al. 2005).

One of the fungi used in the soft-rot method is *Chaetomium globosum*. This fungus causes cavities in the cell wall in only 2–4 weeks. The cavities are relatively long and can be visualized rapidly with Nomarski DIC microscopy (Anagnost et al. 1994). As a word of caution, *C. globosum* can produce skin and nail infections in humans (Serena et al. 2003).

This research is a continuation of a study on *Acer*, in which morphological and chemical differences in the fibers were found (Vazquez-Cooz & Meyer 2004a, 2006). The objectives of this investigation were to measure the MFA in the two fiber types using the soft-rot method and to analyze the fiber’s UV spectra and fluorescence intensity. In our earlier paper we used the terms libriform fiber and fiber-tracheid for what are called fiber types 1 and 2, respectively here (see Discussion).

**MATERIALS AND METHODS**


Variation of microfibril angle between fiber types 1 and 2 of the fifteen *Acer* species was studied using the Anagnost method (Anagnost et al. 2000). Soft-rot decay was induced using the slide culture method described by Anagnost et al. (lit. cit.), which allows the observation of soft-rot development *in vivo*. Slide cultures were prepared by autoclaving separate glass microscope slides, microtomed radial sections of wood (20 μm thick), reduced salts nutrient solutions (RSNS), cotton balls, and cover slips at 121 °C for 15 minutes at 15 psi. Slides for culturing were assembled by placing the radial sections on the slides, soaking them in RSNS and placing them in a plastic Petri dish.
Table 1. Average microfibril angle in degrees, standard deviation, 95% confidence interval, and ANOVA P-values for fiber types 1 and 2 of fifteen *Acer* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>S-number*</th>
<th>Fiber type 2</th>
<th>Fiber type 1</th>
<th>Difference in Avg. MFA (Fiber type 1 – type 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Avg. (n=100)</td>
<td>St. dev. C.I.</td>
<td>Avg. (n=100)</td>
</tr>
<tr>
<td><em>A. barbatum</em></td>
<td>8420</td>
<td>15.1</td>
<td>5.4 ± 1.07</td>
<td>22.3</td>
</tr>
<tr>
<td><em>A. circinatum</em></td>
<td>8620</td>
<td>22.8</td>
<td>6.0 ± 1.20</td>
<td>30.2</td>
</tr>
<tr>
<td><em>A. floridanum</em></td>
<td>8333</td>
<td>11.7</td>
<td>6.0 ± 1.20</td>
<td>23.3</td>
</tr>
<tr>
<td><em>A. glabrum</em></td>
<td>8246</td>
<td>30.6</td>
<td>5.0 ± 1.00</td>
<td>34.5</td>
</tr>
<tr>
<td><em>A. grandidentatum</em></td>
<td>8533</td>
<td>21.6</td>
<td>5.0 ± 1.00</td>
<td>29.8</td>
</tr>
<tr>
<td><em>A. macrophyllum</em></td>
<td>8521</td>
<td>24.3</td>
<td>5.0 ± 1.00</td>
<td>27.1</td>
</tr>
<tr>
<td><em>A. nigrundo</em></td>
<td>8461</td>
<td>16.0</td>
<td>5.3 ± 1.05</td>
<td>24.2</td>
</tr>
<tr>
<td><em>A. nigrum</em></td>
<td>8157</td>
<td>16.4</td>
<td>3.2 ± 0.64</td>
<td>16.4</td>
</tr>
<tr>
<td><em>A. oblongum</em></td>
<td>15176</td>
<td>25.0</td>
<td>5.3 ± 1.06</td>
<td>27.9</td>
</tr>
<tr>
<td><em>A. pennsylvanicum</em></td>
<td>8563</td>
<td>14.9</td>
<td>5.0 ± 1.00</td>
<td>20.3</td>
</tr>
<tr>
<td><em>A. platanoides</em></td>
<td>10971</td>
<td>32.6</td>
<td>4.9 ± 0.98</td>
<td>35.4</td>
</tr>
<tr>
<td><em>A. pseudoplatanus</em></td>
<td>31064</td>
<td>11.8</td>
<td>4.0 ± 0.80</td>
<td>17.2</td>
</tr>
<tr>
<td><em>A. rubrum</em></td>
<td>8277</td>
<td>13.7</td>
<td>4.6 ± 0.91</td>
<td>20.0</td>
</tr>
<tr>
<td><em>A. saccharinum</em></td>
<td>8596</td>
<td>19.7</td>
<td>5.1 ± 1.00</td>
<td>25.6</td>
</tr>
<tr>
<td><em>A. saccharum</em></td>
<td>8003</td>
<td>17.4</td>
<td>6.4 ± 1.28</td>
<td>24.3</td>
</tr>
</tbody>
</table>

*H.P. Brown Memorial Wood Collection accession number (State University of New York, College of Environmental Science and Forestry). All samples from the USA except *A. oblongum* from Taiwan, *A. platanoides* from England, and *A. pseudoplatanus* from Belgium.
The RSNS consisted of 1.5 g NH₄NO₃, 2.5 g KH₂PO₄, 2 g K₂HPO₄, 1 g MgSO₄·7H₂O, and 2.5 g glucose per liter of water (Worrall et al. 1991). Inoculum of Chaetomium globosum S61 (Fig. 1) from a two-week-old culture (2% malt extract agar) was placed adjacent to the cover slip in contact with the wood section to induce decay, and a small ball of cotton soaked in RSNS was placed touching the edge of the section to keep it moist. Additional sterile RSNS was added when needed. The Petri dishes were sealed with parafilm and incubated at 26 °C for three to five weeks. The sections were mounted in lactophenol and observed with a Nikon Eclipse E600W microscope with Nomarski Differential Interference Contrast (DIC) optics.

![Figure 1. Spores and hyphae of the soft-rot-fungus Chaetomium globosum.](image_url)

**Figure 1.** Spores and hyphae of the soft-rot-fungus Chaetomium globosum.

![Figure 2. Radial-longitudinal sections of Acer platanoides showing soft-rot cavities. Microfibril angle was measured by drawing a line parallel to the portion of each soft-rot cavity that was equidistant from the two tangential cell walls of the fibers. A shows the outside face of type 1 fibers (arrows), and B shows the lumen face of type 2 fibers (arrow). The angle is approximately 39° in type 1, and 26° in type 2 fibers.](image_url)

**Figure 2.** Radial-longitudinal sections of Acer platanoides showing soft-rot cavities. Microfibril angle was measured by drawing a line parallel to the portion of each soft-rot cavity that was equidistant from the two tangential cell walls of the fibers. A shows the outside face of type 1 fibers (arrows), and B shows the lumen face of type 2 fibers (arrow). The angle is approximately 39° in type 1, and 26° in type 2 fibers.
**Microfibril angle measurements using soft-rot cavity orientation**

Soft-rot cavities were observed in unstained sections and microfibril angles were measured using the image analysis software Image-Pro Plus 5.0. The longitudinal axis of each fiber was aligned to a $0^\circ$ reference line using a microscope with a rotating stage. Microfibril angles were determined by observing the difference between the cell’s longitudinal axis and the central axis of the soft-rot cavity (Anagnost et al. 2000). The angle was measured on digital images by drawing a line parallel to the portion of each soft-rot cavity which was equidistant from the cell’s two radial walls as shown in Figure 2. Three sections were examined for each species.

To avoid any effect of cell wall curvature on the microfibril angle, only those cavities that extended across the midpoint of the cells were measured, as in Figure 2. On the radial longitudinal sections fibers of type 2 were differentiated from type 1 fibers by using morphological characteristics, such as the distribution of pits and shape of the pit apertures. In fibers of type 2 the pits are distributed along the cell length, whereas in the type 1 fibers they are concentrated toward the median part of the fiber (Vazquez-Cooz & Meyer 2006). The type 2 fibers used to measure the microfibril angle were usually located near to or adjacent to vessel elements.

**Sample size determination and statistical analysis**

To determine sample size, preliminary measurements were performed on radial longitudinal sections of *Acer floridanum* and *A. nigrum* containing soft-rot cavities. The following formula was used to estimate the number of soft-rot cavities to be measured for each species: $n = [(z \times \text{STD})/m]^2$; where $z = 1.96$, $m$ is the desired margin of error for microfibril angle in degrees, and STD is the standard deviation of the microfibril angle. According to data obtained from these preliminary measurements, soft-rot cavities from a sample size of 100 fibers of each type were necessary to provide statistically significant results at a 95% confidence level (Table 1).

ANOVA was used for the analysis of the soft-rot cavity orientation measurements. $F$ statistics and $P$-values were used to test the null hypothesis of no microfibril angle differences between the two fiber types.

**UV spectra analysis**

Unstained transverse sections of *Acer rubrum* and *A. saccharum* were observed using a CRAIC QDI 2000 microspectrophotometer to analyze UV spectra (fluorescence intensity) of fibers at 355–375 nm excitation (max. at 365 nm). Images were captured with a high-resolution color digital image system coupled to the microspectrophotometer. Integrated spectral analysis/instrument control software acquired the fluorescence spectra from 4-μm-square areas of individual cell walls.

**Scanning electron microscopy (SEM)**

Unstained chips of *Acer saccharum* were inoculated with *Ceriporiopsis subvermispora* (white-rot fungus) and after four weeks the chips were oven-dried at 100°C ($\pm 2^\circ$C). The chips were then mounted on aluminum stubs and sputter-coated with gold-palladium. A JEOL-5800 scanning electron microscope (SEM) was used to observe the samples. The chips were photographed digitally and the images processed using Adobe Photoshop Version 8.0.
RESULTS AND DISCUSSION

Bailey and Vestal (1937) showed that the orientation of soft-rot cavities lie parallel to the cellulose microfibrils. Anagnost et al. (2000) demonstrated that the results obtained with this technique are comparable to those obtained using X-ray diffraction and/or iodine staining. In the present study, the results obtained for the microfibril angles in fifteen Acer species, using the orientation of soft-rot cavities, are summarized in Table 1.

On average, type 1 fibers had greater microfibril angles than did type 2 fibers. Figure 3 (A. barbatum) presents an example of this difference. The species with the greatest MFA differences were A. floridanum (11.6°), A. nigrum (10.0°), A. grandidentatum and A. negundo (8.2°). In A. nigrum patches of large cavities oriented almost parallel to the
cell axis and with angular edges were observed in areas of type 2 fibers, and in some cases, the $S_2$ layer was completely lost (Fig. 4). Species with smaller MFA differences were $A. glabrum$ ($3.9^\circ$), $A. oblongum$ ($2.9^\circ$), $A. macrophyllum$ and $A. platanoides$ ($2.8^\circ$). ANOVA showed that differences in MFA are statistically significant in all 15 species studied. The variation in MFA in type 2 fibers was generally less (standard deviations between 3.2 and 6.4) than in type 1 fibers (standard deviations between 4.0 and 8.0).

Type 1 fibers were attacked by the soft-rot fungus ($Chaetomium globosum$) more severely than were type 2 fibers (fewer soft-rot cavities present); see Figure 3. A similar situation was observed when $A. saccharum$ was exposed to Ceriporiopsis subvermispora (a white-rot fungus) for 4 weeks. The degradation in areas of type 1 fibers was considerable in comparison to areas of type 2 fibers (Vazquez-Cooz & Meyer 2004b, personal observations); see Figure 5.

![Figure 5. SEM micrographs of sugar maple ($Acer saccharum$) pulp chips. A shows an untreated chip, and B shows a chip after being exposed to the white-rot fungus Ceriporiopsis subvermispora for 4 weeks. Notice that the area of type 1 fibers (attacked area) is considerably degraded.](image)

The average microfibril angle for type 2 fibers (radial longitudinal sections) of $A. saccharum$ was approximately $17.4^\circ$ and for type $1$ $24.3^\circ$. This result contrasts with the values reported by Anagnost et al. (2005) for the same species; they reported an average microfibril angle of $8^\circ$; even though they also reported values between $10–19^\circ$ (groups with higher frequency distribution). This difference in MFAs could be due to variation within and/or between trees, to tree provenance (growing site) or to silvicultural practices (Addis et al. 1995). Anagnost et al. (2005) did not discriminate between the two types of fibers. In addition, in their study, the inoculation was performed in wood blocks, not in microtomed radial sections as was done in our study.
In a previous study (Vazquez-Cooz & Meyer 2006), we noticed that, when using polarized light to observe unstained transverse sections, areas of type 1 libriform fibers were darker than those of type 2 fibers when the microscope stage was rotated appropriately (Fig. 6). This difference suggested that microfibril angles were different between the two fiber types. The present results indicate a different MFA between these two types of fibers.

The fact that libriform fibers of type 1 have a greater microfibril angle should have an impact on mechanical properties of the wood, particularly in those species of Acer with large volumetric proportions of this fiber type. This may explain why when the wood is machined the areas containing type 1 fibers are easily damaged. For instance, shear failures parallel to the grain were observed in the region of type 1 libriform fibers in A. saccharum (Vazquez-Cooz 2003). Kreitzberg et al. (1976) found that the lignin of the S2 layer in libriform fibers is more friable and the fibers are less lignified than the vessels. Meier (1955) also found that in birch the secondary cell walls of the libriform fibers had less lignin than other wood elements. We observed that type 1 libriform fibers of Acer in the microtomed sections are damaged easily. This probably is caused by the fact that these libriform fibers are lignified differently than type 2 libriform fibers.

We know from histochemical analysis using the Vazquez-Meyer astra blue-safranin double-staining technique (Vazquez-Cooz & Meyer 2002) and from differences in fluorescence microscopy that libriform fibers of type 1 have different lignin chemistry in comparison with type 2 fibers (Vazquez-Cooz & Meyer 2004a). Lignin emits energy in the form of autofluorescence when illuminated by ultraviolet light, with a maximum emission at 358 nm (Lundquist et al. 1978). UV absorbance analysis is useful to find differences in wood chemical composition (Yoshinaga et al. 1997). Our spectra analyses within 4-μm-square areas of single cells of the two fiber types showed differences in fluorescence intensity, peak positions, and spectra shape (Fig. 7 & 8). Morphological differences allow the identification of the two types of fibers with a microscope. The spectral analyses indicate that for both A. rubrum and A. saccharum, there are not only
morphological differences, but also differences in chemical composition between them. Some authors have found that in oak (*Quercus mongolica*) differences in the proportion of syringyl units tend to increase as the cell function varies from water conduction (vessels) to mechanical support (fibers); libriform fibers have a relatively high syringyl content, whereas fiber-tracheids have a high guaiacyl content (Yoshinaga *et al.* 1997; Schwarze 2000). In *Acer* the fibers of type 2 (initially termed fiber-tracheids by us; Vazquez-Cooz & Meyer 2004a) tend to resemble fiber-tracheids, despite their lack of distinctly bordered pits.

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Figure 7. Micrograph of a transverse section of unstained red maple (*Acer rubrum*) observed using ultraviolet light (excitation 365 nm) and fluorescence spectra. Notice in *A* that areas of type 1 fibers have less fluorescence (encircled area) than the type 2 fibers in *B*. The black squares (outlined in white) show the area where the fluorescence spectra (below) were taken (×200). For type 1 fibers (vertical arrow) the maximum number of counts (fluorescence intensity) was 247.90 and the wavelength was 486.99 nm. For type 2 fibers (horizontal arrow) the maximum number of counts was 383.67 for the same wavelength. The UV fluorescence spectra obtained for the two fiber types show differences in intensity and peak positions. The normalized spectra (shown above) also displayed different shapes. This indicates that the type 1 fibers have different lignin composition from the type 2 fibers.
Controversy about how to distinguish libriform fibers from fiber-tracheids based on pit size and morphology has been common for about seven decades (Carlquist 2001). In some species (for instance, *Ormosia paramensis*) the pits of libriform fibers are minutely bordered while fiber-tracheid pits are distinctly bordered (Baas 1986). We observed distribution patterns in cell size and shape in cross sections of *Acer* (Vazquez-Cooz & Meyer 2004a). It is significant to add here that Wheeler observed similar patterns in petrified specimens of *Acer*, demonstrating that morphological differences between libriform fibers and fiber-tracheids have existed for millions of years (Wheeler, personal communication 2006).

Figure 8. Micrograph of a transverse section of sugar maple (*Acer saccharum*) observed using ultraviolet light (excitation 365 nm) and fluorescence spectra. Notice in A that areas of type 1 fibers have less fluorescence (encircled area) than the type 2 fibers in B. The black squares (outlined in white) show the area where the fluorescence spectra (below) were taken (×200). For type 1 fibers (vertical arrow) the maximum number of counts (fluorescence intensity) was 305.86 and the wavelength was 464.82 nm. For type 2 fibers (horizontal arrow) the maximum number of counts was 530.35 for the same wavelength. Notice that the spectra show differences in fluorescence intensity, peak positions, and in shape, suggesting different lignin compositions between fibers of type 1 and 2.
In this paper we report differences in microfibril angle between fibers of type 1 and 2 for fifteen Acer species. In addition, we report differences in the UV spectra and fluorescence intensity between these distinct fiber types for Acer rubrum and A. saccharum excited using ultraviolet wavelengths in the region where lignin fluoresces. The UV and fluorescence data imply a difference in lignin chemistry between the two types of fibers for these species, which substantiates the histochemical differences we reported earlier.

In our previous studies on Acer (Vazquez-Cooz & Meyer 2002, 2004a, 2006), we found several differences between the two types of fibers. The differences we found were consistent within and between all fifteen species. The differences are:

– A blue staining reaction occurs only for the type 1 libriform fibers with the safranin-O and astra blue double staining technique when using alcohol solutions;
– Fibers of type 1 do not fluoresce when excited at 355–375 nm;
– Fibers of type 1 are rounder in transverse sections, while type 2 fibers are more square;
– The peripheries of pit apertures in type 1 fibers are rounder, while in type 2 fibers they are more elliptical;
– Intercellular spaces are common and generally quite pronounced among type 1 fibers.

Now, we can add the following differences between these fiber types:

– The microfibril angles of type 1 fibers are greater than the microfibril angles of type 2 fibers;
– Lignin chemistry of type 1 fibers differs from the chemistry of type 2 fibers – type 1 fibers contain more syringyl lignin.

Most authors, such as Wheeler (2007), do not recognize fibers with distinctly bordered pits in Acer. We also failed to demonstrate significant differences between the generally simple to minutely bordered pits of both fiber types, and therefore have abandoned the terms fiber-tracheid and libriform fiber for fiber types 1 and 2, used in our earlier study (Vazquez-Cooz & Meyer 2004a): basically fiber types 1 and 2 in Acer are considered to be different sub-types of libriform fibers in current fiber terminology (IAWA 1989).

It would be of interest to compare the two types of libriform fibers reported here for Acer with the fiber dimorphism reported among libriform fibers in some species of Asteraceae, Melastomataceae, Oleaceae, Lythraceae and Sapindaceae with parenchyma-like fiber-bands (cf. IAWA 1989). Differences in fiber wall thickness and cell shape in those taxa might also be associated with ultrastructural and biochemical differences.

CONCLUSIONS

There are fundamental differences between the two types of fibers found in Acer.

Type 1 fibers fluoresce with less intensity than type 2 fibers. Differences in fluorescence and differences in UV spectra suggest that fibers of type 1 have a greater proportion of syringyl lignin in comparison with type 2 fibers.
Compared to type 2 fibers, type 1 libriform fibers have significantly larger microfibril angles in all 15 Acer species studied. This factor should be considered when analyzing and predicting mechanical properties of Acer, particularly in those species with large volumetric proportions of type 1 fibers. Differences in microfibril angle and in lignification may explain why type 1 fibers are more susceptible to damage than type 2 fibers when wood is machined.

The observations in this work provide further evidence of differences between two types of libriform fibers in Acer. Type 1 fibers in the species studied differ from type 2 fibers not only morphologically but also in their chemical composition.

ACKNOWLEDGEMENTS

With gratitude the authors thank Dr. Paul Martin for his benevolence in allowing us to use the CRAIC Technologies facilities. The authors greatly appreciate the helpfulness of Dr. Susan Anagnost who generously provided Chaetomium globosum cultures used in this study. We also appreciate the help of graduate student Haowen Xu for taking the micrographs used in Figure 5.

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