Variations in Topochemistry and Micromechanics between Opposite and Tension Wood Fiber from *Populus Nigra*

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Abstract

Chemical imaging by confocal Raman microscopy has been used for the comparison of the topochemical distribution of cellulose and lignin, in particular with regard to coniferyl alcohol and coniferaldehyde (Lignin-CAA) between opposite wood (OW) and tension wood (TW) fiber. In the Raman image of the ratio of the 1655 cm⁻¹ to the 1605 cm⁻¹ band, the homogeneous distribution of Lignin-CAA was visualized both in OW and TW fiber S2. In situ Raman images also revealed that highest cellulose concentration was found in the OW fiber S2 and fewest in CC as well as CML. By comparison in TW fibers high cellulose concentration spots were confined mostly to fiber S2 and GL. Besides variation in chemical composition, OW and TW fiber also showed micromechanical differences reflected by the band shift at 1097 cm⁻¹. The band at 1097 cm⁻¹ in OW fiber S2 shifted to 1095 cm⁻¹ in TW fiber S2 and GL which demonstrated that TW fiber S2 as well as GL still loaded tensile strength even after the stress was released. The localization of lignin and cellulose together with the micromechanical variation at sub-cellular level will contribute to the further understanding of tensional stress origin in TW.

Keywords: *Populus nigra*, Opposite wood, Tension wood, Topochemistry, Micromechanics, Confocal Raman microscopy
Introduction

In response to mechanical stress such as wind and gravity the stems and branches of trees can bend or lean and the trees are able to progressively straighten their leaning trunks or branches by an active mechanical action driven by cambium, which produces a special type of wood, called reaction wood (Sinnot 1952). Reaction wood characteristics and location differ between angiosperm and gymnosperm trees. In gymnosperm trees, such as pine, the reaction wood is named compression wood (CW) and develops at the lower side of leaning stems and branches, whereas in angiosperm trees, such as poplar, it is named tension wood (TW) and occurs at the upper side. The morphology and chemical composition of reaction wood differ markedly from those of opposite wood (OW). In gymnosperms, CW fibers typically have a round shape, intercellular spaces and cracks in the cell wall (Timell 1986). Their wall is thick and heavily lignified, and the microfibrils are oriented at a wide angle with respect to the fiber axis. Among angiosperms, TW is commonly characterized by the presence of specialized G-fibers with a particular morphology, chemical composition and micromechanics due to the development of the so called gelatinous layer (GL). The GL has been variously described as filling the cell lumen, as being attached to the S3 layer (S1+S2+S3+GL), as replacing the S3-layer (S1+S2+GL) or partly or entirely substituting the S2 layer (S1+GL) (Dadwell and Wardrop 1955).

It has been reported that the GL is composed of axial orientated microfibrils and high crystallinity of cellulose (Norberg and Meier 1966, Pilate 2004). However, in addition to cellulose this layer also contains polysaccharides, including pectin and hemicelluloses. In sweetgum and hackberry TW, a number of antibodies that recognize arabinogalactan proteins and RG I-type pectin molecules bound to the GL (Bowling and Vaughn 2008). Using immunohistochemical method, (1, 4)-β-galactan in cell walls of poplar TW G-fibers was detected and found it to be mainly restricted to the interface between the GL and the adjacent secondary cell wall (Arend 2008). Evidence of xyloglucan and xyloglucan-synthesizing proteins in the GL has also been reported (Nishikubo et al. 2007). Furthermore, previous literatures have demonstrated the presence of aromatic compounds in the GL using different microscopic techniques (Gierlinger and Schwanninger 2006, Joseleau et al. 2004, Lehringer et al. 2008, Lehringer et al. 2009).

Although it has been well established that the GL with its microfibril angle parallel or nearly parallel to the longitudinal axis is the driving force of the tensile stress generated in TW (Fujita et al. 1974, Clair et al. 2011, Goswami et al. 2008, Chang et al. 2009, Clair and Thibaut 2005, Yoshida et al. 2002), the underlying mechanism at the origin of tensional stress in TW has been the subject of a lot of controversy. It has been proposed that it would be due to the swelling of the wood matrix substance during lignification, the angle of cellulose microfibrils controlling the anisotropy of the resulting stress (Yamamoto et al. 1990). Different hypotheses have been proposed to explain the mechanism, such as the contraction of amorphous zones within the cellulose microfibrils (Yamamoto 2004), the action of xyloglucans during the formation of microfibril aggregates, or the effect of changes in moisture content stimulated by pectin-like substances. However, no literature has focused on
the difference in micromechanics at the sub-cellular level using the confocal Raman microscopy.

Here, we use confocal Raman microscopy to acquire and compare chemical images between OW and TW fiber from *Populus nigra*. Differences in cellulose and lignin localization in the cell walls are visualized without staining of tissues. Furthermore, the miromechanical variations between and within cell wall layers were reflected by Raman band shift.

**Material and methods**

**Plant material and preparation.** An inclined 5-year-old *P. nigra* tree, which exhibited crooked growth in the lower parts of the stems, was provided by the arboretum of Northwest Agricultural and Forestry University, China. Specimens from fresh disks of inclined stem were collected. Without any embedding routine, 30-μm-thick cross-sections for chemical imaging were cut on a sliding microtome (Leica 2010R). For chemical imaging, samples were placed on a glass slide with a drop of D2O, covered by a coverslip (0.17 mm thickness) and sealed with nail-polish to prevent evaporation during measurement.

**Confocal Raman microscopy.** Raman spectra were acquired with a LabRam Xplora confocal Raman microscope. In order to achieve high spatial resolution, measurements were conducted with a high numerical aperture (NA) microscope objective from Olympus (100×, oil, NA=1.40) and a linear-polarized 532-nm laser excitation was focused with a diffraction-limited spot size (1.22λ/NA). The software (Labspec) was used for measurement setup and image processing.

**Results**

**Variations in topochemistry between OW and TW fiber.** The topochemical analysis of fibers in OW and TW reveals the distribution of cellulose and lignin within the morphologically distinct cell wall layers. The basic morphology of the measured cell walls in the OW (Fig. 1a) and TW (Fig. 1b) becomes apparent in the chemical images based on the C-H stretching bands (3000-2771 cm⁻¹), in which all cell wall polymers (cellulose, hemicellulose, pectin, lignin) contribute to the Raman signal (Atalla and Agarwal 1984, Wiley and Atalla 1987). In TW the GL was readily differentiated from the S2 and appeared as wavy structures with very irregular lumens. The formation of GL appeared to affect the development of S2 layer, which appears consistently thinner than that in OW.

Besides the cell wall structures, the distribution of cell components (cellulose and lignin) were also visualized. The characteristic cellulose vibrations can be seen in the region from 2900-2750 cm⁻¹, assigned to CH and CH2 stretching vibration (Agarwal and Ralph 1997, Agarwal 1999, Edwards et al. 1997). In OW fibers highest intensity was found in the secondary wall and fewest in the CC as well as CML and in TW fibers high cellulose concentration spots were confined mostly to S2 and GL (Figs. 1c and 1d).
The chemical image based on the O-D stretching band (integrating the intensity from 2775-2200 cm\(^{-1}\)) is shown for OW fiber (Fig. 1e). Highest intensity was found in the S2 followed by the CC and CML and the fewest in the S3. As expected, in TW fiber high intensity is observed in the GL. The O-D signal, albeit much weaker, is also observed in the S2 wall layer, the CC and in some parts of the CML (Fig. 1f). As a possible explanation, the higher concentration of D2O in the GL could have related to the porosity or the hydrophilicity of the various structures. As was reported earlier (Chang et al 2009), the results of adsorption-desorption isotherms revealed that the TW has much higher porosity and suggested that high porosity is an attribute of the GL itself.

Finally, lignin distribution was visualized by integrating over the 1600 cm\(^{-1}\) band, assigned to phenyl groups in lignin (Agarwal 2006, Agarwal and Ralph 2008). In OW fiber, there is strong contrast between morphologically distinct cell wall regions due to different lignin signal intensity (Fig. 2a). The higher lignin signal intensity was observed in the cell corner (CC) and somewhat lower in other regions of the compound middle lamella (CML).
the S2 wall layer of the fibers less, yet not insubstantial, amounts of lignin are observed. By contrast, there are marked differences for the TW fibers (Fig. 2b). While the relative lignin signal intensity is still higher in the CC and CML than in the S2 layer, there is a clear overall increase in the S2 layer of the fibers. This is further illustrated by semi-quantitative spectra analysis.

Owing to the in situ chemical sensitivity and the specific chemical attributions of the lignin bands, it is possible to further dissect the spatial lignin distribution both within and between samples. While the 1,605 cm\(^{-1}\) band is due to aryl ring stretching and thus is a more general lignin marker, the 1,665 cm\(^{-1}\) band is indicative of coniferyl alcohol and coniferaldehyde. In order to gain more subtle differences, images for band ratios were obtained. In the OW and TW image for the ratio of the 1,665 cm\(^{-1}\) to the 1,605 cm\(^{-1}\) band (Figs. 2c and 2d), the intensity distribution of Lignin-CAA is homogeneous. The same results were also reported previously (Schmidt 2009), which presented that both in wild type and transgenic *Populus trichocarpa* the distribution of CAA was relatively even.

![Image](image_url)

Figure 2. Raman images calculated by integrating over Raman bands attributed to different functional groups of cell wall polymers. The lignin distribution of OW (a) and TW (b) fiber, 1,641-1,546 cm\(^{-1}\); The coniferyl alcohol and coniferaldehyde distribution of OW (c) and TW (d) fiber, 1,670-1,645 cm\(^{-1}\).

**Variations in micromechanics between OW and TW fiber.** The mechanism for tree orientation in angiosperms is based on the production of high tensile stress on the upper side of the inclined axis. In many species, the stress level has relations to the presence of the GL. In the TW the fibrils in the GL are parallel to the fiber axis, which enable them to deform approximately the same order of magnitude as the fiber.

When covalent bonds in a fibrous polymer are loaded in tension, their stretching vibrational bands in the infrared or Raman spectrum shift to lower frequency (Wool 1975). In order to...
investigate the band shift at 1097 cm\(^{-1}\) attributed to C-C and C-O stretching of cellulose in TW fiber, the spectra range from 1250-1000 cm\(^{-1}\) was extracted. The resulting spectra were deconvoluted at the different frequency ranges. After the spectra fitting, we found besides changes in the overall Raman intensity, that some Raman bands showed obvious changes in band positions (Fig. 3). The band at 1097 cm\(^{-1}\) in the OW fiber S2 shifted to lower wavenumbers in TW fiber S2 (1095 cm\(^{-1}\)) and GL (1095 cm\(^{-1}\)) (-2.0 cm\(^{-1}\) at its center). Negative band shifts are expected for covalent bonds aligned and stretched along the fiber axis. The same result was also reported that there exists a strong correlation between the shift of the band at 1097 cm\(^{-1}\) corresponding to the stretching of the cellulose ring structure and the applied strain (r=0.99). Along with increasing strain, the band at 1097 cm\(^{-1}\) shifted remarkably to lower wavenumbers (1091 cm\(^{-1}\)) (Gierlinger 2006). Thus, it is reasonable to assume that in comparison with OW and TW fiber S2 as well as GL still loaded tensile strength even after the stress was released.

Figure 3. Average Raman spectra acquired from the OW fiber S2 (OW-S2), the TW fiber S2 (TW-S2) and the GL showing the bandshift of C-O-C.

The mechanism at the origin of tensile stress has been explored according to different hypothesis (Fujita et al. 1974, Clair et al. 2011, Goswami et al. 2008, Chang et al. 2009, Clair and Thibaut 2005, Yoshida et al. 2002). The G-layer swelling hypothesis proposed that the tensional deformation originates in the swelling of the GL and is transmitted to the adjacent secondary layers, where the larger MFAs allow an efficient conversion of lateral stress into axial tensile stress. Cellulose tension hypothesis postulated that tensional stress develops in the G-layer, which then drives shrinkage of the S-layer. Both hypothesis assumed that the generation of longitudinal shrinkage in the TW fiber S2 was induced by the GL. The negative band shift of TW fiber S2 and GL in our experiment revealed that after the fibers are transversally cut, the TW fiber S2 and GL kept the permanent tensional deformation. The tensional deformation of the TW fiber S2 may be due to its being in a state of tension during the TW formation. Thus, we can assume that the generation of longitudinal shrinkage in the TW fiber was the combination of S2 and GL.
Conclusion

High resolution confocal Raman microscopy proved to be a useful method for investigating topochemical and micromechanical characteristics of OW and TW fibers from *P. nigra*. In situ Raman images revealed that lignin-CAA was homogeneously distributed in OW and TW fiber S2. Furthermore, the Raman band shift reflected that the cellulose fibrils in TW fiber S2 and GL still loaded tensile strength even after the stress was released. The localization of lignin and cellulose together with the micromechanical variation at sub-cellular level will contribute to the further understanding of tensional stress origin in TW.

Acknowledgments

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