

Wood Growth/Morphology

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Cell wall structure of tensile flexure wood fibers in *Populus x euramericana*

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Abstract: In response to gravitropic environmental stimuli, woody angiosperms develop tension wood (TW). Typical TW fibers are characterized by an inner gelatinous cell wall (c.w.) layer (G-layer). On the other hand, in response to mechanical stimuli, woody plants develop flexure wood. Flexure wood formed under tension in angiosperms is termed tensile flexure wood (TFW). Similarly to TW, TFW represents an increased source of non-recalcitrant cellulose for biofuel production. Histochemical, SEM, and Raman microspectroscopic analyses of TFW of juvenile *Populus x euramericana* formed in response to severe long-term static bending were performed. The presence of lignin in the G-layer, higher syringyl/guaiacyl ratio, and differences in lignin structure in TFW compared to normal wood, revealed by histochemical analysis, was confirmed by Raman microspectroscopy. Additionally, Raman microspectroscopy indicated differences in cellulose and hemicellulose structure and pectin methylesterification. The G-layer and compound middle lamella contribute to the response to bending by opposite shifts of the orientation-sensitive cellulose band

attributed to the glycosidic C–O–C bond. SEM micrographs revealed the c.w. ultrastructure of TFW fibers. The presented findings encourage further investigation of TFW in terms of both changes in c.w. structure as a mechanical acclimation to stem bending and its potential for biofuel production.

Keywords: cell wall; gelatinous fibers; *Populus x euramericana* cl. NS 11-8; tensile flexure wood

Abbreviations

c.w.	cell wall
CML	compound middle lamella
GL	gelatinous layer
NW	normal wood
SCW	secondary cell wall
SL	S ₂ layer of SCW in NW
TFW	tensile flexure wood

1 Introduction

In response to gravitropic environmental stimuli in stems and branches, woody plants develop a reaction wood to overcome stem lean (Scurfield 1973; Timell 1969). Reaction wood in angiosperms is called tension wood (TW). TW contains more cellulose and less lignin. TW fibers, gelatinous fibers (G-fibers), differ anatomically from normal wood (NW) fibers formed in the absence of stimuli or from opposite wood (OW) fibers located opposite to the TW in the same growth ring. Typical G-fibers are characterized by an inner gelatinous cell wall (c.w.) layer (G-layer). The G-layer may be formed inside the S₃ layer or replace the S₃ layer and a part or the whole S₂ layer (Abedini et al. 2015; Dadswell and Wardrop 1955). On the other hand, in response to mechanical stimuli (such as wind or artificial bending treatments), woody plants develop “flexure wood” (Telewski 1989), which shows some differences compared to reaction wood. Recently, for “flexure wood” formed under tension in angiosperms, the term “tensile flexure wood” (TFW) was proposed (Roignant et al. 2018).

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Compared to TW fibers, TFW fibers show a thinner G-layer (with a higher microfibrillar angle) and a thinner S-layer (Niez et al. 2020; Roignant et al. 2018). Similarly to TW (Donaldson and Singh 2016), TFW represents an increased source of non-recalcitrant cellulose for biofuel production (Urbancsok et al. 2023).

Further studies of wood c.w. differentiation as a response to bending were suggested (Roignant et al. 2018). There is no data on the alterations in the structure/composition of c.w. polymers in TFW fibers. Histochemical, SEM, and Raman microspectroscopic analysis of TFW fibers in juvenile *Populus x euramericana* cl. NS 11-8 trees formed in response to severe long-term static bending were performed and discussed regarding both changes in c.w. structure as a mechanical acclimation to stem bending and its potential for biofuel production.

2 Materials and methods

2.1 Plant material and sample collection

Populus x euramericana cl. NS 11-8 juvenile trees, were grown from 20 cm long cuttings, a gift from the Institute of Lowland Forestry and Environment (ILFE), University of Novi Sad. The cuttings were grown in plastic pots 20 × 20 × 20 cm from March 2011 to April 2012 (shoots were about 1.2 m high), outdoors in Belgrade, Serbia (44° 49'N, 20° 29'E). Bending was applied by wiring from April to October 2012 (the bending angle was about 90°, Prokopijević et al. 2022). Control plants of the same age were grown under the same conditions as the bent plants but without the bending treatment. Stem samples at stem base from both control and bent trees were collected in October 2012.

2.2 Histochemical analysis

For histochemical analysis unfixed, untreated, dried stem cross-sections, 70 µm thick, of control and bent trees were soaked in distilled water for 2 h before staining. After imbibition, sections were stained in 10 % phloroglucinol-HCl (Jensen 1962) for 1 h, in 0.05 % toluidine blue O (O'Brien et al. 1964) for 15 min, or in Mäule reagent for 10 min (Soukup 2019). Unstained imbibed sections were observed under UV light – excited (358 nm) autofluorescence (Vazquez-Cooz and Meyer 2004). All sections prepared for histochemical analysis were mounted in water, examined, and photographed by a Zeiss Axiovert light-fluorescence microscope (Carl Zeiss GmbH, Göttingen, Germany).

2.3 Scanning electron microscopy (SEM)

Cross sections of dried stems, 100 µm thick, were sputter-coated with a thin layer of gold and examined using a TESCAN VEGA 5130MM scanning electron microscope (SEM, TESCAN, Brno, Czech Republic) in secondary electron imaging mode at an accelerating voltage of 20 kV.

2.4 Raman microspectroscopy

Raman microspectroscopy spectra were recorded using XploRA Raman spectrometer (Horiba Jobin Yvon, Palaiseau, France) equipped with microscope Olympus BX51 (Tokyo, Japan), 50 LWD objective (Olympus, Tokyo, Japan). Raman spectra for fiber c.w.s were recorded using a laser at a wavelength of 532 nm equipped with a 1,200 lines mm⁻¹ grating; spectra were acquired by applying exposure time 10 s and scanning 9–10 cells per sample, using 10 % filter. In NW samples of control *P. x euramericana* trees Raman spectra were recorded for CML at cell corners and for SCW at the mid region (which corresponds to S₂ as the thickest layer of the SCW). In TFW of bent trees, Raman spectra were recorded for CML at cell corners and for the G-layer at the mid region of the G-layer as the thickest c.w. layer. The spectral resolution was about 3 cm⁻¹ and autocalibration was done each time before recording of spectra by 520.47 cm⁻¹ line of silicon.

2.4.1 Spectroscopic data pre-processing and analysis

The Origin Pro 2018 software was used to preprocess the Raman spectra, including baseline correction, Savitzky–Golay smoothing (10 data points, second-order polynomial), and normalization via Standard Normal Variate (SNV).

3 Results and discussion

3.1 Histochemical and ultrastructural analysis of *P. x euramericana* NW and TFW

Autofluorescence and different staining methods were used to determine lignin distribution in different c.w. layers of *P. x euramericana* fibers (Figure 1).

NW fibers exhibit high UV excited autofluorescence intensity (Figure 1A and B), intense violet-red coloration after the phloroglucinol staining (Figure 1D and E), orange coloration after Mäule staining (Figure 1H and I), and dark blue coloration after the toluidine blue O staining (Figure 1L and

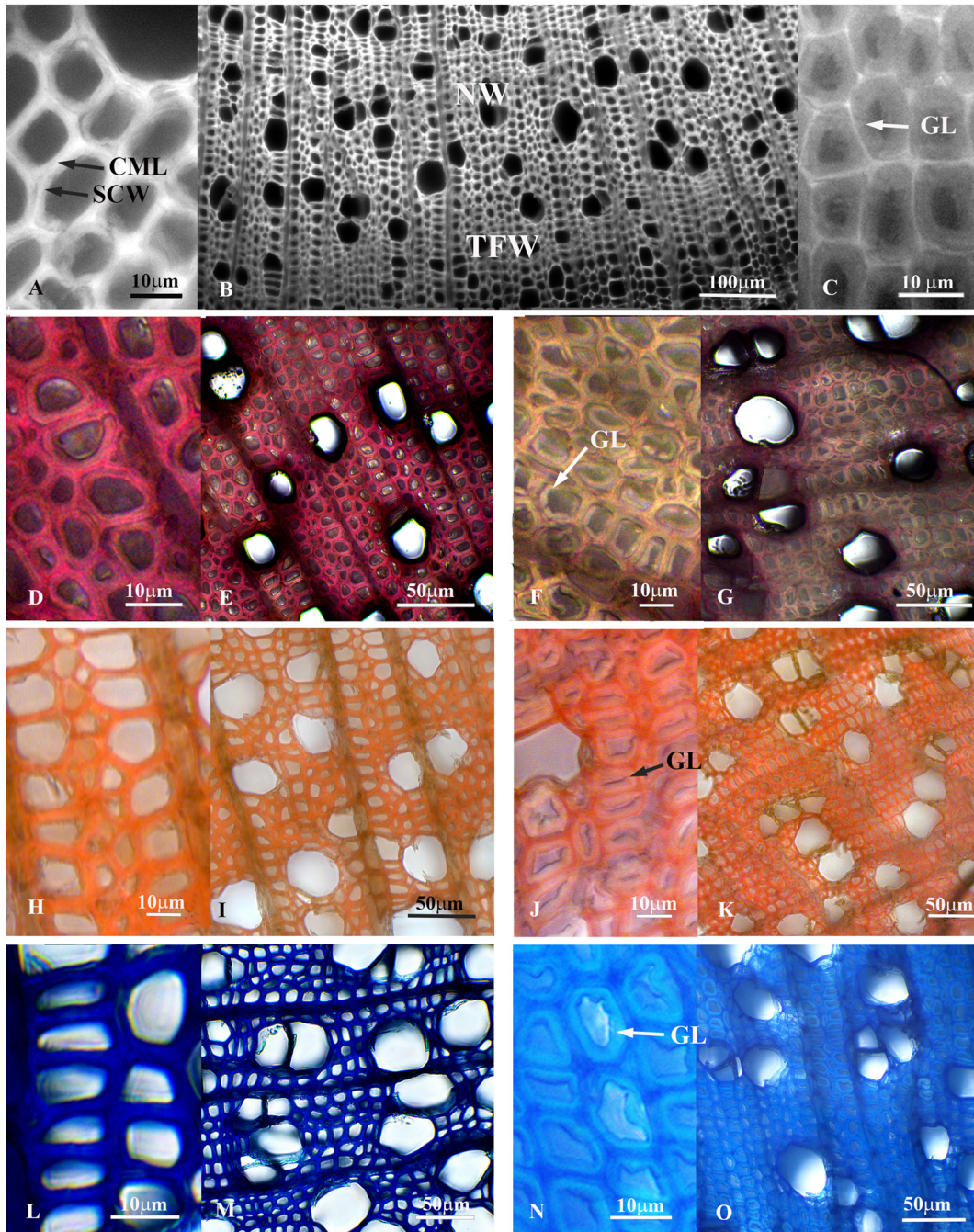


Figure 1: *Populus x euramericana* cl. NS 11-8 stem cross sections of control and bent trees. (A, B, D, E, H, I, L, M) NW of control trees; (B, C, F, G, J, K, N, O) TFW of bent trees; (B) last two growth rings from bent trees showing the difference in autofluorescence intensity between NW formed before bending treatment was performed, and TFW formed in response to the bending treatment. (A, B, C) UV-excited autofluorescence; (D, E, F, G) phloroglucinol staining; (H, I, J, K) Mäule staining; (L, M, N, O) toluidine blue O staining. NW, normal wood; TFW, tensile flexure wood; CML, compound middle lamellae; SCW, secondary cell wall; GL, gelatinous layer.

M), across the entire c.w., indicating the presence of lignin in all c.w. layers. CML, particularly at cell corners, shows to some extent higher autofluorescence intensity (Figure 1A and B), darker violet-red after the phloroglucinol staining (Figure 1D and E), intense orange coloration after Mäule

staining (Figure 1H and I), and darker blue color after the toluidine blue O staining (Figure 1L and M), suggesting higher lignin content in CML compared to SCW. The highest lignin content, guaiacyl-rich and highly condensed, in CML of NW fibers, particularly at cell corners, compared to inner

c.w. layers was demonstrated by various methods, from the Klason and acetyl bromide method to Raman scattering microscopy in fibers of divergent origins (Meshitsuka and Nakano 1985; Zeng et al. 2017; Zhang et al. 2022). In addition, Mäule staining reveals a higher syringyl/guaiacyl (S/G) ratio in *P. x euramericana* NW fibers (colored orange) compared to the ray parenchyma and vessels (colored brown) (Figure 1H and I). The difference in lignin composition between vessel and fiber cell walls was established in *Populus deltoides* using confocal fluorescence microscopy: G-rich lignin characterizes vessel cell walls (both CML and SCW), whereas a mixture of S and G lignin characterizes the fiber walls (Donaldson 2013).

In addition, Mäule staining reveals a higher S/G ratio in TFW (colored red-orange, Figure 1J) compared to NW (colored orange, Figure 1H).

In poplar TW fibers the G-layer replaces the S_3 and a part of the S_2 layer (Ghislain and Clair 2017; Wardrop and Dadswell 1955), while in TFW fibers, a decrease in S-layer thickness compared to TW is shown (Roignant et al. 2018). Consequently, in *P. x euramericana* TFW fibers, CML and the thin S-layer on micrographs are visible as one highly lignified c.w. layer (Figure 1B, C, F, G, J, K, N and O), rich in G units (high UV excited autofluorescence intensity, violet-red coloration after phloroglucinol staining, red-orange coloration after Mäule staining, Figure 1B, C, F, G, J and K). In contrast, the G-layer shows weak UV excited autofluorescence (Figure 1C) and almost no coloration after phloroglucinol staining (Figure 1F), indicating that the G-layer contains little to no G lignin. At the same time, weak UV excited autofluorescence (Figure 1C) of the G-layer may represent the presence of S lignin. Fluorescence spectra of G-fibers in *P. deltoides* showed G-rich lignin in the S_2 layer and S-rich lignin at the inner margin of the G-layer (Donaldson 2013). The presence of the S lignin in the G-layer is also indicated by the pale pink coloration after Mäule staining (Figure 1J and K). The light blue coloration after toluidine blue O staining (Figure 1N and O) indicates the presence of phenolic or lignin like substances in the G-layer, whereas the dark blue coloration of the thin border on the lumen surface (Figure 1N) indicates the difference in lignin/phenolic composition compared to the integral G-layer. The dark blue to light blue coloration (Figure 1L–O) of CML, S, and G-layer, respectively, indicates the differences in lignin/phenol structure/content in these c.w. layers of TFW fibers in *P. x euramericana*. Bowling and Vaughn (2008) used toluidine blue O to demonstrate the presence of an unligified G-layer colored purple in the G-fibers of *Liquidambar styraciflua* L., while the lignified G-layer of sclerenchyma cells in the woody stem of monocotyledonous liana *Dioscorea balcanica* Košanin showed a light blue coloration (Simonović Radosavljević et al. 2017).

Roussel and Clair (2015) showed that the G-layer either has high cellulose content and no lignin or is lignified later during the maturation process in some species. The presence of lignin or lignin-like substances in the G-layer or on its lumen surface has been demonstrated in different tree species (mainly diverse hybrid poplars) using complex techniques. Using transmission electron microscopy, Joseleau et al. (2004) demonstrated that in *P. deltoides* S lignin units are abundant in the G-layer. Raman microspectrometry (Gierlinger and Schwanninger 2006) in *Populus nigra x Populus deltoides* showed the presence of small amounts of lignin within the G-layer, but its increase toward the lumen surface. Label-free *in situ* confocal Raman microscopy in *P. nigra* (Ma et al. 2013) revealed the presence of a lignin related band at $1,603\text{ cm}^{-1}$ in the G-layer, though not in high enough concentration to be visualized by the imaging approach. Fluorescence spectroscopy (Donaldson 2013) suggested the presence of syringyl p-hydroxybenzoate-rich lignins at the inner margin of the G-layer of *P. deltoides*, while Zhao et al. (2021) revealed a unique adaptive mechanism of poplar species to the mechanical stress/gravistimulation, which includes S-lignin-bound p-hydroxybenzoate accumulation in TFW/TW.

SEM micrographs (Figure 2) reveal the differences in the ultrastructure between *P. x euramericana* NW (Figure 2A–C) and TFW (Figure 2B–D) fibers.

NW fibers show relatively thin cell walls with slightly distinguishable CML and SCW (Figure 2A–C).

In TFW fibers, the G-layer loosened from the S-layer is visible, while CML and the thin S-layer are visible as one layer. The cracks within the G-layer (Figure 2D) and the differences in cellulose microfibrils orientation between the S-layer of NW and G-layer of TFW are observable (Figure 2C and D). These features, the weak adhesion of the G-layer to the preceding S-layer and the cracks within the G-layer, are considered the main features of G-fibers in TW (Clair et al. 2005). The main differences in the orientation of cellulose microfibrils in different c.w. layers can be observed using scanning electron microscopy (SEM), including both conventional and field-emission types, as demonstrated by Simonović Radosavljević et al. (2017) and Ruelle et al. (2007).

3.2 Raman microspectroscopy of *P. x euramericana* NW and TFW

Raman microspectroscopy analysis focused on direct measurements of fiber's cell walls on *P. x euramericana* stem cross sections (Figure 3). The CML was measured at the cell corners in both NW fibers of control trees and TFW fibers of bent trees. Although thin c.w. layers (like CML) can be

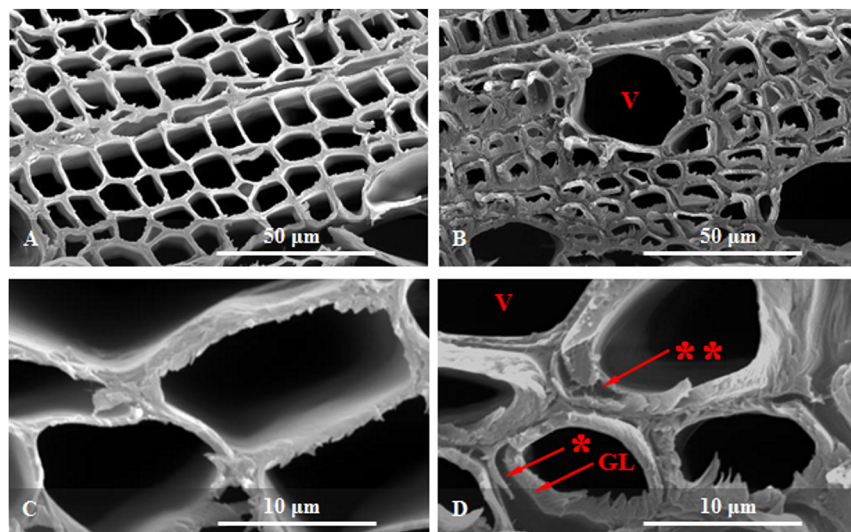


Figure 2: SEM micrographs of *Populus x euramericana* cl. NS 11-8 stem cross sections of control and bent trees: (A, C) normal wood of control trees, and (B, D) tensile flexure wood of bent trees. NW, normal wood; TFW, tensile flexure wood; GL, gelatinous layer; *, detachment of the thick GL from the thin S_2 layer of secondary cell wall; **, cracks within the GL due to the shrinkage of the GL; V, vessel.

distinguished using Raman microspectroscopy, spectral contributions from the neighboring c.w. layers may overlap (Gierlinger and Schwanninger 2006). In NW fibers, SCW was examined at the mid-region, corresponding to the S_2 layer (SL). In TFW fibers, the G-layer (GL) was measured at the mid-region (Figures 1 and 2).

Figure 3 presents overlaid averaged Raman spectra for the 500–1,800 cm^{-1} region, derived from 9 to 10 fiber cell walls per sample of NW (CML and SL) and TFW (CML and GL).

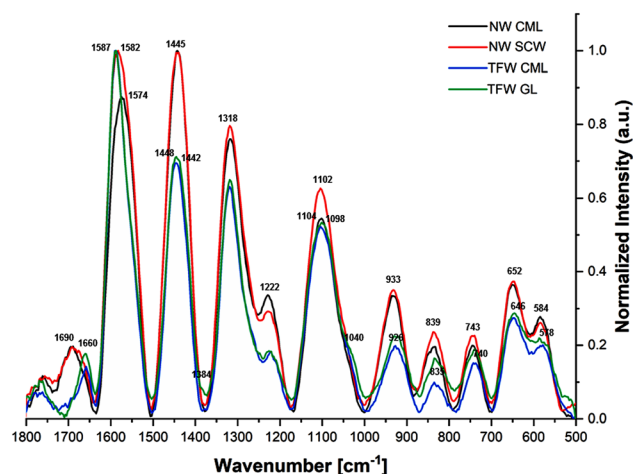


Figure 3: Averaged Raman spectra of *Populus x euramericana* NW and TFW fiber cell walls recorded in the spectral range from 500 to 1,800 cm^{-1} . NW CML, compound middle lamella of normal wood fibers (measured at cell corners); NW SL, secondary cell wall of normal wood fibers (measured at the mid region, corresponding to the S_2 layer, as the thickest layer of the SCW in NW fibers); TFW CML, compound middle lamella of tensile flexure wood fibers (measured at cell corners); TFW GL, gelatinous layer of tensile flexure wood fibers (measured at the mid region). Assignments of the bands characteristic of c.w. polymers and the corresponding references for assignment are provided in Table 1.

Table 1 provides the assignments of the bands characteristic for c.w. polymers and the corresponding references for assignment.

The region between 1,200 and 1,700 cm^{-1} reflects the spectral contribution of lignin or lignin-like compounds. The 600–1,100 cm^{-1} region is dominated by cellulose, hemicellulose, and pectin.

Small changes in band positions and intensities can provide insights into alterations in polymer composition and orientation. Several factors, including the amount, composition, orientation, crystallinity, and linkages to other components, contribute to the spectral changes (Gierlinger and Burgert 2006). Shifts in peak position arise from variations in the length of chemical bonds or the positions of the atoms. Shifts to lower wavenumbers result from longer bond length (and tensile strain), and vice versa shifts to higher wavenumbers suggest shorter bond length (compressive strain, higher strength) (Tuschel 2019). On the other hand, variations in band width reflect the uniformity of bond lengths; narrowing band widths suggests structural arrangement or order – the weaker the chemical interaction, the narrower the band will be (Tuschel 2019).

The region around 1,600 cm^{-1} contains the main marker band of lignin assigned to symmetric aromatic ring stretching vibrations (Table 1). It informs on lignin structure and ring orientation or the lignin microenvironment, the alignment of S and G units, and it differs in different wood species (Larsen and Barsberg 2010). In this region, two bands characterize *P. x euramericana* NW, 1,574 cm^{-1} (CML) and 1,582 cm^{-1} (S-layer), suggesting the difference in lignin structure; a shorter bond length and higher strength of lignin structure characterize the S-layer compared to the CML. In TFW, shift to the higher wavenumber (1,587 cm^{-1}) compared to NW is visible in both

Table 1: Assignment of the Raman bands characteristic for the polymers in the cell walls of *Populus x euramericana*, and the corresponding references used for assignment.

Wavenumber (cm ⁻¹)	Vibrational mode	Chemical moiety	Reference sources
1,690, 1,660	C=C in coniferyl alcohol or C=O in coniferyl aldehyde	Lignin	Agarwal (2006), Agarwal et al. (2011), Gao et al. (2021), Zhang (2021) and Zhu et al. (2018)
~1,585, 1,574	Symmetric aromatic ring stretching vibrations	Lignin	Agarwal (2006), Agarwal et al. (2011), Gao et al. (2021), Gierlinger et al. (2008)
~1,455, 1,442	O-CH ₃ deformation; CH ₂ scissoring; guaiacyl/syringyl ring vibration	Lignin	Agarwal et al. (2011), Agarwal (2019), Gierlinger and Schwanninger (2006), and Larsen and Barsberg (2010)
1,384	C-O-C stretching	Cellulose	Agarwal (2019) and Gierlinger et al. (2012)
1,318	Aromatic ethers stretching	S Unit	Agarwal and Atalla (2010)
1,306	G ring mode with C=O group	Coniferyl alcohol	Agarwal et al. (2011) and Gao et al. (2021)
~1,222	Aryl-O of aryl-OH and aryl-O-CH ₃ ; guaiacyl/syringyl ring (with C=O group) mode	G ring mode of lignin	Agarwal et al. (2011) and Gierlinger et al. (2008)
1,098–1,104	Glycosidic C-O-C asymmetric and symmetric stretching vibrations	Cellulose or hemicellulose	Agarwal (2006), Gao et al. (2021), Gierlinger et al. (2008), Gierlinger and Schwanninger (2007), Szymańska-Chargot et al. (2015), and Zhang (2021)
1,036	C-O of aryl O-CH ₃ and aryl OH, C-O stretching vibration	Cellulose	Agarwal et al. (2011), Gao et al. (2021), Gierlinger et al. (2008), and Gierlinger and Schwanninger (2006)
1,040	OC stretching; ring deformation, CH ₃ wagging	Lignin	Agarwal et al. (2011) and Synytsya et al. (2003)
939–925	C-C-H wagging vibration, C-O-H bending vibration	Lignin	Agarwal et al. (2011) and Synytsya et al. (2003)
848–833	C-O-C skeletal modes	Pectin, hemicellulose	Agarwal (2014) and Synytsya et al. (2003)

Table 1: (continued)

Wavenumber (cm ⁻¹)	Vibrational mode	Chemical moiety	Reference sources
750–736	Skeletal deformation	Lignin	Agarwal et al. (2011)
658–652, 635	C-O-C skeletal modes	Pectin	Szymańska-Chargot et al. (2015)
584–578	Skeletal deformation	Lignin	Agarwal (2014) and Agarwal et al. (2011)

CML and GL (Figure 3, Table 1). However, the narrowing of the band at 1,587 cm⁻¹ characterizing TFW, despite its shift to a higher wavenumber, suggests weaker chemical interactions relative to the shift.

The main marker band of lignin is accompanied by the band at 1,657 cm⁻¹ assigned to coniferyl alcohol or coniferyl aldehyde, precursors of lignin. In hardwood lignin, this band may exhibit different contributions from sinapyl alcohol and sinapaldehyde units. In *Populus euramericana* TFW this band shifts to the lower wavenumber (1,660 cm⁻¹) and became narrower compared to NW (1,690 cm⁻¹), suggesting tensile strain, and weaker chemical interactions.

As opposed to *P. euramericana* TFW (Figure 3), in grounded samples of TW of the inclined stem of *P. euramericana* Raman spectroscopy (Guan et al. 2021) showed no shift in the main marker band of lignin, assigned to symmetric aromatic ring stretching vibrations, while the band assigned to coniferyl alcohol and coniferyl aldehyde shifted to the lower wavenumber in TW compared to NW. In contrast, in TW of the inclined stem of *P. nigra* label-free *in situ* Raman microspectroscopy revealed no shifts in band positions in the region 1,700–1,500 cm⁻¹ region compared to OW (Ma et al. 2013).

The high intensity band assigned to guaiacyl/syringyl ring vibration (1,445 cm⁻¹) decreased considerably in *P. euramericana* TFW compared to NW (Figure 3, Table 1), while in the spectra of the G-layer, a doublet at 1,448 cm⁻¹ and 1,442 cm⁻¹ is visible.

A shoulder at 1,384 cm⁻¹, a non-orientation-sensitive cellulose band (Agarwal 2019; Gierlinger et al. 2012), is visible only in the G-layer of TFW.

The bands at 1,222 and 1,318 cm⁻¹, characteristic of G and S lignin units, respectively, indicate the higher content of S compared to G unit in both NW and TFW, and suggest the higher S/G ratio in TFW compared to NW fibers (Figure 3, Table 1), already indicated by Mäule staining (Figure 1H–K). Similarly to TFW (Figure 3, Table 1), lower lignin content and higher S/G ratio were shown in TW of *P. euramericana* compared to NW (Guan et al. 2021).

he bands in the region from 1,090 to 1,120 cm^{-1} , characteristic for glycosidic C–O–C asymmetric and symmetric stretching vibrations in cellulose or hemicellulose, mainly xyloglucan and glucomannan, represent the most orientation-sensitive cellulose band. This broad band includes both carbohydrate concentration and orientation changes (Gierlinger and Schwanninger 2006). Consequently, changes in the amount cannot be accurately investigated by the band integration, as they include changes in cellulose microfibril orientation and/or crystallinity (Gierlinger 2017). A strong correlation between the shift of this band and the applied strain was found (Gierlinger 2017); with the increasing strain, this band shifted remarkably to lower wavenumbers (Gierlinger et al. 2006). Ma et al. (2013) showed that in *P. nigra*, this band in the OW fiber S_2 layer (at 1,097 cm^{-1}) shifted to lower wavenumbers in the TW fibers, both S_2 layer (1,094.4 cm^{-1}) and G-layer (1,094 cm^{-1}), meaning that both S_2 and G-layer respond to leaning by the changes of the same direction in cellulose microfibril orientation (tensional deformations of the glycosidic C–O–C bond of cellulose).

In juvenile *P. x euramericana* (Figure 3, Table 1), this band at 1,102 cm^{-1} in NW fibers (both CML and S-layer) shifted to a lower wavenumber (1,098 cm^{-1}) in the G-layer of TFW, suggesting tensional deformations. However, in the CML of TFW (it should be noted that in thin c.w. layers, the spectral contributions from the neighboring c.w. layers may overlap; Gierlinger and Schwanninger 2006), this band shifted to a higher wavenumber (1,104 cm^{-1}), suggesting a shorter bond length and compressive strain (higher strength) in the glycosidic C–O–C bond of cellulose. In other words, CML and G-layer of *P. euramericana* TFW fibers respond to long-term severe static bending by opposite changes in cellulose microfibril orientation; compressive strain (higher strength) characterizes the glycosidic C–O–C bond of cellulose in CML, opposed to tensile strain (weaker chemical interactions) in the G-layer.

A shoulder at 1,040 cm^{-1} , visible only in the G-layer of TFW (Figure 3, Table 1), is suggested to be linked to lignin (Gao et al. 2021; Gierlinger and Schwanninger 2006).

The band at 939 cm^{-1} , characteristic of C–C–H wagging vibration, or C–O–H bending vibration in lignin (Figure 3, Table 1), shifted to lower wavenumbers (926 cm^{-1}) in TFW. Also, bands at 750–736 cm^{-1} and 584–578 cm^{-1} , characteristic of skeletal deformations in lignin, shifted to lower wavenumbers in TFW compared to NW (Figure 3, Table 1).

The bands at around 835 and 655 cm^{-1} characteristic for pectin (Table 1) are the most prominent Raman markers for pectin polysaccharides, without overlapping with other c.w. polymers. The wavenumber positions of these bands are shifted for pectin with a different degree of methylesterification (Synytsya et al. 2003; Szymańska-Chargot et al. 2015;

Zhu et al. 2018). The band at 839 cm^{-1} in *P. x euramericana* NW fibers shifted to lower wavenumbers (835 cm^{-1}) in TFW (Figure 3), while the band at 652 cm^{-1} in NW fibers shifted to 646 cm^{-1} in TFW. These shifts suggest the differences in pectin methylesterification (Synytsya et al. 2003; Szymańska-Chargot et al. 2015; Zhu et al. 2018) in TFW compared to NW (Figure 3).

3.3 The difference in cell wall structure of TFW (Figures 1, 2, and 3) and TW (literature data)

The difference in c.w. structure of TFW fibers compared to TW fibers, a thinner G-layer (with a higher microfibrillar angle), and a thinner S-layer (Nie et al. 2020; Roignant et al. 2018), are determined by structural differences of c.w. polymers. Heinrich and Gärtner (2008), Robards (1966) and Wilson and Gartner (1996) suggested that the tension wood severity in angiosperms, similar to compression wood severity in gymnosperms, is related to the degree of lean from the vertical or to the severity of bending treatments. However, Urbancsok et al. (2023) showed that the transcriptional program of TFW only partially overlapped the TW program.

Comparison of c.w. structural differences between TW formed in the inclined stem of *P. euramericana* (Raman spectroscopy, Guan et al. 2021) and *P. nigra* (label-free *in situ* Raman microspectroscopy, Ma et al. 2013) with presented Raman microspectroscopy results on TFW of *P. euramericana* (Figure 3) formed as a response to long term static bending treatment, suggest significant difference in c.w. structure between TW and TFW.

In TW of *P. euramericana* (grounded samples, Guan et al. 2021) and *P. nigra* (S_2 and G-layer, Ma et al. 2013), the difference in the structure of c.w. polymers was only visible in the shifts to the lower wavenumbers of the bands assigned to coniferyl alcohol and coniferyl aldehyde (compared to NW, Guan et al. 2021) and bands assigned to glycosidic C–O–C stretching vibrations, the most orientation-sensitive cellulose band (compared to OW, Ma et al. 2013).

In contrast, in TFW of *P. euramericana* (Figure 3, Table 1), differences in the structure of lignin, cellulose, hemicellulose, and pectin are suggested compared to NW.

The difference in the structure of lignin in TFW compared to NW is suggested based on the shifts of the bands: at 1,587 with the dominant spectral contribution of lignin, at 1,687 cm^{-1} characteristic for coniferyl alcohol or coniferaldehyde, at 933, 750, and 584 cm^{-1} characteristic for wagging vibrations and skeletal deformations of lignin, as well as the shoulder at 1,040 cm^{-1} visible only in G-layer.

The difference in the structure of cellulose and hemicellulose in TFW compared to NW is suggested based on the shifts of the bands at around $1,100\text{ cm}^{-1}$ (the most orientation-sensitive cellulose band), and the shoulder at $1,384\text{ cm}^{-1}$ visible only in G-layer (a non-orientation-sensitive cellulose band).

The difference in methyl esterification of pectin in TFW compared to NW is suggested by the shifts of the bands at 839 and 652 cm^{-1} .

In this regard, a comprehensive and detailed study of TW formation as a response to precisely controlled leaning treatments, as well as TFW formation as a response to precisely controlled bending treatments, would be of great importance for understanding the similarities and differences in the structure of c.w. polymers between TW and TFW.

3.4 TFW as an increased source of non-recalcitrant cellulose for biofuel production

Urbancsok et al. (2023) showed that low-intensity stem flexing stimulates growth, while induced TFW, similarly to TW (Donaldson and Singh 2016), represents an increased source of non-recalcitrant cellulose for biofuel production. The efficiency of bioethanol production depends on lignocellulose composition. It increases with higher cellulose content (Serapiglia. et al. 2013), lower cellulose crystallinity (hemicellulose/cellulose ratio) (Li et al. 2014), pectin acetylation levels (Xiao and Anderson 2013), lower lignin content (Li et al. 2014; Serapiglia. et al. 2013), higher syringyl (S) to guaiacyl (G) ratio (Kang et al. 2014), higher coniferaldehyde content (Yamamoto et al. 2020), and lower ash content (Kang et al. 2014).

Presented preliminary data suggest the difference in the structure of lignin, coniferyl alcohol and coniferaldehyde, cellulose, hemicellulose, and pectin methyl esterification, as well as the higher S/G ratio in TFW compared to NW (Figures 1 and 3). These findings encourage further investigation on *P. euramericana* TFW for biofuel production. Planting *P. euramericana*, a fast-growing tree species, as windbreak forests to block the prevailing winds could provide TFW as an increased source of non-recalcitrant cellulose for biofuel production.

4 Conclusions

The presence of lignin in the G-layer, higher S/G ratio, as well as the differences in lignin structure in different c.w. layers

between TFW and NW fibers in *P. x euramericana*, indicated by histochemical analysis, was confirmed by Raman microspectroscopy. Additionally, Raman microspectroscopy suggested the difference in cellulose and hemicellulose structure and pectin methylesterification in TFW compared to NW. The G-layer and CML contribute to the response to long-term severe static bending by opposite shifts of the band attributed to the glycosidic C–O–C bond, the most orientation sensitive cellulose band, suggesting opposite changes in cellulose microfibril orientation; higher strength characterizes cellulose in CML, opposed to weaker chemical interactions in the G-layer.

SEM showed the c.w. ultrastructure of TFW fibers: G-layer loosened from the thin S-layer, cracks within the G-layer, and the differences in cellulose microfibrils orientation/arrangement compared to S-layer of NW.

These preliminary data encourage further investigation of TFW, focusing on both changes in c.w. structure as a mechanical acclimation to stem bending and its potential for biofuel production.

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