Chemical responses to modified lignin composition in tension wood of hybrid poplar (*Populus tremula × Populus alba*)

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The effect of altering the expression level of the *F5H* gene was investigated in three wood tissues (normal, opposite and tension wood) in 1-year-old hybrid poplar clone 717 (*Populus tremula × Populus alba* L.), containing the *F5H* gene under the control of the *C4H* promoter. Elevated expression of the *F5H* gene in poplar has been previously reported to increase the percent syringyl content of lignin. The wild-type and three transgenic lines were inclined 45° for 3 months to induce tension wood formation. Tension and opposite wood from inclined trees, along with normal wood from control trees, were analyzed separately for carbohydrates, lignin, cellulose crystallinity and microfibril angle (MFA). In the wild-type poplar, the lignin in tension wood contained a significantly higher percentage of syringyl than normal wood or opposite wood. However, there was no significant difference in the percent syringyl content of the three wood types within each of the transgenic lines. Increasing the *F5H* gene expression caused an increase in the percent syringyl content and a slight decrease in the total lignin in normal wood. In tension wood, the addition of a gelatinous layer in the fiber walls resulted in a consistently lower percentage of total lignin in the tissue. Acid-soluble lignin was observed to increase by up to 2.3-fold in the transgenic lines. Compared with normal wood and opposite wood, cell wall crystallinity in tension wood was higher and the MFA was smaller, as expected, with no evidence of an effect from modifying the syringyl monomer ratio. Tension wood in all the lines contained consistently higher total sugar and glucose percentages when compared with normal wood within the respective lines. However, both sugar and glucose percentages were lower in the tension wood of transgenic lines when compared with the tension wood of wild-type trees. Evaluating the response of trees with altered syringyl content to gravity will improve our understanding of the changes in cell wall chemistry and ultrastructural properties of normal, opposite and tension wood in plants.

**Keywords:** cellulose, crystallinity, ferulate 5-hydroxylase, guaiacyl, hemicelluloses, lignin, microfibril angle, monosaccharides, *Populus*, sugars, syringyl, tension wood.

**Introduction**

Gravity induces the formation of a special type of wood called tension wood in the branches and displaced stems of porous wood in angiosperms. In inclined trunks, it is formed only on the upper side of the stem, whereas wood formed on the lower side is termed opposite wood. Generally, tension wood has more cellulose and less lignin and hemicelluloses than normal wood (Timell 1969). Tension wood is also characterized anatomically by wood with more fibers and fewer vessels of smaller diameter. In most woody angiosperms, tension wood fibers have secondary cell walls with up to two S-layers and an additional layer of gelatinous appearance (G-layer). In the G-layer, highly crystalline microfibrils are oriented almost parallel to the longitudinal axis of the fiber or what is referred to as ‘approaching zero degrees’ (Pilate et al. 2004). The microfibrils are of high tensile strength and the angle they form...
correlates with shrinkage events in the tension wood at maturation (Boyd 1977). The major components of the G-layer in the tension wood of woody dicots are, by dry weight of the acid-insoluble residue (AIR), ~70% cellulose, ~15% xyloglucan, ~2% mannan, ≤8% pectin and traces of lignin. In normal wood, the secondary cell walls have three S-layers, S1, S2 and S3. In these layers, cellulose constitutes 50% of the dry weight of the AIR. Other major components include 20–30% xylan, ~5% glucomannan and 10–20% lignin. Composition summaries of wood tissue and cell wall layers can be found in Nishikubo et al. (2007) and Mellerowicz and Gorshkova (2012).

Lignins are important phenolic polymers that are abundant in the middle lamellae and secondary cell walls of terrestrial plants (Boerjan et al. 2003). Generally, they have been perceived to provide rigidity and a hydrophobic surface to conductive cells in the xylem (Hacke et al. 2001), contributing to the mechanical and physiological importance in woody tissues. The mechanical strengths of lignin in the opposite wood and cellulose in the gelatinous fibers of tension wood are necessary for angiosperm trees to establish the gravitropic response (Fratzl et al. 2008). Lignins are derived from the random polymerization of three monolignols — coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol — and are commonly referred to as guaiacyl (G), syringyl (S) and p-hydroxyphenyl (H) units once incorporated into the lignin polymer. Across the plant kingdom, where lignin is synthesized, the composition and monomer ratios vary substantially (reviewed in Vanholme et al. 2010). These ratios have been found to change in response to external stimuli such as wind (Koehler and Telewski 2006) and gravity (Baillères et al. 1995). To understand the contribution of the monomers to tree form and function, several studies have looked at the differing ratios of monomers in different tissues or plant species (Yoshinaga et al. 1992, Chabbert et al. 1997, Hoffmann et al. 2003). However, the combined effect of other variables such as anatomy, cell wall structure and the chemical composition of these tissues and species complicates the interpretation of the effect of lignin monomer composition on structure and function. Investigating the effect of lignin composition on transgenic lines of the same species minimizes the possible bias of other structural and anatomical parameters introduced when different plant species or different growth conditions or plant species (α1 radiation (1.54 Å); the X-ray source was fit with a 0.5 mm collimator and the scattered photon collected by a GADDS detector. Both the X-ray source and the detector were set to θ = 0° for MFA determination, whereas the 2θ (source) was set to 17° for wood crystallinity determination. The average T-value of the two 002 diffraction arc peaks was used for MFA calculations, as per the method of Ukrainetz et al. (2007), whereas crystallinity was determined by mathematically fitting the data by the method of Vonk (1973). Two radii were taken from each tree and these values were averaged for each tree.

Materials and methods

Plant material and sampling

This study was conducted using wild-type hybrid poplar clone 717 (Populus tremula L. × P. alba L.) and three lines of transgenic trees (F5H37, F5H64 and F5H82), previously described by Franke et al. (2000), over-expressing the Arabidopsis F5H gene. The syringyl monomer abundance in the normal wood ranged from 68.5 mol% in the wild-type to a high of 93.4 mol% in F5H64 mutant. Young trees of verified lignin composition were grown under greenhouse conditions at Michigan State University. Pots (18-liter) with a soil-less medium (Baccto, Michigan Peat Company, Houston, TX, USA) were used with no additional fertilization or supplemental lighting. Trees with 100–120-cm-long stems were inclined 45° from the vertical to induce tension wood formation, and were grown for 3 months along with paired upright controls. For chemical analyses, stem samples were taken 15 cm above the root collar. They were debarked and air-dried at 60 °C to achieve a constant weight. Stem segments, taken from the inclined trees, were cut longitudinally to separate tension wood from opposite wood for independent chemical analyses.

Crystallinity and microfibril angle

Microfibril angle (MFA) and cell wall crystallinity were determined by X-ray diffraction according to Coleman et al. (2009) using a Bruker D8 Discover X-ray diffraction unit equipped with an area array detector (GADDS). Briefly, diffractograms were obtained from samples of radial face wood sections precision cut (1.69 mm) from the growing stem isolated 15 cm above the root collar. Wide-angle diffraction was used in the transmission mode, and the measurements were made with CuKα1 radiation (λ = 1.54 Å); the X-ray source was fit with a 0.5 mm collimator and the scattered photon collected by a GADDS detector. Both the X-ray source and the detector were set to θ = 0° for MFA determination, whereas the 2θ (source) was set to 17° for wood crystallinity determination. The average T-value of the two 002 diffraction arc peaks was used for MFA calculations, as per the method of Ukrainetz et al. (2007), whereas crystallinity was determined by mathematically fitting the data by the method of Vonk (1973). Two radii were taken from each tree and these values were averaged for each tree.
Lignin and carbohydrate analysis

Extractive-free ground wood was prepared by grinding representative samples of wood in a Wiley mill to pass through a 40-mesh sieve, and Soxhlet extracted overnight with acetone at 70 °C. The extract-free wood was then dried over P₂O₅ and retained for complete chemical analysis. A modified Klason method was employed to determine total carbohydrate and lignin ratios (Huntley et al. 2003). The AIR was quantified gravimetrically after acid hydrolysis, whereas the acid-soluble lignin was quantified using absorption spectroscopy at 205 nm (TAPPI Useful Method UM-250 1991). Carbohydrate concentration was determined by high-performance liquid chromatography (Dionex BioLC, Dionex, CA, USA) equipped with an ion exchange PA1 column, a pulsed amperometric detector with a gold electrode and an AS50 auto-injector. Prior to injection, samples were filtered through 0.45 mm filters (Millipore, Bedford, MA, USA). A 20 ml volume was loaded on the column equilibrated with 250 mM NaOH and eluted with de-ionized water at a flow rate of 1.0 ml min⁻¹, followed by a post column addition of 200 mM NaOH at a flow rate of 0.5 ml min⁻¹. Each sample was determined in triplicate.

The ratios of syringyl to guaiacyl to p-hydroxyphenyl were determined by thioacidolysis (Robinson and Mansfield 2009) using 10 mg samples of extractive-free wood. Gas chromatography analyses were performed on an HP 5890 Series II fit with a 15 m × 0.25 mm DB-5 column (J&W Scientific, 91 Blue Ravine Rd., Folsom, CA, USA) and an FID detector. The GC method used a 2.0 μl injection volume, an initial injector temperature of 250 °C and a detector temperature of 270 °C. The initial oven temperature was set to 130 °C (held for 3 min) and thereafter ramped at a rate of 3 °C min⁻¹ to 260 °C and held for 5 min. The syringyl content is reported here as the percent syringyl monomer of the total monomer content in lignin.

Data analysis

Three individual trees for each genetic line and treatment were sampled for statistical analyses. Data were analyzed using the software R (D Development Core Team 2007). Wood type and line effects were evaluated by a two-way analysis of variance. Assumptions of normality and homogeneity of variances were tested.

Results

Effects of genetic and environmental modifications on total lignin and lignin components

Inclined hybrid poplar trees of wild-type origin and the three F5H over-expression lines formed tension wood on the upper side of the leaning stems. When compared with normal wood, the tension wood of wild-type (non-transformed controls) poplar stems had a 6.4 mol% increase (P = 0.04) in percent syringyl monomer at the expense of guaiacyl monomer (Figure 1a). Transgenic line F5H64 had a statistically similar percentage of ~93 mol% in all three wood tissues. In wild-type hybrid poplar, percent total lignin in tension wood was significantly lower (P = 0.011) than in both normal or opposite wood (Figure 1b). Among normal wood samples, percent total lignin was 2.6% higher in wild-type trees than in transgenic trees (P = 0.005, correlation coefficient = −0.56). Both wood type and percent syringyl content affected the percent acid-soluble and -insoluble lignins (Figure 1c and d). On a wood sample weight basis, in addition to a lower total lignin, tension wood consistently had a lower percentage of soluble and insoluble lignins when compared with normal or opposite wood (both P < 0.001). In the normal wood of trees expressing an increasing ratio of syringyl monomer, lignin acid solubility increased by 2.3-fold (P < 0.001, correlation coefficient = 0.90), whereas acid-insoluble lignin (Klason) decreased by as much as 5.5% (P < 0.001, correlation coefficient = −0.88).

Effect on cellulose MFA and cellulose crystallinity

Both the wild-type and transgenic poplar trees that were displaced with respect to the gravitational vector produced a typical gravitropic response. This included the production of tension wood on the upper side of the stem, resulting in the reorientation of the displaced stem to the vertical position (Figure 2). Within any wood tissue, increased percent syringyl content did not affect the cellulose MFA (Figure 3a), while tension wood had a significantly lower MFA (P < 0.001). Cell wall crystallinity, which does not appear to be affected by changes in lignin composition, was significantly higher in tension wood (P < 0.001) compared with opposite or normal wood (Figure 3b).

Effect on wood carbohydrate content

In wild-type and transgenic hybrid poplar lines, normal wood had a significantly lower percentage of total sugars (P < 0.001) than tension or opposite wood (Figure 4a). Interestingly, there was a slight, yet significant, interaction between percent syringyl monomer in the lignin fraction and percent total sugar (to dry mass) in tension wood (P = 0.038, correlation coefficient = −0.63); a 19.1 mol% increase in syringyl monomer abundance in tension wood was accompanied by a 3.6% decrease in total sugar relative abundance. A similar trend was observed when the lignin modification was accompanied by a 4.1% decrease in glucose relative abundance (Figure 4b). On average, percent galactose was higher in tension wood than in normal wood (P = 0.038) or opposite wood (P = 0.004; Figure 4c). However, percent galactose in tension wood decreased with increasing abundance of syringyl and equalled its level in normal wood. The overall average percent xylose in tension wood was 2.2% lower than in normal wood (P < 0.001). Altering the percent syringyl content did not significantly affect xylose relative abundance in the three wood tissues (Figure 4d), whereas a significant interaction was shown between percent...
xylene and percent total lignin in any of the wood tissues ($P \leq 0.006$, see Figure S1 available as Supplementary Data at Tree Physiology Online). The average rhamnose percentage was higher in normal wood ($P < 0.001$) than in either tension or opposite wood. However, in spite of the differing monomer ratios in their lignin, the relative abundance of rhamnose within each wood type remained constant (Figure 4e). Manipulating the percent syringyl content in poplar trees did not affect the relative abundance of mannose (Figure 4f) or arabinose (data not shown) in the three wood types.

Discussion

Environmental stimuli, such as gravity, alter the shape and form of angiosperm trees, often resulting in stem reorientation to a new upright position driven by the tensile force of specialized xylem tissue called tension wood (Pilate et al. 2004). Different approaches have been adopted to induce tension wood formation. Andersson-Gunnerås et al. (2006) and Foston et al. (2011) induced the formation of tension wood by bending the stems (a higher mechanical stress), in contrast with displacing...
Figure 3. Effect of varying mole percent syringyl on (a) MFA and (b) cell wall crystallinity in hybrid poplar clone 717 (P. tremula × P. alba) expressing C4Hprom::F5H at different levels. Trees were kept upright or inclined with respect to gravity to form tension wood. Wood samples of normal, opposite and tension wood were dried and analyzed separately. Error bars represent means ± SE (n = 3 trees).

Figure 4. Effect of different levels of mole percent syringyl on the percentages, to dry mass, of (a) total sugars, (b) total glucose, (c) galactose, (d) xylose, (e) rhamnose and (f) mannose in hybrid poplar clone 717 (P. tremula × P. alba) expressing C4Hprom::F5H at different levels. Trees were kept upright or inclined with respect to gravity to form tension wood. Wood samples of normal, opposite and tension wood were dried and analyzed separately. Error bars represent means ± SE (n = 3 trees).
the trees with respect to gravity (a lower mechanical stress), a method we employed in this study. The inclination of unsupported angiosperm stems is usually followed by tension wood formation on the upper side of the lower stem leading to re-orientation of the stems to the vertical position. Bending of the stems leads to the formation of a wider spread of homogeneous tension wood on the side under tension. The effect of stem bending is analogous to that of snow build-up on the stem or branches, and the effect of tree inclination is similar to that of tilting trees due to sudden changes to the land surface following a landslide or stem displacement due to wind-throw (Telewski 1995).

A major anatomical characteristic of tension wood in poplars is a fiber with an additional non-lignified cell wall layer of gelatinous appearance (G-layer) that is mostly composed of highly crystalline cellulose microfibrils. Previous analyses using *P. tremula* × *P. alba* reported 24% lignin in tension wood compared with 27% in opposite or normal wood (Foston et al. 2011). Despite the fact that tension wood presents a problem to the lumber industry, its low lignin and high cellulose characteristics are of interest as a feedstock for alternative energy production (Ragauskas et al. 2006).

**Effect on lignin**

Sarkanen and Hergert (1971) reported an increase in syringyl propane units in tension wood when compared with normal wood, in birch (*Betula* sp.) and madrona (*Arbutus* sp.). Foston et al. (2011) reported an increase in the percent syringyl content in tension wood when compared with normal wood in hybrid poplar (*P. tremula* × *P. alba*), a change that is equivalent to 12.5% based on NMR data. Other studies observed a similar trend (Bland and Scurfield 1964, Baillères et al. 1995, Yoshida et al. 2002, Aguayo et al. 2010).

The presented results in hybrid poplar concur with these reports, and show a typical response to the gravitropic stimulus. Wild-type poplar had an average of 6.4% increase in the percent syringyl content in tension wood when compared with normal wood and an average of 7.6% increase when compared with the paired opposite wood section. A similar increase in the percent syringyl content was also reported for the same type of hybrid poplar trees as a result of mechanically perturbing them to simulate wind sway (Koehler and Telewski, 2006). Gravity-induced increase in the percent syringyl content in tension wood diminished in the transgenic trees utilized in this study. Exogenous C4Hpromo:FSH over-expression in these trees resulted in a lignin nearly saturated with the syringyl monomer, effectively preventing any additional increase in the percent syringyl content in tension wood.

A negative correlation existed between the percent syringyl content and the percent total lignin in normal wood in poplar stems. In a study using 13 different poplar clones (*Populus deltoides* Bartr., *P. deltoides* × *Populus maximowiczii* A. Henry F1 hybrids, *P. deltoides* × *Populus nigra* L. F1 hybrids and undisclosed *Populus* species), it was found that a strong negative correlation existed between the percent syringyl content and the lignin content (Bose et al. 2009). In the present study, the percent total lignin (soluble and insoluble) was significantly higher in normal and opposite wood when compared with the paired tension wood samples. Tension wood incorporates a mostly cel lullosic layer that adds to the total mass of the cell wall. A study employing eucalyptus showed similar results, where tension wood formation had impaired lignification compared with normal wood (Aguayo et al. 2010). In spite of the slight decrease in the percent total lignin in normal wood of the over-expression lines used in the present study, acid-soluble lignin percentage increased by up to 2.3-fold in the same tissue. The increase in lignin acid solubility was an expected outcome and is in agreement with previous studies because of the syringyl moieties being more acid labile than guaiacyl (Stewart et al. 2006). As a result of the positive correlation between lignin acid solubility and syringyl monomer abundance, increasing the percent syringyl content of lignin resulted in >60% reduction in pulping time and/or a concurrent reduction in the processing chemicals (Huntley et al. 2003).

**Effect on cellulose MFA and cellulose crystallinity**

Cellulose MFA and crystallinity were found to correlate with total lignin, growth stresses and shrinkage events in tree stems (Boyd 1977, Baillères et al. 1995, Yoshida et al. 2002). In the hybrid poplar trees that were evaluated in this study, the MFA decreased (closer to the parallel with the fiber axis) in tension wood and the cellulose crystallinity increased. These two ultrastructural changes can be correlated with the formation of the cellulose-rich gelatinous layer. These findings are consistent with previously reported characteristics of tension wood (Boyd 1977, Pilate et al. 2004). Altering the percent syringyl content in the transgenic lines did not influence secondary cell wall MFA or crystallinity in the different wood tissues. The results reported here indicate that the lignin monomer composition of angiosperm wood does not affect these ultrastructural traits.

**Effect on wood monosaccharides**

Notable differences in cell wall composition between tension wood and normal wood were found when the different cell wall layers were analyzed separately. In most woody angiosperms, the G-layer of tension wood fiber, which replaces the S3-layer in normal wood fiber, has a distinct cellulose ultrastructure, non-cellulose carbohydrates and lignin content (reviewed in Mellerowicz and Gorkowka 2012). These features give tension wood its distinct chemical and structural properties.

Of the saccharide component, hemicelluloses help in maintaining the structure of the primary and secondary cell walls. Several models have been proposed on how this can be achieved, including cross-linking the cellulose microfibrils.
(Atalla et al. 1993, Cosgrove 2000), covalently linking to pectins (Keegstra et al. 1973) and/or interacting with lignin (Sugino et al. 1994).

In *P. alba*, xylose is a component of xylglucan, a hemicellulose that exists in the G-layer of tension wood fibers (Kim and Daniel 2012). Xylglucan constitutes 10–15% of the layer dry mass, making it the most abundant non-cellulose carbohydrate of the layer (Nishikubo et al. 2007, Mellerowicz et al. 2008, Kaku et al. 2009, Mellerowicz and Gershkova 2012). The data presented in this paper show a significantly lower xylose percentage in tension wood (16.6% of dry mass) when compared with opposite (18.5%) or normal wood (19.8%). These results are similar to previous reports (Fuji et al. 1982, Foston et al. 2011). However, some other studies showed higher xylose in developing tension wood of poplar (Andersson-Gunnerås et al. 2006) and in 8-year-old eucalyptus (Aguayo et al. 2010). The latter study also found an increase in the content of hemicelluloses in tension wood, contrary to an earlier report by Timell (1969). In some cases the same plant yielded different results when analyzed at different developmental stages. Mature, light-grown pea (*Pisum sativum* L. cv. Alaska) stems yielded less xylglucan than elongating, dark-grown stems, indicating either less xylglucan later in the tissue or less accessibility to extraction enzymes due to an altered structure (Pauly et al. 2001). Similarly, tension wood samples may yield differing results when analyzed at different developmental stages. Andersson-Gunnerås et al. (2006) reported higher xylose in developing tension wood of field-grown hybrid aspen (*P. tremula*) 11 days after bending, whereas samples of fully developed tension wood of hybrid poplar (*P. tremula* × *P. alba*) bent for 60 days showed significantly lower xylose than those of normal wood (Foston et al. 2011). This would indicate that standardization for tissue age might be required for performing comparative studies of cell wall composition.

A decrease in mannose and 1,4-β-mannan in tension wood compared with normal wood of aspen was reported previously (Andersson-Gunnerås et al. 2006, Hedenström et al. 2009), whereas no significant change in percent mannose was observed in the two tissues in 8-year-old eucalyptus (*Eucalyptus globulus* Labill.) sapwood (Aguayo et al. 2010). The mannose quantification results of the present study are inconclusive due to the high variance among the replicates.

Galactose is a major component of several polymers in plant cell walls. In the G-layer, galactose and arabinose are components of both arabinogalactan II and xylglucan side chains. The latter were reported to cross-link the G-layer to the adjacent Sinnerlayer (Nishikubo et al. 2007). Similar to the results presented here for hybrid poplar, galactose was found to be higher in tension wood than in normal wood of beech species, and therefore has been proposed to be an indicator of the presence of tension wood (Ruel and Barnoud 1978, Azuma et al. 1983). However, a significant decrease in percent galactose was observed in tension wood of 8-year-old eucalyptus sapwood (Aguayo et al. 2010), indicating contrasting results from different species or tissues. The increase in syringyl lignin abundance in poplar in this study was correlated with a slight decrease in galactose abundance in tension wood. This decrease may be a result of a reduced galactose binding to xylglucan and/or arabinogalactan II in the G-layer because of a lower galactosyltransferase activity or due to reduced epimerization of glucose into galactose.

Similarly to galactose, different studies have reported contrasting results for rhamnose; one fraction of alkaline cell-wall extracts was found to contain a higher level of rhamnose in tension wood than in normal wood of Japanese beech (*Fagus crenata* Blume) (Azuma et al. 1983), whereas lower levels were found in tension wood in eucalyptus sapwood (Aguayo et al. 2010). In poplar (*P. alba*), rhamnose was reported to exist in isolated G-layer of tension wood (Nishikubo et al. 2007). The presence of rhamnose in tension wood of hybrid poplar reported in this study supports the findings of Nishikubo et al. (2007), and its occurrence in tension wood and opposite wood at lower levels than in normal wood supports the findings of Aguayo et al. (2010). Rhamnose and galacturonic acid make up the backbone of rhamnogalacturonan I (RG I) pectins. In American beech (*Fagus grandifolia* Ehrh.), rhamnose was part of galactan in the G-fiber of tension wood, suggesting the presence of RG I in these fibers (Kuo and Timell 1969). Using immunocytochemical labeling, Bowling and Vaughn (2008) reported the presence of RG I in the tension wood of sweet gum (*Liquidambar styraciflua* L.) and hackberry (*Celtis occidentalis* L.). They propose that the arabinogalactan proteins and RG I-type pectin may be responsible for creating the contractile forces within the cellulose matrix of tension wood required to bend the stem in a gravitropic response. However, according to Mellerowicz and Gershkova (2012), conclusive bio chemical proof of the presence of RG I in tension wood is still lacking.

Glucose abundance was also found to slightly decrease with increasing syringyl monomer abundance in the same samples of tension wood. The estimated glucose percentages in this study take into account the abundance present in both cellulose and hemicelluloses. In tension wood, a slight decrease in extractable glucose would be expected due to the previously reported decrease in hemicelluloses (Timell 1969). This decrease is usually offset by the increase in cellulose deposition in the G-layer, which explains the higher percent glucose in tension wood when compared with normal wood. However, the slight, yet statistically significant, decrease in tension wood glucose and overall sugar percentages in response to syringyl monomer enhancement raises further questions as to whether the ferulate 5-hydroxylase (FSH) enzyme or any of its products is involved in the signaling process for tension wood formation or whether the elevated expression of FSH protein interferes with the conversion of sucrose into glucose. Given
that these percentages are relative abundances, a quantitative change in any structural component results in a cascade shift in the calculated percentages of the remaining components.

**Conclusion**

This study confirms the results of previous studies on tension wood and provides new insight into the effect that altering the syringyl content of lignin has on the cell wall chemistry of hybrid poplar in normal, tension and opposite wood. Both wild-type and transgenic trees responded to gravity by producing tension wood on the upper side of the stem, leading to a corrective growth that returned the displaced stems to the vertical position. Earlier studies are confirmed by the following observations: tension wood in the wild-type poplar is characterized by being higher in percent glucose, cellulose crystallinity and percent syringyl monomer, and being lower in MFA and percent total lignin when compared with normal wood. Increasing the syringyl lignin by over-expressing C4Hprom::F5H in hybrid poplar in the transgenic lines also increased the acid solubility of lignin. However, increasing the syringyl lignin did not alter cellulose crystallinity or MFA in tension wood, opposite wood or normal wood compared with the wood in the wild-type line where lignin was unmodified. Similar to the wild-type line, normal wood of the transgenic lines also had lower percent total sugar than tension or opposite wood. In contrast to the wild-type line, transgenic lines exhibited no additional change in the monolignol percentage when comparing the three different types of wood (tension, opposite and normal). In normal wood, the total lignin percentage in wild-type trees was higher than that in the transgenic lines.

The results reported here focus new attention on the carbohydrate content of the different wood tissues of the tested poplar lines. With a few exceptions, the data on the cell wall composition of tension wood vs. normal and opposite wood reported here support earlier studies. Some of the conflicting results reported in earlier studies and reviewed in this study could be explained by the different experimental designs used to induce tension wood formation and in the timing of tissue harvest resulting in the analysis of tissues that are developmentally different. In general, increasing the syringyl monomer abundance in lignin does not significantly affect cellulose crystallinity or MFA when compared with the wild-type hybrid line. However, increasing the percent syringyl lignin does inhibit further enrichment of the syringyl monomer in tension wood. This could be the result of saturating the sinapyl alcohol biosynthetic pathway. What is unclear is why increasing the syringyl lignin reduced the relative abundances of total sugars and glucose, or why galactose percentage decreased in tension wood with increasing the syringyl lignin. These trends in carbohydrate chemistry might provide some insight into the role of monosaccharides as a possible link between different monolignols within the complex lignin molecule and the cellulose matrix of the secondary cell wall.

**Supplementary data**

Supplementary data for this article are available at Tree Physiology Online.

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**Conflict of interest**

None declared.

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