THERMAL SOFTENING OF TRANSGENIC ASPEN

Balazs Horvath,[a] Perry Peralta,[a] Charles Frazier,[b] and Ilona Peszlen [a,*]

Studies on the softening behavior of *in situ* lignin of normal wood in a given species have never been performed before due to the relatively narrow lignin content and lignin structural variation within one species. Using transgenic trees with different levels of lignin content and/or syringyl to guaiacyl propane (S/G) ratio helped us to overcome this problem. Submersion three-point bending and parallel-plate compression-torsion dynamic mechanical analyses were conducted on one-year-old wild type and transgenic aspen (*Populus tremuloides* Michx.). The different genetic modifications included groups with reduced lignin content, increased S/G ratio, and both reduced lignin content and increased S/G ratio. Measurements with both methods revealed a statistically significant decrease in glass transition temperature in the reduced-lignin genetic group compared to the wild-type. Increase in the S/G ratio did not affect the thermo-mechanical properties; these results contradict claims that increasing the methoxyl groups would reduce lignin cross-linking and the glass transition temperature.

Keywords: Genetically modified trees; Transgenic aspen; Dynamic mechanical analysis; Compression-torsion, Glass transition temperature, Loss modulus, Storage modulus, Tan delta

*Contact information: a: Department of Forest Biomaterials, North Carolina State University, Raleigh, NC 27695-8005 USA; b: Department of Wood Science and Forest Products, Virginia Tech, Blacksburg, VA 24061-0323 USA; * Corresponding author: ilona_peszlen@ncsu.edu*

INTRODUCTION

Many properties of wood are affected when it is heated. Of particular interest during the manufacture of wood and wood products is when wood changes from a stiff and glassy to a soft and rubbery material as a result of heating. In this case, wood is said to have gone through its glass transition temperature (T_g). Wood is composed of semi-crystalline cellulose microfibrils embedded in a matrix of amorphous lignin and hemicelluloses. These wood polymers have different softening properties (Goring 1963). When dry, the differences in glass transition temperature are not huge: 200 to 250 °C for the amorphous region of cellulose, 150 to 220 °C for hemicelluloses, and 205 °C for lignin (Back and Salmén 1982). An increase in the moisture content lowers the glass transition temperatures (Goring 1963) and sharpens the main transition of the wood components (Placet et al. 2007). At the water-saturated condition, the T_g of isolated amorphous cellulose and of hemicelluloses decrease to subambient temperatures (Back and Salmén 1982). Isolated water-saturated lignin has a softening temperature of ca. 100 °C (Salmén 1984). The major glass transition of water-saturated wood also occurs near 100 °C, and so the softening of wood is often attributed to the glass transition of *in situ* lignin (Salmén 1984). Because of this major influence of lignin on the viscoelastic

properties of wood, the softening of lignin has become the target of process engineering during pulping, ethanol production, and manufacture of wood composites.

The effect of various parameters on the viscoelasticity of lignin has been studied by different investigators. Relying on the fact that the lignin content of earlywood is somewhat higher than that of latewood, Wennerblom et al. (1996) studied the difference in the softening of these two parts of a growth ring. Their results showed no such difference. Birkinshaw et al. (1986) found that whereas chemical composition was important, microstructural variations from species to species and indeed from softwood to hardwood were not important in determining the softening behavior. The *in situ* lignin glass transition of hardwood was found by Olsson and Salmen (1992, 1997) to be lower than that of softwood due to the higher amount of methoxyl groups present in hardwoods. They explained that the methoxyl groups tend to block the cross-linking between aromatic units during biosynthesis of the wood, thereby resulting in a more flexible network with a lower glass transition temperature. In addition to lignin type, differences in hardwood and softwood lignin glass transition are affected by many other factors, such as lignin content and anatomical structure. Studies on the softening behavior of *in situ* lignin of normal wood in a single species have never been performed before due to the relatively narrow lignin content and lignin structural variation within a given species. Using transgenic trees with different levels of lignin content and/or syringyl to guaiacyl propane (S/G) ratio helped us overcome this problem.

Among the hardwoods, poplars are favorable for mechanical pulping mainly due to their relatively low lignin content of about 20% (Blechschmidt et al. 1986a, 1986b). This was one of the driving forces for forest geneticists to modify the lignin biosynthetic pathway of aspen (*Populus tremuloides* Michx.). Using genetic engineering, the lignin content of this species has been successfully reduced by the insertion of antisense 4CL gene (Hu et al. 1999), and its S/G ratio has been increased by the transfer and expression of sense CAld5H gene (Li et al. 2001). Simultaneous insertion of these genes has resulted in both a reduction in lignin content and an increase in the S/G ratio. These transgenic trees provide a great opportunity for gaining scientific understanding of the relationship between lignin content/structure and thermo-mechanical properties. Genetic engineering pushes the manipulation of the amorphous nature of lignin upstream to the time period when the chemical component is being laid down by the tree.

The objective of this study was to evaluate the thermal softening behavior of genetically modified aspen trees with reduced lignin content and/or increased S/G ratio. The specific objective was to determine the storage and loss modulus, tan delta, and glass transition temperature of transgenic wood by dynamic mechanical analysis using two different submersion methods.

EXPERIMENTAL

Dynamic mechanical analysis (DMA) is a well-established technique for measuring the *in situ* thermomechanical transitions of polymers (Birkinshaw et al. 1986) and is one of the most powerful tools available for the study of structure-property relationship in wood (Rials and Glasser 1984). In this study, submersion three-point

bending and parallel plate compression-torsion dynamic mechanical analysis methods were used.

Materials

One-year-old wild-type and transgenic aspen (*Populus tremuloides* Michx.) were tested (Li et al. 2003). Eight genetic lines from four genetic groups were studied: (1) genetic group PtrWT as wild type with one genetic line (line 271); (2) genetic group Ptr4CL with reduced lignin content, which included three genetic lines (line 21, 23, 37); (3) genetic group PtrCAld5H with increased S/G ratio, which included two genetic lines (line 94 and 96); and (4) genetic group Ptr4CL/CAld5H with both reduced lignin content and increased S/G ratio, which included two genetic lines (line 72 and 141). The chemical compositions of these genetic groups can be seen in Table 1. Genetic lines Ptr4CL/CAld5H-72 and Ptr4CL/CAld5H-141 were handled as separate genetic groups due to the differences in their chemical composition.

Five sample trees were obtained from each genetic line. Specimens 25 mm long, 1 to 5 mm wide (Tangential), and 1 mm thick (Radial) were prepared from each genetic line using a micro circular saw. All specimens were dried at room temperature over phosphorous pentoxide (P_2O_5) under vacuum for at least seven days. After drying, the specimens were submerged in ethylene glycol (EG) using vacuum/pressure treatment and were kept saturated throughout all measurements.

Determination of the Linear Viscoelastic Region

Preliminary isothermal strain-sweep experiments were conducted at the temperature extremes on a representative sample set to determine the linear viscoelastic region (LVR). This region is defined as inclusive of the strain values that caused a 5% or less reduction in the initial storage modulus (Sun et al. 2007). We established an LVR strain limit of 0.07% for our specimens, and so subsequent dynamic mechanical analysis experiments were performed in such a way as to not exceed this value.

Submersion Three-point Bending in a Dynamic Mechanical Analyzer

A submersion three-point bending clamp set to a span of 15 mm was used to conduct the viscoelastic experiments in a TA Instruments DMA Q800 (Horvath et al. 2010b). The samples were oriented with the longitudinal direction parallel to the span. The sample was kept submerged in ethylene glycol during the measurement. Two temperature scans at a heating rate of 2°C/min from 30 °C to 95 °C were performed at 0.05 Hz frequency and 0.05% strain. The thermal history of each specimen was removed during the first temperature scan. The first heating stage was followed by cooling to room temperature at a rate of 10 °C/minute. During the temperature scans, storage modulus, loss modulus, and tan delta were recorded.

Parallel Plate Compression-torsion in a Rheometer

The viscoelastic properties of the genetic lines in compression-torsion were measured as described in previous studies by Lopez-Suevos and Frazier (2005, 2006). The applicability of this method to the characterization of small biomass specimens was validated by Chowhudry et al. (2010). The same samples that were used in the

submersion three-point bending test were used in this part of the study. The experiments were performed in a TA Instruments AR-2000 rheometer fitted with 8-mm-diameter stainless steel parallel plates. The bottom plate was inside a stainless steel cup that contained the specimen and ethylene glycol. The specimens were measured in the TR grain orientation, that is, the tangential surfaces [T] were in contact with the parallel plates while the torsion axis was in the radial direction [R] during the compression-torsion measurements. To ensure good contact between the specimen and the plates, a normal force of 5±0.1N was applied on the specimen. Experiments were conducted under strain control at a strain value of 0.07%. Two temperature scans were conducted from 0 °C to 130 °C at a heating rate of 3 °C/min and frequency of 1Hz. The first temperature scan was used to remove the thermal history of the specimens. Tan delta curves were recorded during the temperature scans.

Experimental Data Analysis

The storage modulus, loss modulus, and tan delta curves were recorded as a function of temperature using Data Analysis v.5.4.8 software from TA Instruments. Six- to eight-order polynomials were fitted to these curves and were used to predict the mean and standard deviation values for each of the genetic groups. The temperature corresponding to the peak in the polynomial curve fitted to the tan delta data was used to determine the glass transition temperature (T_g). To test the effect of genetic modification on the measured properties, SAS Enterprise Guide 4.1 (SAS Institute Inc, 2006) was used. Due to the large diameter growth differences between genetic groups (Horvath *et al.* 2010a), stem diameter was used as a covariate in the general linear model,

$$Y_{ijk} = \mu + \beta D_{ijk} + G_i + L_j(G_i) + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} = subject of interest (measured properties), μ = overall mean, β = coefficient related to diameter, D_{ijk} = stem diameter of the k^{th} tree in the j^{th} genetic line of the i^{th} genetic group, G_i = effect of i^{th} genetic group, $L_j(G_i)$ = effect of j^{th} genetic line in a genetic group, and ε_{ijk} = random error with $E(0, \sigma^2)$. Duncan multiple range tests were used to determine significant differences between genetic groups for each of the measured properties at α=0.05.

RESULTS AND DISCUSSION

On analysis of the results of the general linear model, it was found that stem diameter and genetic lines within genetic groups had no significant effect, but genetic groups had a significant effect on the measured properties. Submersion three-point bending DMA measurements showed that the storage modulus of wild-type, genetic group PtrCAld5H with increased S/G ratio, and genetic line Ptr4CL/CAld5H-141 with both reduced lignin content and increased S/G ratio did not differ statistically (Table 1); however, genetic group Ptr4CL with reduced lignin content and genetic line Ptr4CL/CAld5H-72 with both reduced lignin content and increased S/G ratio had significantly lower storage modulus. These results are in general agreement with the elastic moduli of the transgenic aspen measured using a micromechanical bending test

(Horvath et al. 2010c), an ultrasonic dynamic test, and static DMA tests in water and ethylene glycol (Horvath et al. 2010b). Examining the second heat temperature scans, the wild-type and the genetic group with increased S/G ratio demonstrated similar softening behavior. The genetic group with reduced lignin content had lower glass transition temperature (72°C) and a different curve shape than the wild-type (84°C) and other transgenics (Fig. 1).

Table 1. Glass Transition Measurements for Wild-Type and Transgenic Aspen Clones Using a Dynamic Mechanical Analyzer in Submersion 3-Point Bending Mode

Genetic Group	Description	Genetic Line	Lignin Content[1] (%)	S/G Ratio[2]	Stem Diameter (mm)	Thermo-mechanical Properties			
						N	Storage Modulus at 30°C (MPa)	Tan Delta Peak	Glass Transition (°C)
PtrWT	Wild-type (unchanged)	271	23.2	2.2	9.3	7	3266 [a] (16.1)	0.21 [a] (8.6)	84 [bc] (4.0)
Ptr4CL	Reduced lignin content	21 & 23 & 37	17.5	2.1	9.6	10	1903 [b] (12.0)	0.22 [a] (12.3)	72 [d] (5.4)
PtrCAld5H	Increased S/G ratio	94 & 96	21.9	5.2	7.4	8	2791 [a] (15.8)	0.19 [b] (7.0)	85 [ab] (4.9)
Ptr4CL/CAld5H	Reduced lignin content, Increase S/G ratio	72 *	17.0	3.6	9.2	6	1544 [b] (18.4)	0.20 [ab] (4.1)	88 [a] (3.3)
		141 *	21.2	2.7	9.6	7	2791 [a] (13.0)	0.20 [ab] (3.0)	80 [c] (3.1)

[1] Actual lignin content of sample trees measured by Horvath *et al.* (2010c);
[2] S/G ratio represents the syringyl to guaiacyl lignin content ratio of each genetic line adopted from Li *et al.* (2003).
[3] Common superscript letter(s) indicate(s) values that are not significantly different from each other as determined by Duncan multiple range test at α=0.05. Values in parentheses are percent coefficient of variation.
[4] Genetic line Ptr4CL/CAld5H-72 and genetic line Ptr4CL/CAld5H-141 were handled as separate genetic groups because of the large lignin content differences within genetic group Ptr4CL/CAld5H.

Parallel plate compression-torsion measurements (Table 2) indicated that the glass transition temperatures of the genetic group PtrCAld5H with increase S/G ratio and genetic line Ptr4CL/CAld5H-72 did not differ statistically from that of the wild-type. Similar to the results for the submersion three-point bending measurements, the genetic group with reduced lignin content had lower glass transition temperature (82°C) for the second temperature scan than the wild-type (89°C). However, its T_g and the shape of its tan delta curve were closer to those of the other transgenics (Fig. 2).

The results of the glass transition temperature measurements obtained with both methods were highly reproducible, ranged between 71 and 96°C, and were in agreement with those reported elsewhere. Olsson and Salmén (1992) reported for aspen (*Populus tremula*) a glass transition temperature of 68 °C at 0.6 Hz frequency under water-saturated condition. For the same species, a glass transition temperature of 80±2 °C at 1 Hz frequency was reported by Olson and Salmén (1997). Bjurhager et al. (2010) studied transgenic *Populus* with 30% less lignin content and found lower glass transition

temperature for the transgenic, but the difference between wild type (69±0°C) and transgenic (67±0.5°C) was not statistically significant. Differences in target enzymes, loading mode, plasticizer, frequency, heating rate, and method of determining T_g could have caused the difference in the results between our study and that of Bjurgaher et al. (2010).

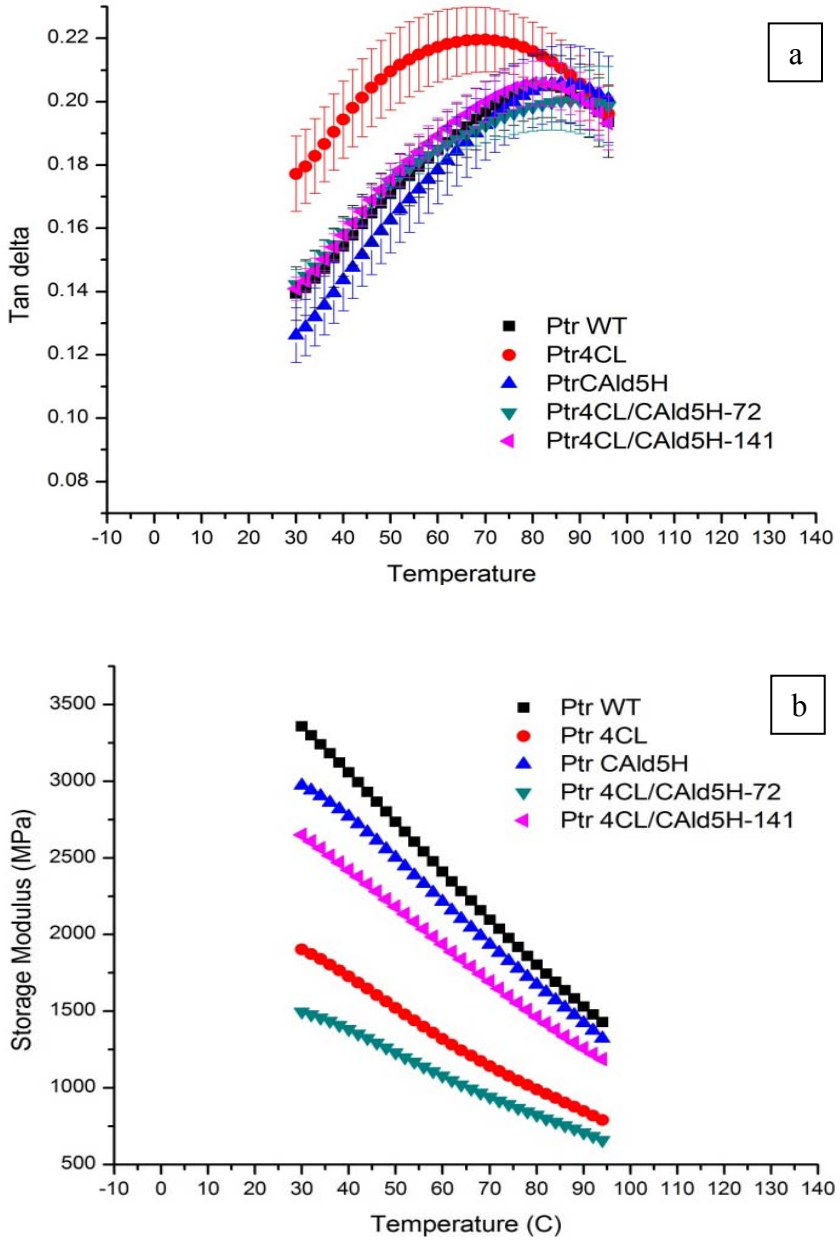

Fig. 1. Tan delta (a) and storage modulus (b) curves from the second heat of the submersion three-point bending dynamic mechanical analysis using 0.05Hz frequency and heat rate of 2°C/min. Each point represents the average value of several measurements. In Part (a), whiskers represent the standard deviation. Standard deviation whiskers for part (b) are too small to be visible on the graph.

Table 2. Glass Transition Measurements for Wild-Type and Transgenic Aspen Clones Using a Rheometer in Compression-Torsion Mode

Genetic Group	Description	Genetic Line	Thermo-mechanical Properties				
			First Heat			Second Heat	
			N	Glass Transition Temperature[1] (°C)	Tan Delta Peak[1]	Glass Transition Temperature[1] (°C)	Tan Delta Peak[1]
PtrWT	Wild-type (unchanged)	271	4	91 ^{a} $_{A}$ (3.7)	0.18 ^{a} $_{A}$ (13.3)	89 ^{a} $_{A}$ (7.4)	0.16 ^{a} $_{A}$ (13.9)
Ptr4CL	Reduced lignin content	21 & 23 & 37	10	81 ^{c} $_{A}$ (7.6)	0.18 ^{a} $_{A}$ (4.2)	82 ^{b} $_{A}$ (3.8)	0.15 ^{ab} $_{B}$ (7.6)
PtrCAld5H	Increased S/G ratio	94 & 96	6	85 ^{ab} $_{A}$ (4.3)	0.18 ^{a} $_{A}$ (7.3)	85 ^{ab} $_{A}$ (4.3)	0.15 ^{ab} $_{B}$ (3.5)
Ptr4CL/CAld5H	Reduced lignin content, Increase S/G ratio	72[2]	3	83 ^{bc} $_{A}$ (6.1)	0.18 ^{a} $_{A}$ (7.3)	86 ^{ab} $_{A}$ (7.4)	0.14 ^{b} $_{B}$ (2.3)
		141[2]	4	89 ^{ab} $_{A}$ (1.3)	0.17 ^{a} $_{A}$ (8.9)	82 ^{b} $_{B}$ (3.3)	0.14 ^{b} $_{B}$ (5.4)

[1]Differences among genetic groups for each property are denoted by lowercase superscript letters as determined by Duncan multiple range test at α=0.05. Difference between the first and second heat property within a genetic group are denoted by uppercase subscript letters as determined by Duncan multiple range test at α=0.05. Values in parentheses are percent coefficient of variation.
[2]Genetic line Ptr4CL/CAld5H-72 and genetic line Ptr4CL/CAld5H-141 were handled as separate genetic groups, because of the large lignin content differences within genetic group Ptr4CL/CAld5H.

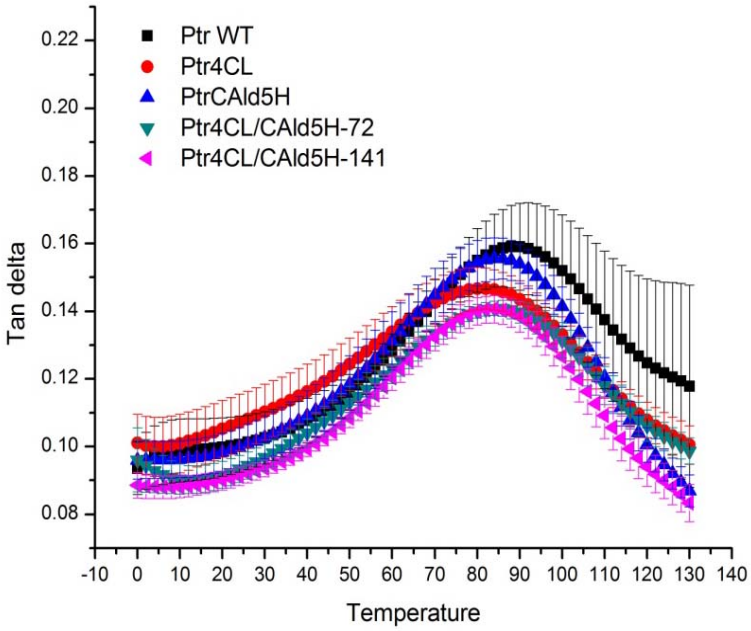

Fig. 2. Tan delta curves from the second heat of the parallel plate compression-torsion dynamic mechanical analysis using 1Hz frequency and a heating rate of 3 °C/min. Each point represents the average value of several measurements. Whiskers represent the standard deviation.

Our results demonstrate that an increase in the S/G ratio did not have a significant effect on the glass transition temperature. This finding suggests that the proportionally higher content of methoxyl groups and thus less cross-linked lignin in genetic group CAld5H did not alter the softening behavior. This contradicts the argument of other researchers that a high amount of methoxyl groups in hardwoods results in less cross-linking and in lower softening temperature compared to softwoods (Olsson and Salmen 1992, 1997; Placet et al. 2007). It needs to be noted that their comparisons were based on hardwood lignin and softwood lignin having structural differences. Lignin content differences were not taken into consideration. In their measurements, the lower glass transition temperature of hardwood lignin may have been affected by both the lower lignin content and the higher content of methoxyl groups within the lignin that was present. Due to the relatively low lignin content and structural variation within one species, these effects (lignin content and structure) on the softening behavior of *in situ* lignin has not been studied before. Using transgenic trees with different levels of lignin content and/or structure (S/G ratio) helps us to overcome this problem and makes it clear that lignin glass transition temperature is only affected by the lignin content changes and not by the structural changes.

Based on the current investigation, reduction in lignin content resulted in a decrease in the glass transition temperature. The hypothesis is that with lower lignin content there is more free volume present between the polymers. This could allow more translational and rotational movements of the polymers whose motions become progressively activated with increasing temperature. With increasing free volume and internal motions, the glass transition shifts to a lower temperature (Irvine 1985).

Both the submersion three-point bending and the parallel plate compression-torsion proved useful in characterizing the thermal softening of the transgenics. Although these methods showed similar trends for glass transition temperatures and tan delta between genetic groups, direct data comparison should be avoided due to differences in stress modes and clamping method as explained by Chowdhury et al. (2010). Since this study's objective was to evaluate the effect of lignin modification on the thermal softening behavior of transgenics, the compression-torsion method seems more appropriate. The samples used in this study came from trees with diameter of about 10 mm, and so sample configuration was a limiting factor in designing the experiments. Compression-torsion DMA has been proven effective in probing the lignin glass/rubber transition of very small biomass specimens and/or specimens with poor mechanical integrity (Chowdhury et al. 2010). In three-point bending parallel to the grain, cellulose has a significant role in imparting stiffness to the wood and might be masking the subtle effect of lignin content and structure modification on the softening of wood. Compression-torsion minimizes this and is able to better probe the role of lignin as a matrix material.

CONCLUSIONS

1. Investigating transgenic trees with different levels of lignin content and/or S/G ratio provided the opportunity to explore how the thermal softening behavior of *in situ* lignin is affected by changes in the amount and structure of lignin.

2. Results showed that a reduction in lignin content decreased the glass transition temperature of aspen. However, an increase in the S/G ratio did not affect the glass transition temperature.
3. The similar softening behavior of the wild type group and the group with increased S/G ratio contradicts the argument that a high amount of methoxyl groups should result in a lowering of the lignin glass transition temperature.
4. Both submersion three-point bending and compression-torsion dynamical mechanical analyses proved useful in evaluating thermal softening behavior of *in situ* lignin in one-year-old transgenic aspen.

ACKNOWLEDGMENTS

This project was supported by the National Research Initiative of the US Department of Agriculture – Cooperative State Research, Education, and Extension Service, grant number 2005-35504-16145. The plants and greenhouse facilities were provided by Dr. Vincent Chiang, North Carolina State University, Forest Biotechnology Group.

REFERENCES CITED

Back, E. L., and Salmén, N. L. (1982). "Glass transitions of wood components hold implications for molding and pulping processes," *Tappi* 65,107-110.

Birkinshaw, C., Buggy, M., and Henn, G. G. (1986). "Dynamic mechanical analysis of wood," *J. Material Sci*. Lett 5, 898-900.

Bjurhager I., Olsson, A. M., Zhang, B., Gerber, L., Kumar, M., Berglund, L. A., Burgert, I., Sundberg, B., and Salmén, L. (2010). "Ultrastructure and mechanical properties of *Populus* wood with reduced lignin content caused by transgenic down-regulation of cinnamate 4-hydroxylase," *Biomacromolecules* 11(9), 2359-2365.

Blechschmidt, J., Engert, P., and Stephan, M. (1986a). "The glass transition of wood from the viewpoint of mechanical pulping," *Wood Sci. Technol.* 20, 263-272.

Blechschmidt, J., Engert, P., and Stephan, M. (1986b). "Thermal softening of wood – Fundamentals for mechanical pulping," *Cellulose Chem. Technol.* 20, 257-266.

Chowdhury, S., Fabiyi, J., and Frazier, C. E. (2010). "Advancing the dynamic analysis of biomass: Comparison of tensile-torsion and compressive-torsion wood DMA," *Holzforschung* 64, 747-756.

Goring, D. A. I. (1963). "Thermal softening of lignin, hemicellulose and cellulose," *Pulp Pap. Mag. Can.* 64, 517-527.

Horvath, B., Peszlen, I., Peralta, P., Kasal, B., and Li, L. (2010a). "Effect of lignin genetic modification on wood anatomy of aspen trees," *IAWA J.* 31(1), 29-38.

Horvath, B., Peszlen, I., Peralta, P., Horvath, L., Kasal, B., and Li, L. (2010b). "Elastic modulus determination of transgenic aspen using a dynamical mechanical analyzer in static bending mode" *For. Prod. J.* 60(3), 296-300.

Horvath, L., Peszlen, I., Peralta, P., Kasal, B., and Li, L. (2010c). "Mechanical properties of genetically engineered young aspen with modified lignin content and/or structure," *Wood Fiber Sci.* 42(3), 310-317.

Hu, W. J., Harding, S. A., Lung, J., Popko, J. L., Ralph, J., Stokke, D. D., Tsai, C.J., and Chiang, V. L. (1999). "Repression of lignin biosynthesis promotes cellulose accumulation and growth in transgenic trees," *Nat. Biotechnol.* 17, 808-812.

Irvine, G. M. (1985). "The significance of the glass transition of lignin in thermomechanical pulping," *Wood Sci. Technol.* 19, 139-149.

Li, L., Cheng, X. F., Leshkevich, J., Umezawa, T., Harding, S. A., and Chiang, V. L. (2001). "The last step of syringyl monolignol biosynthesis in angiosperms is regulated by a novel gene encoding sinapyl alcohol dehydrogenase," *The Plant Cell* 13, 1567-1585.

Li, L., Zhou, Y., Cheng, X., Sun, J., Marita, J. M., Ralph, J., and Chang, V. L. (2003). "Combinatorial modification of multiple lignin traits in trees through multigene cotransformation," *Proc. Natl. Acad. Sci. USA* 100, 4939-4944.

López-Suevos, F., and Frazier, C.E. (2005). "Parallel-plate rheology of latex films bonded to wood," *Holzforschung* 59, 435-440.

López-Suevos, F., and Frazier, C. E. (2006). "Rheology of latex films bonded to wood: Influence of cross-linking," *Holzforschung* 60, 47-52.

Olsson, A. M., and Salmén, L. (1992). "Viscoelasticity of in situ lignin as affected by structure softwood vs. hardwood," *ACS Symp. Series USA* 489, 133-149.

Olsson, A. M., and Salmén, L. (1997). "The effect of lignin composition on the viscoelastic properties of wood," *Nordic Pulp Pap. Res. J.* 12, 140-144.

Placet, V., Passard, J., and Perré, P. (2007). "Viscoelastic properties of green wood across the grain measured by harmonic tests in the range 0-95°C: Hardwood vs. softwood and normal wood vs. reaction wood," *Holzforshung* 61, 548-557.

Rials, T. G., and Glasser, W. G. (1984). "Characterizing wood components as network polymers by dynamic mechanical analysis," *Wood Fiber Sci.* 16, 537-542.

Salmén, L. (1984). "Viscoelastic properties of in situ lignin under water-saturated conditions," *J. Mater. Sci.* 19, 3090-3096.

SAS Institute Inc. (2006) SAS Enterprise Guide 4.1. SAS Institute Inc., Cary, North Carolina.

Sun, N., Das, S., and Frazier, C. (2007). "Dynamic mechanical analysis of dry wood: Linear viscoelastic response region and effects of minor moisture changes. *Holzforschung* 61, 28-33.

Wennerblom, M., Olsson, A. M., and Salmén, L. (1996). "Softening properties of earlywood and latewood of spruce," *Nordic Pulp Pap. Res. J*. 4, 279-280.

Article submitted: March 6, 2011; Peer review completed: April 2, 2011; Revised version received and accepted: April 22, 2011; Published: April 25, 2011.