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ELASTIC MODULUS OF TRANSGENIC ASPEN

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ABSTRACT

Changing lignin content and structure in trees by manipulating genes encoding key enzymes in various lignin biosynthetic pathways provides a great potential to efficiently utilize woody biomass in pulp and paper making and ethanol production. Solid wood and composite board applications could also be possible for genetically engineered trees, where mechanical properties are crucial. In this work, the elastic modulus of wild-type and transgenic aspen (*Populus tremuloides* Michx.) with reduced lignin content and increased syringyl to guaiacyl ratio was investigated. A total of fifty sample trees were harvested from the green house after 2.5-years of growth and used to measure the static and dynamic modulus of elasticity (MOE). Dynamic MOE was determined by Fakopp Microsecond Timer; while static MOE was measured by three-point bending using micromechanical testing. Based on the results, a reduction in the lignin content reduced both the dynamic and static MOE. An increase in the syringyl to guaiacyl ratio also resulted in a decrease in the elastic moduli but to a lesser extent. However, the combined influence of lignin content and structural changes showed the most obvious negative effect on the elastic properties. Dynamic MOE and static MOE values showed similar trend across genetic groups, thus the Fakopp Microsecond Timer can be used to predict the elastic modulus of small diameter trees.

KEY WORDS: nondestructive evaluation, Quaking aspen, *Populus tremuloides* Michx., genetic engineering, lignin content, syringyl to guaiacyl ratio, Fakopp Microsecond Timer

INTRODUCTION

Advances in forest genetic engineering have enabled scientists to change the chemical composition of wood. Trees with different amounts and types of lignin have already been developed by manipulating the lignin biosynthetic pathway with the expression of various sense and antisense genes (Li et al. 2003). Transgenic wood with reduced lignin content and changed syringyl to guaiacyl ratio (S/G ratio) can potentially decrease the amount of chemicals and energy used in wood pulping and bio-ethanol conversion resulting in cost savings and less pollution (Talukder 2006). On the other hand, the genetic modification of lignin could negatively impact wood mechanical properties which are critical to solid wood or composite board applications. Therefore, testing the mechanical properties of young transgenic trees (1-3 years old) is essential to answer the question of how these trees will perform in different applications (Kasal et al. 2007) and also during field trials (Chiang 2006).

Traditional assessment of mechanical properties requires certain dimensions of test specimens as specified by American Society for Testing and Materials (ASTM) standards. These relatively large specimens cannot be prepared from young trees with a diameter of 8-14mm. Micromechanical testing was developed to evaluate mechanical properties of young trees by Kasal et al. (2007), where special equipment and procedures were designed to measure static modulus of elasticity (MOE). Sample preparation and the mechanical testing of non-standard specimens are time consuming, shape dependent (longitudinal curvature of the stem), and require expensive testing machines.

Nondestructive evaluation (NDE) by acoustic method provides a fast and simple prediction of mechanical properties at modest equipment cost by measuring the velocity of sound propagation along a piece of wood (Lindstrom et al. 2002, Huang et al. 2003). Numerous studies have been conducted to find ways to assess wood properties such as stiffness, degradation, microfibril angle through nondestructive techniques (Ross and Pellerin 1994, Bucur 1996, Huang et al. 2003, Wang et al. 2004a). Most of the research works address softwoods because of their structural applications, where nondestructive testing can have advantages. Limited research has focused on fast-growing hardwood species and no information is available on young trees with small diameter.

In this study, the overall objective is to evaluate the effect of lignin content and structure change on the elastic modulus of young (2.5-year-old) transgenic aspen trees with small diameters (8-14 mm). A specific objective is to compare dynamic MOE measured by a newly developed Fakopp Microsecond Timer and static MOE determined by micromechanical testing.

MATERIALS AND METHODS

Wild-type (clone 271) and transgenic quaking aspen (*Populus tremuloides* Michx.) groups with reduced lignin content (Ptr4CL), increased S/G ratio (PtrCAld5H), and both reduced lignin content and increased S/G ratio (Ptr4CL/CAld5H) were investigated (Tab. 1) (Li et al. 2003).

Genetic group Ptr4CL had three genetic lines (line 21, 23, 37), PtrCAld5H had two genetic lines (line 94, 96) and Ptr4CL/CAld5H had also two genetic lines (72, 141). Due to the considerable differences in chemical composition between genetic lines 72 and 141 (Tab. 1), these two lines were handled as separate genetic groups in the statistical analysis. A total of fifty sample trees were harvested from the green house of the Forest Biotechnology Group, North Carolina State University after 2.5-years of growth.

Tab. 1: Chemical composition of wild-type and transgenic aspen

	Ptr WT Wild-type	Ptr 4CL Reduced Lignin Content			Ptr CAld5H Increased S/G Ratio		Ptr 4CL/CAld5H Reduced Lignin Content, Increased S/G Ratio	
	271	21	23	37	94	96	72	141
Number of Sample Trees	8	6	8	6	7	5	3	7
Stem Diameter with Bark (mm)	13.9	13.9	13.1	12.6	9.8	9.8	13.0	13.3
Lignin Content [1] (%)	21.3	16.0	14.4	14.3	19.9	19.6	16.8	19.3
Syringyl/Guaiacyl Ratio [2]	2.2	2.1	2.2	2.1	4.9	5.5	3.6	2.7

[1] Actual lignin content measured for sample trees used in this study by Horvath et al. (2010); [2] Adopted S/G ratio values from Li et al. (2003).

Dynamic Modulus of Elasticity Measurements

A Fakopp Microsecond Timer was used to measure the ultrasonic transmission time between two piezo sensors on wood specimens at the green condition (Fig. 1). No coupling medium was used; the sensors were pressed to the sample surface to eliminate air gap between sample and sensor. One sensor was excited by a 300 V impulse for a duration of 20 microseconds. The result of the excitation was a 45 kHz frequency ultrasonic pulse.

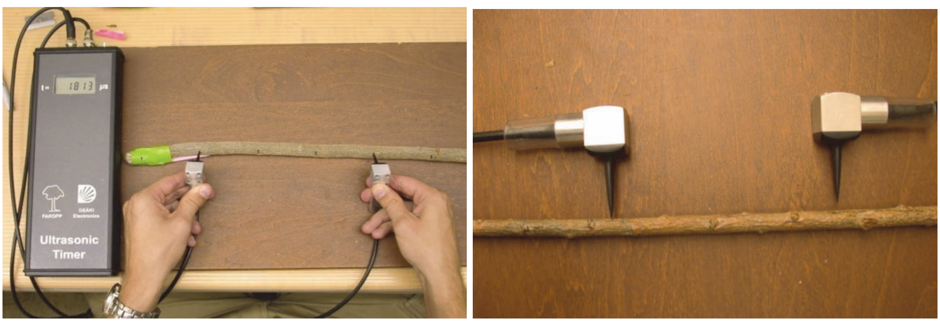

Fig. 1: Measuring ultrasonic transmission time between two piezo sensors using a Fakopp Microsecond Timer

The excitation signal started the timer of the instrument. The timer was stopped by the receiver sensor when the pressure wave arrived. The time resolution of the instrument was 1 microsecond and the pulse repetition rate was 1 Hz. One sensor remained stationary at one end; the other sensor was moved up to six different locations of equal distance depending on the stem length (i. e. for a stem length of 600 mm, the distances of the sensors were set to 100, 200, 300, 400, 500, and 600 mm from each other). The velocity of sound propagation (V) was calculated from the slope of the ultrasonic transmission time versus distance linear regression line. After the ultrasonic transmission time measurements, 20 mm thick disks were cut from the base of each stem and used to measure the moisture content and the green specific gravity of the sample trees using a standard water displacement method. The density of each sample tree at the given moisture content was calculated. The dynamic MOE of each sample tree was determined using Christoffel's fundamental equation:

$$\text{Dynamic MOE} = \rho_{MC} \cdot V^2 \tag{1}$$

where Dynamic MOE = dynamic modulus of elasticity (Pa), ρ_{MC} = density of specimen at given moisture content ($kg.m^{-3}$), V = velocity of sound propagation ($m.s^{-1}$).

Static modulus of elasticity measurements

Static modulus of elasticity was measured at the green condition by three-point bending using a modified ASTM D143 standard (Kasal et al. 2007) on the same samples trees that were used for dynamic MOE determination. Fixed roller supports were used to support the cylindrical-shaped specimens, keeping a span-to-diameter ratio of 15. The specimen had a 10 mm overhang at each support. A specially designed bearing block was used to avoid surface crushing of the specimens (Fig. 2). An MTS Alliance RF/300 mechanical testing machine with an MTS Testwork system was used to obtain the load-deflection curve.

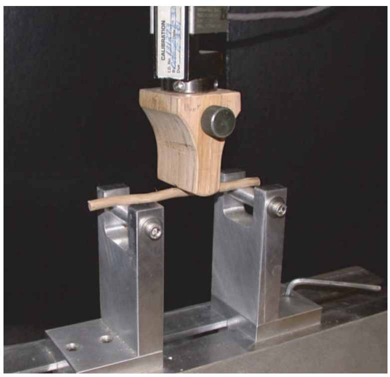

Fig. 2: Static three-point bending test with a special bearing block

The static MOE was calculated as follows:

$$\text{Static MOE} = s \cdot \frac{4 \cdot L^3}{3 \cdot \pi \cdot \left(D^4 - d^4\right)} \tag{2}$$

where Static MOE = static modulus of elasticity (MPa), s = slope of the linear portion of the load-deflection diagram ($N.mm^{-1}$), L = span distance between the two supports (mm), D = diameter of the specimen at mid-length (mm), d = diameter of the pith (mm).

Experimental data analysis

Descriptive statistics and general linear model (GLM) procedure were used to test the effect of genetic modification and genetic line within genetic groups on dynamic MOE and static MOE using SAS® Enterprise Guide 4.1 (SAS Institute Inc, 2006). During harvest, large diameter growth differences were observed between genetic groups, thus stem diameter was used as a covariate in the statistical model:

$$Y_{ijk} = \mu + \beta D_{ijk} + G_i + L_j (G_i) + \varepsilon_{ijk} \tag{3}$$

where Y_{ijk} = subject of interest (measured properties), μ = overall mean, β = coefficient related to diameter, D_{ijk} = stem diameter of k^{th} tree in j^{th} genetic line of 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, ε_{ijk} = random error with E(0, σ^2).

Duncan multiple range tests were used to determine significant differences between genetic groups for dynamic MOE and static MOE at α=0.01. Bartlett`s tests were used to verify the assumption of equal variances.

RESULTS AND DISCUSSION

The green specific gravity values of wild-type and transgenic aspen ranged from 0.32 to 0.38 (Tab. 2). Other investigators reported similar values. Klasnja et al. (2003) found a specific gravity value of 0.38 for 4-year-old *Populus deltoides* clones, Zhang et al. (2003) measured specific gravity values between 0.28 and 0.48 for 3-year-old hybrid poplar clones, Hernandez et al. (1998) stated a green specific gravity of 0.35 for 9-year-old hybrid poplar clones, and Olson et al. (1985) obtained a green specific gravity of 0.33 for 3-year-old *Populus deltoides*.

Compared to the wild-type, significantly lower green specific gravity values were measured for the genetic group Ptr4CL with reduced lignin content and genetic line Ptr4CL/CAld5H-72. Genetic group PtrCAld5H with increased S/G ratio and genetic line Ptr4CL/CAld5H-141 had the same green specific gravity value as the wild-type. These relatively high specific gravity values for genetic group PtrCAld5H and genetic line Ptr4CL/CAld5H-141 can be explained on one hand by the slower diameter growth for the increased S/G ratio (Tab. 1) group and on the other hand by the chemical composition of Ptr4CL/CAld5H-141.

Tab. 2: Elastic modulus and selected physical properties of wild-type and transgenic aspen groups

	Ptr WT Wild-type	Ptr 4CL Reduced Lignin Content	Ptr CAld5H Increased S/G Ratio	Ptr 4CL/CAld5H Reduced Lignin Content & Increased S/G Ratio	
	271	21 & 23 & 37	94 & 96	72 *	141 *
Green Specific Gravity (-)	0.38 ^{a} (6.3)	0.35 ^{b} (3.0)	0.37 ^{ab} (4.84)	0.32 ^{c} (3.3)	0.37 ^{a} (4.1)
Moisture Content (%)	94.1 ^{a} (37.3)	90.5 ^{a} (15.3)	81.1 ^{a} (15.1)	100.6 ^{a} (14.2)	72.9 ^{a} (16.1)
Density at given MC ($kg.m^{-3}$)	726.5 (15.3)	664.8 (7.7)	669.6 (5.6)	652.1 (9.8)	645.9 (8.9)
Velocity of Sound Propagation ($m.s^{-1}$)	3146 ^{a} (11.1)	2334 ^{c} (6.1)	2692 ^{b} (6.3)	2269 ^{c} (4.9)	3247 ^{a} (7.2)
Dynamic MOE (MPa)	7084 ^{a} (12.1)	3623 ^{c} (12.1)	4860 ^{b} (11.8)	3344 ^{c} (3.9)	6786 ^{a} (10.1)
Static MOE (MPa)	5203 ^{a} (10.6)	2928 ^{b} (12.4)	4653 ^{a} (10.5)	2058 ^{c} (14.2)	4814 ^{a} (9.4)

Notes: Results are presented as mean values. Common superscript letter(s) indicate(s) values that are not significantly different from each other as determined by Duncan multiple range test at α = 0.01. Letter `a` shows the highest value(s). Values in parentheses are coefficient of variation in percentage.

* Genetic line Ptr4CL/CAld5H-72 and genetic line Ptr4CL/CAld5H-141 were handled as separate genetic groups, because of the high lignin content differences within genetic group Ptr4CL/CAld5H

The velocity of sound propagation at the green condition in wild-type and transgenic aspen varied between 2,269 $m.s^{-1}$ and 3,247 $m.s^{-1}$ (Tab. 2). These values were in range with those reported elsewhere. Velocity of sound propagation in the longitudinal direction of wood is about 3,800 $m.s^{-1}$ for an air-dried wood sample with MOE of 12.4MPa and a density of 480 $kg.m^{-3}$ (Wood Handbook 1999). Grabianowski et al. (2006) reported velocity of sound propagation values of 2,466 $m.s^{-1}$ for air-dried radiata pine logs. Smulski (1991) found values ranging from 4,504 $m.s^{-1}$ to 4,993 $m.s^{-1}$ for four eastern hardwoods. Our results showed that velocity of sound propagation in wild–type and genetic line Ptr4CL/CAld5H-141 were not different, but genetic group Ptr4CL, genetic group PtrCAld5H, and genetic line Ptr4CL/CAld5H -72 showed significantly lower values.

Dynamic MOE values calculated based on the density and velocity of sound propagation determined by the Fakopp nondestructive technique ranged from 3,344 MPa to 7,084 MPa (Tab. 2). Information on dynamic MOE values is limited in the literature for fast-growing hardwood species. Hernandez et al. (1998) found dynamic MOE values of 8,065 MPa for air-dried fast-growing poplar clones. Dynamic MOE of genetic line Ptr4CL/CAld5H-141 was not statistically different from the wild-type, but genetic groups Ptr4CL, genetic group PtrCAld5H, and genetic line Ptr4CL/CAld5H-72 showed significantly lower values.

Static MOE measured by three-point bending ranged from 2,058 to 5,203 MPa. In the Wood Handbook (1999), quaking aspen is listed with a static MOE value of 5,900 MPa for standard sized specimens at the green condition. Kasal et al. (2007) found a static MOE of 7,143-8,100 MPa for 1-year-old transgenic tree stems at air-dried condition using micromechanical testing. Bjurhager et al. (2008) measured 4,800-6,000 MPa for static MOE of small diameter (10-14mm) hybrid aspen stems at green condition. The lowest static MOE values of 1,000-2,500MPa were reported for poplar clones in wet condition by Peszlen (1998).

Static MOE of the wild-type group, genetic group PtrCAld5H, and genetic line Ptr4CL/CAld5H-141 were not statistically different. Genetic groups Ptr4CL and genetic line Ptr4CL/CAld5H-72 had significantly lower static MOE.

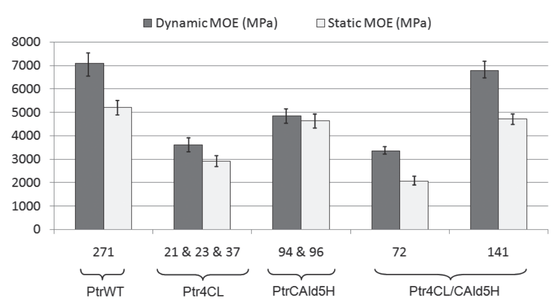

Fig. 3: Dynamic and static modulus of elasticity of wild-type and transgenic aspen clones for the different genetic groups: PtrWT = wild type, Ptr4CL = reduced lignin content, PtrCAld5H = increased syringyl to guaiacyl ratio, Ptr4CL/CAld5H = both reduced lignin content and increased syringyl to guaiacyl ratio. Whiskers represent the standard deviation

The dynamic MOE and static MOE values showed similar trend across genetic groups (Fig. 3.). However, dynamic MOE was about 5-40 % higher than the corresponding static MOE which can be explained by the different nature of the testing techniques. Interestingly, relatively smaller differences (4-18 %) were observed between dynamic and static MOE for genetic groups Ptr4CL and genetic group PtrCAld5H compared to the other genetic groups. Spycher et al. (2008) observed 5.3 % difference between dynamic and static MOE for sycamore, Karlinasari et al. (2008) reported a 9-11 % difference for Gmelina wood, and Ilic (2001) found an average 29 % difference for *Eucalyptus delegatenis* R. Baker.

Investigating the relationship between the different variables, a weak correlation was found between velocity of sound propagation and specific gravity (R^2=0.60), while moderate correlations were obtained between dynamic MOE and static MOE (R^2=0.78), and between static MOE and specific gravity (R^2=0.80). Other investigators showed high correlation between dynamic MOE and static MOE for jack and red pine logs (Spycher et al. 2008), for various softwood species (Wang et al. 2004b), for gmelina wood (Karlinasari et al. 2008), for *Eucalyptus delegatenis* R. Baker (Ilic 2001), and for fast-growing poplar clones (Funck et al. 1979, Hernandez et al. 1998). In previous studies, there were contradictory findings regarding the correlation between green specific gravity and static MOE. No correlation was found between static MOE and density for 4-year-old radiata pine clones by Lindstrom et al. (2002), moderate correlation was shown for 9-year-old fast-growing poplar clones (Hernandez et al. 1998), and high correlation was reported for *Eucalyptus delegatenis* R. Baker (Ilic 2001). Considering all the measured variables, green specific gravity still can be a good predictor for static MOE.

CONCLUSIONS

Dynamic MOE using a nondestructive technique and static MOE using micromechanical testing method were investigated for young (2.5-year-old) small diameter (8-14 mm) transgenic aspen clones. Result from this study suggests that a reduction in the lignin content reduce both the dynamic and static MOE. However, an increase in the syringyl to guaiacyl ratio results in only a slight decrease in the dynamic MOE and static MOE. The combined influence of lignin content and syringyl to guaiacyl ratio changes shows the most obvious negative effect on both the dynamic MOE and static MOE. Dynamic MOE and static MOE values showed similar trend across genetic groups; therefore, the Fakopp Microsecond Timer can be a convenient and efficient tool for predicting elastic modulus of small diameter trees and could be used in tree improvement programs. However, the fact that dynamic MOE overestimated the static MOE should be considered.

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