Elastic Modulus Determination of Transgenic Aspen Using a Dynamic Mechanical Analyzer in Static Bending Mode

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Abstract

The applicability of a dynamic mechanical analyzer (DMA) in determining the modulus of elasticity (MOE) of 2.5-year-old transgenic aspen (*Populus tremuloides* Michx.) was investigated. Fifty sample trees with diameters ranging from 8 to 14 mm were harvested from the greenhouse. The trees were from one wild-type group and three transgenic groups. DMA was used in static bending mode to determine the MOE of samples soaked in two different plasticizers: water and ethylene glycol. In addition, dynamic MOE by a nondestructive method and static MOE by a micromechanical test were determined. Results showed that DMA measurements were accurate in showing significant differences between the genetic groups. Although notably higher MOE values were obtained for dynamic MOE and static MOE compared with the DMA measurements, the trend of elastic moduli change across the genetic groups was the same for all three methods.

Advances in genetic engineering have enabled scientists to change the content and structure of lignin in aspen trees. By manipulating the lignin biosynthetic pathway with sense and antisense genes, lignin content of quaking aspen can be reduced (Hu et al. 1999) or the syringyl/guaiacyl lignin ratio (S/G ratio) can be increased significantly (Li et al. 2001). In addition, simultaneous transfer and expression of specific genes results in both decreased lignin content and increased S/G ratio. These genetic modifications have the potential to reduce chemical consumption, energy use, and environmental impact, thus providing advantages in pulp and paper manufacture and bioethanol production (Baucher et al. 2003). However, it is not clear how these modifications will influence mechanical properties, which are crucial for solid wood and engineered wood applications. Therefore, testing the mechanical properties of young trees (1 to 3 y old) with small diameters (8 to 14 mm) is essential in answering fundamental questions of how lignin genetic engineering changes the mechanical characteristics of transgenic wood (Kasal et al. 2007) and in providing timely feedback to geneticists during field trials (Chiang 2006).

Longitudinal modulus of elasticity (MOE), one of the basic properties of wood, is usually obtained through a standard three-point bending test, and calculated from the deformation of wood under low stress in the elastic region (US Department of Agriculture Forest Service, Forest Products Laboratory 1999). Bending is an easy way to measure MOE because sample preparation does not require tedious work and it simulates the important stresses that occur in most product applications and in living trees subjected to wind load. Standard procedures for bending by ASTM D143-09 (ASTM International 2010a) require relatively large sample dimensions, which are not suitable for cylindrical materials with small diameters. To overcome this problem, micromechanical testing was developed for small-diameter trees by modifying the ASTM D143-94 standard (Kasal et al. 2007). Mechanical testing of these nonstandard specimens is usually shape dependent and requires an expensive testing machine.

Elastic modulus can also be determined by a dynamic mechanical analyzer (DMA) in static mode using submersion clamps. DMA already has enabled scientists to

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Forest Prod. J. 60(3):296–300.

determine thermal properties (i.e., glass transition temperature) of small wood samples in dynamic mode (Menard 2008), but limited research has been focused on static measurements. Many thermal properties were measured on samples that were submerged in water or in ethylene glycol (EG; Salmen 1984); therefore these plasticizers should be the most convenient to use for static measurements as well.

The objective of this study was to evaluate the applicability of a dynamic mechanical analyzer in static mode in determining the modulus of elasticity of young transgenic aspen clones with small diameters. The results of the measurements were compared with values obtained using a micromechanical testing method and a nondestructive evaluation technique.

Materials and Methods

Aspen (*Populus tremuloides* Michx.) trees were grown under controlled conditions in the greenhouse of the Forest Biotechnology Group at North Carolina State University. After two and a half years of growth, a total of 50 sample trees were harvested from four genetic groups that included a wild-type group and three transgenic groups (Li et al. 2003). The descriptions of the genetic groups along with their chemical compositions are shown in Table 1.

Nondestructive evaluation

Ultrasonic transmission time between two piezo sensors was measured for each of the sample trees at the green condition using a Fakopp Ultrasonic Timer. This particular device was specifically designed for small-diameter stems (Horvath et al. 2010a). One piezo sensor remained stationary near one end and was excited by a 300-V impulse. The result of this excitation was a 45-kHz ultrasonic pulse that was captured by a receiver piezo sensor. The receiver sensor was moved to six different locations at equal incremental distances. Since the lengths of the stems varied between 200 and 800 mm, the incremental distances also varied from stem to stem. The velocity of sound propagation was calculated from the regression line relating ultrasonic transmission time versus distance. After the velocity of sound propagation measurements, the density of each sample tree was determined by the conventional water displacement method described in ASTM D2395-07 (ASTM International 2010b). With the velocity of sound propagation and density values, the dynamic MOE was determined by Christoffel's fundamental equation (Ross and Pellerin 1994):

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

where

ρ_{MC} = density of the specimen at the given moisture content (kg/m^3), and

V = velocity of sound propagation (m/s).

Micromechanical testing

A modified ASTM D143-09 method (Kasal et al. 2007) was used to measure the static bending modulus of elasticity at the green condition on the same sample trees that were used for nondestructive evaluation. The cylindrical specimens were supported in three-point bending by fixed roller supports that kept a span-to-diameter ratio of 15. The bearing block was designed to avoid surface crushing of the specimens (Fig. 1). The load-deflection curve was obtained using an MTS Alliance RF/300 mechanical testing machine with an MTS Testworks system. The static MOE was calculated as follows:

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

where

s = slope of the linear portion of the load-deflection diagram (N/mm),

L = distance between the two supports (mm),

Table 1.—Comparison of the mean values of the modulus of elasticity (MOE) of different genetic groups using nondestructive evaluation (dynamic MOE), micromechanical testing (static MOE), and dynamic mechanical analysis in static mode for samples soaked in water (DMA water MOE) and in ethylene glycol (DMA EG MOE).

						MOE[c]					
									DMA	
Genetic group	Description	Genetic line	Lignin content (%)[a]	S/G ratio[b]	Stem diameter (mm)	n_1	Dynamic (MPa)	Static (MPa)	n_2	Water (MPa)	EG (MPa)
PtrWT	Wild type (unchanged)	271	21.3	2.2	13.9	8	7,084 A (12.1)	5,203 A (10.6)	7	2,368 A (8.2)	3,266 A (16.1)
Ptr4CL	Reduced lignin content	21 & 23 & 37	14.8	2.1	13.3	20	3,623 C (12.1)	2,928 B (12.4)	10	1,063 C (26.2)	1,903 C (12.0)
PtrCAld5H	Increased S/G ratio	94 & 96	19.7	5.2	9.8	12	4,860 B (11.8)	4,653 A (10.5)	8	1,825 B (11.4)	2,791 B (15.8)
Ptr4CL/CAld5H	Reduced lignin content, increased S/G ratio	72[d]	16.8	3.6	13.0	3	3,344 C (3.9)	2,058 C (14.2)	6	644 D (24.6)	1,544 C (18.4)
		141[d]	19.3	2.7	13.3	7	6,786 A (10.1)	4,814 A (9.4)	7	1,620 B (21.3)	2,790 B (13.0)

[a] Actual lignin content of sample trees measured by Horvath et al. (2010b).

[b] S/G ratio represents the syringyl to guaiacyl lignin content ratio of each genetic line as reported by Li et al. (2003).

[c] n_1 = number of sample trees used to measure dynamic MOE and static MOE; n_2 = number of DMA strips used to measure MOE of samples soaked in water and in EG. Differences among genetic groups for each measurement method are indicated by different letters as determined by Duncan multiple range tests at $\alpha = 0.05$. The letter A denotes the highest value. Numbers in parentheses are the percent coefficient of variation.

[d] Genetic line Ptr4CL/CAld5H-72 and genetic line Ptr4CL/CAld5H-141 were handled as separate genetic groups because of the high lignin content difference within genetic group Ptr4CL/CAld5H.

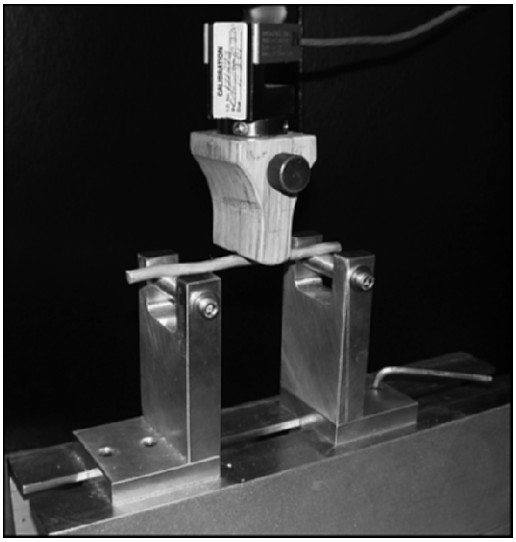

Figure 1.—Micromechanical testing of small-diameter stem in three-point bending. The span was 130 mm.

D = diameter of the specimen at midlength (mm), and

d = diameter of the pith (mm).

The pith was factored out since it did not have a load-bearing capacity.

Dynamic mechanical analyzer in static mode

After the nondestructive evaluation and the micromechanical testing, a DMA was used in static mode to measure MOE in two different plasticizers: water and EG. The stems were cut into 25-mm-long pieces and glued to the end of a medium density fiberboard to ensure safe sample preparation. Approximately 1-mm-thick by 20-mm-long by 1- to 5-mm-wide parallel-sided specimens (excluding the pith and any defects) were prepared using a microcircular saw. These strips were placed in deionized water for 24 hours before testing. A DMA Q800 (TA Instruments) equipped with a 15-mm span submersion three-point bending clamp (Fig. 2) was used to obtain the load-deflection curve of each sample while submerged in water. The MOE calculated for the water-submerged strips will hereinafter be designated as the DMA water MOE. For further testing, the strips were dried over phosphorus pentoxide (P_2O_5) in a desiccator at room temperature. After 1 week of drying, the strips were placed in EG and kept under vacuum until the strips sank in the plasticizer. The DMA measurements were repeated for these strips using EG as the submersion fluid. The calculated MOE will hereinafter be designated as the DMA EG MOE.

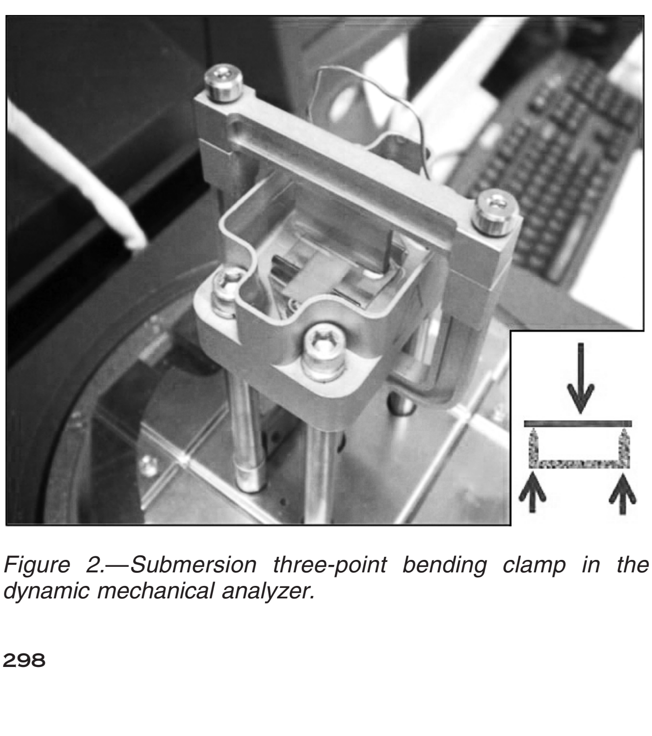

Figure 2.—Submersion three-point bending clamp in the dynamic mechanical analyzer.

Experimental data analysis

To test the effect of genetic modification and genetic lines within genetic groups on the different elastic moduli, descriptive statistics and the general linear model procedure were used in SAS Enterprise Guide 4.1 (SAS Institute Inc. 2006). Large diameter growth differences were observed between genetic groups during harvest; thus, stem diameter was used as a covariate in the statistical model:

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

where

Y_{ijk} = subject of interest (measured property),

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, 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 a level of significance $\alpha = 0.05$.

Results and Discussion

On analysis of the results of the general linear models, it was found that there were no significant stem diameter effects on any of the measured properties. In addition, there were no significant genetic line effects within genetic groups, but genetic groups always had a significant effect on the measured properties.

In all measurement techniques, the elastic moduli of the transgenics were lower compared with the wild type (Table 1). Reduction in the lignin content resulted in a significant decrease in all MOEs. An increase in the S/G ratio resulted in only a slight decrease in MOE. The mean MOE for this group was not significantly different from that of the wild type when the static bending method was used, although the difference was significant when the other three methods were employed. The combined influence of lignin content and S/G ratio changes in genetic line Ptr4CL/CAld5H-72 showed the most obvious negative effect on MOE, while genetic line Ptr4CL/CAld5H-141 showed only a slight decrease in the MOE compared with the wild type.

The measured MOE values were in range with those reported elsewhere for aspen of different ages (Bendtsen and Senft 1986, Hernandez et al. 1998, Peszlen 1998, Kasal et al. 2007, Bjurhager et al. 2008). In addition, Roos et al. (1990) measured a static MOE of 3,438 MPa for the micro three-point bending of young quaking aspen (*P. tremuloides*) at the green condition. Another study from Coutand et al. (2004) reported an MOE of 2,103 MPa for 1-year-old poplar (*Populus* cv. I4551) using three-point bending at the green condition.

When we compared the different measurement techniques, both DMA water MOE and DMA EG MOE measurements showed notably lower values than the other two techniques, where dynamic MOE exhibited the highest MOE values followed by static MOE (Table 1). Several studies reported that dynamic MOE is higher than static MOE in general (Ross and Pellerin 1991, Halabe et al. 1997, Ilic 2001, Karlinasari et al. 2008, Spycher et al. 2008). On the other hand, the large difference between the DMA measurements and other two techniques can be explained by the nature of the sample preparation procedure and the measurement techniques. Dynamic MOE and static MOE were measured on the stem of the sample trees, while DMA strips were manufactured out of these stems and had cut fibers on the surfaces, which could significantly decrease the cross-sectional area and the MOE. Also, submerging the DMA strips in water and in EG kept the materials always fully saturated and the surfaces wet, thereby resulting in a decreased MOE.

Comparing the DMA MOE measurements in water and in EG as plasticizers, strips submerged in water showed lower MOE than strips submerged in EG (Table 1). Compression, tension, and shear stresses develop within the material during bending, but bending MOE is mainly determined by the tensile properties of the material. Cellulose, which can be plasticized by water, is primarily responsible for the tensile strength. On the other hand, EG plasticizes the lignin not the cellulose, and so the effect of this chemical on bending MOE is not as substantial as that of water.

On further study of the MOE values determined by the different techniques, good linear correlation was found between the DMA water MOE and the DMA EG MOE ($R^2 = 0.75$). This shows the good relationship between the measurements obtained using the two different plasticizers and shows the reliability of the DMA measurements. In addition, good linear correlation was found between the dynamic MOE and the static MOE ($R^2 = 0.78$) measured on the stems of the sample trees, which similarly proves the reliability of these techniques. Many investigators have reported correlations between dynamic MOE and static MOE. High correlations were shown by Funck et al. (1979) and Hernandez et al. (1998) between dynamic MOE and static MOE of fast-growing poplar clones. Similar good correlations were found for *Eucalyptus delegatenis* R. Baker (Ilic 2001), for various softwood species (Wang et al. 2004, Spycher et al. 2008), and for gmelina wood (Karlinasari et al. 2008).

All of the measurement techniques detected similar differences between genetic groups as determined by Duncan multiple range tests (Table 1). Across the genetic groups, the trend was the same for all the measurement techniques (Figure 3), proving that DMA in static bending mode can be applied to identify differences in elastic modulus of small-diameter stems.

Conclusions

A dynamic mechanical analyzer in static bending mode was used to determine the elastic modulus of 2.5-year-old wild-type and transgenic aspen submerged in water and in EG. DMA MOE values were compared with those assessed by nondestructive evaluation and micromechanical testing. Across the genetic groups, the different measurement methods showed the same trend and detected similar differences. This proves that DMA in static mode can be applied in tree improvement programs to identify differences in MOE of young stems with small diameters. DMA measurements showed notably lower MOE values than the other techniques, but this is not of major concern in tree improvement research, since work in this area is not meant to obtain absolute values for a given property but rather to assess whether a prescribed treatment shows a significant effect on the subject trees. Relative comparisons are therefore the norm. The fact that the DMA method is able to detect MOE differences and to discriminate between treatments proves its usefulness in tree improvement research. It can provide geneticists timely feedback on the effect of plant modifications on the mechanical property of

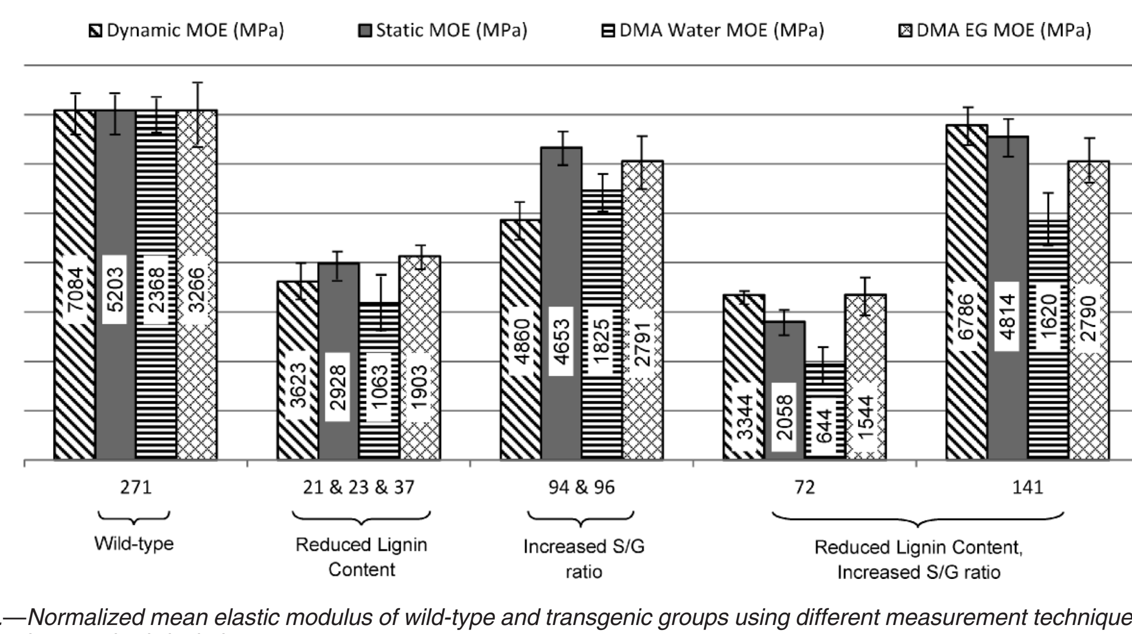

Figure 3.—Normalized mean elastic modulus of wild-type and transgenic groups using different measurement techniques. Whiskers represent the standard deviation.

wood. The method is founded on a strong materials science footing and has proved useful in many areas of research. It is particularly applicable to small samples and is versatile in its ability to provide properties related to thermal treatment, static and dynamic loading, and rheological phenomena.

Acknowledgments

This project was supported by the National Research Initiative of the US Department of Agriculture–Cooperative State Research, Education, and Extension Service, grant no. 2005-35504-16145. The plants and greenhouse facilities were provided by Dr. Vincent Chiang, North Carolina State University, Forest Biotechnology Group. The authors thank Dr. Ferenc Divos at the University of West Hungary for making the Fakopp Ultrasonic Timer available and Dr. Fikret Isik at North Carolina State University for his advice on statistical analysis.

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