CLONAL VARIATION IN WOOD PROPERTIES OF *PINUS TAEDA L.*

by

BENJAMIN S. HORNSBY

(Under the direction of Richard F. Daniels and Laurence R. Schimleck)

ABSTRACT

The use of clonal varieties in forestry offers great potential to improve growth traits (quantity) and wood properties (quality) of loblolly pine (*Pinus taeda* L.). Loblolly pine plantings established via somatic embryogenesis (clones), full-sib zygotic crosses, and half-sib zygotic open pollination were sampled to identify variation of wood properties among and within clonal lines and controls. Properties measured included breast height diameter, total height, wood density (specific gravity), latewood proportion, stem oven-dried weight, and microfibril angle (MFA). MFA was predicted using near infrared spectroscopy. Mixed model analysis showed properties were influenced by the random effect of planting location, there were no significant differences in growth characteristics by method of propagation, while clones and full-sib zygotic trees illustrated superior wood quality characteristics compared to half-sib zygotic trees. No differences were detected with respect to MFA. Georgia locations were superior to those in Mississippi with respect to growth and quality characteristics.

INDEX WORDS: Loblolly pine, Specific gravity, Latewood, Microfibril angle, Near infrared spectroscopy

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# DEDICATION

This thesis is dedicated to all who have been a part of my life for the past two and a half years, but above all to my mother and father, Mrs. Margaret and Mr. Jeff Hornsby. Thank you for the love and support you have so graciously granted me through all my endeavors.

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# CHAPTER 1

# INTRODUCTION

The Southern region of the United States of America plays an important role in national timber production, being responsible for 58% of the wood production within the U.S. and 16% of wood production worldwide (Wear and Greis 2002). This region is also responsible for approximately 80% of the nation's tree planting activities, with the majority of seedlings (84%) planted being loblolly pine (McKeand *et al*. 2003). Timber models forecast that between the years 1995 and 2040 timber production in the U.S. will increase by approximately one-third (Wear and Greis 2002). The South is forecast to accommodate the national surge by increasing production of softwoods by 56% (Wear and Greis 2002).

Intensively managed plantations utilizing genetically improved planting stock in collaboration with advanced silvicultural practices, are believed to be the best strategies to meet the future demands, rather than managing more acres of forest (Li *et al*. 1999). The combination of intensive management and genetically superior trees produces improved growth rates and decreased rotation ages, which facilitate harvesting of merchantable size trees at younger ages (Atwood *et al*. 2002). Rapid early growth also produces a large juvenile core with inferior wood properties compared to mature wood, which is produced in the outer portion of the stem as the tree ages. Genetically improved planting stock not only has the potential for improved growth, but also for improvements in quality traits like stem straightness and wood quality (Li *et al*. 1999). This creates an opportunity to address wood quality as well as wood quantity. The research performed here investigated the variation between different levels of genetically

improved planting stocks (full-sib zygotic seedlings, half-sib zygotic seedlings, and somatic seedlings) with respect to wood quality parameters.

OBJECTIVES

The objectives of the study were:

- to examine and quantify the variation in wood properties among and within clones of loblolly pine established via somatic embryogenesis.
    - properties measured included diameter at breast height (DBH), total height, wood density (specific gravity), by earlywood and latewood, proportion of latewood, stem oven-dried weight, and microfibril angle.
- test for significant differences among the fixed effect of seedling type with respect to the variables previously mentioned.
- identify variance components associated with random effects described in the model (Chapter 3).
- to assess the inclusion of wood properties into early genetic / clonal screening using x-ray densitometry and NIR spectroscopy.

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# CHAPTER 2

# LITERATURE REVIEW

## WOOD QUALITY DEFINITION

Wood quality includes a number of properties of a combination of anatomical and chemical characteristics of wood. The term "wood quality" is not easily defined and perceptions of what constitutes quality can vary among the different divisions of the forestry and wood using industries (Kliger *et al*. 1994). Ultimately definitions of "wood quality" are subjective as they can change as the end product for which the tree is intended change (MacDonald and Hubert 2002). Therefore, "wood quality" is more of a concept that emphasizes particular wood properties, which have a positive influence on a specific end product.

## WITHIN-TREE WOOD FORMATION

Wood that is produced by a young tree is referred to as juvenile wood, and is the wood first produced near the center of a tree. Juvenile wood is produced by a tree at all ages, since the cambium is a continuous sheath around the stem. In an older tree juvenile wood is produced near the crown and mature wood at its base (Zobel and van Buijtenen 1989). Within a single year there is no absolute shift from juvenile to mature wood, with the change transitioning over several years. Therefore, the age of determination between juvenile and mature wood is difficult to determine as the transition in loblolly pine is gradual and not abrupt (Clark *et. al*. 2006). The wood formed during this period is known as transition wood (Saranpää 2003). The period of juvenile wood formation in southern pines typically lasts 6-12 years. Loblolly pine, for example, generally requires 7-10 years of growth before the onset of mature wood production (Megraw

1985). Clark *et. al*. (2006) found the transition from juvenile to mature wood in loblolly pine varied by physiographic region. With respect to specific gravity, they found the length of juvenility was shorter in the Atlantic Coastal Plain, ranging from 5.5 to 7.9 years compared to that in the Hilly Coastal Plain and Piedmont that ranged from 10.4 to 13.6 years. They also found that the age of transition from juvenile to mature wood changes depending on the trait examined (*e.g*. when using microfibril angle as an indicator the length of juvenility is longer). Bendtsen and Senft (1986) estimated the proportion of juvenile wood in loblolly pine trees to be approximately 60% at age 20 and 25% at age 40. Juvenile wood has properties that are less desirable than mature wood (Neale *et al*. 2002; Larson *et al*. 2001; McAlister and Clark 1992; Clark and Saucier 1989; Cregg *et al*. 1988). Unfavorable characteristics of juvenile wood, as compared to mature wood, include: lower specific gravity, decreased cellulose content, increased hemicellulose and lignin contents, thinner cell walls, shorter tracheids, greater microfibril angle, low percentage of latewood in the annual ring, and a greater amount of compression wood (Haygreen and Bower 1996; Saranpää 2003). Compression wood is formed in conifers when a stress is present, for example in a leaning stem. Ultimately, lower specific gravity observed in juvenile wood is due to wide annual rings, having a low proportion of latewood and short fibers with thin walls. Mature wood has higher specific gravity due to narrower growth rings with a higher proportion of latewood (Saranpää 2003). Neale *et al*. (2002), proposed that increased juvenile wood content in trees harvested from short rotations emphasizes the need to improve wood quality of the juvenile wood, as well as mature wood. Both could be addressed simultaneously through genetic improvement in forest breeding programs.

## WITHIN-RING WOOD FORMATION

Southern pines show a large degree of variation within one year's growth or across an annual ring. Growth begins rapidly in early spring and slows in late summer before ceasing in the fall. This growth pattern results in different kinds of wood being formed within the various seasons of the year. This variation is due to seasonal climatic changes (for example, water availability) and the formation of latewood (Saranpää 2003). Wood produced in the spring and early summer is referred to as earlywood or springwood, while wood produced late in the growing season is referred to as latewood or summerwood. The latewood is characterized by thick-walled, small-lumen tracheids with small radial dimensions, compared to thin-walled, large lumen tracheids in the earlywood that have larger radial dimensions (Butterfield 2003). Latewood has higher average specific gravity than earlywood owing to these differences in structure. The main function of earlywood cells is to transport water and nutrients from root to stem while the cells of latewood provide strength to the new growth sheath and support for the expanding crown (Larson *et al*. 2001). In softwoods, the width of individual rings and the proportion of latewood within each ring both contribute to overall wood stiffness, and hence wood quality (Butterfield 2003).

## SPECIFIC GRAVITY DEFINITION

Wood density can be used as an effective indicator of wood quality. Wood density can be simply defined as the weight or mass per unit volume. The structure of wood can be simplified into solid material (cell walls) and voids (cell lumens), so consequently wood structure determines the ultimate density. Specific gravity with respect to wood quality within a tree is the ratio of cellular material per unit volume (or density) compared to the density of pure water at $4^{o}$C (Megraw 1985).

## SPECIFIC GRAVITY AS A WOOD QUALITY INDICATOR

Wood density or specific gravity is a useful indicator of wood quality because it has a major effect on both the yield and quality of fibrous and solid wood products. In addition, it can be altered through sivilcultural and genetic manipulation (Zobel and van Buijtenen 1989). Wood density in softwoods has a strong correlation with pulp yield, pulp quality, and the strength and stiffness of wood (Saranpää 2003). Prior research shows that high specific gravity values are positively correlated with wood stiffness and strength, both important properties for pine lumber (Saranpää 2003; Clark and Daniels 2002; Stamm and Sanders 1966). Beyond the primary emphasis on growth traits (*e.g*. height), genetic improvement of southern pine has predominately concentrated on improving specific gravity with limited emphasis on other wood quality traits (Zobel and Jett 1995). This can be credited to the fact that specific gravity is relatively easy to measure while most other traits are both difficult and expensive to measure.

## MICROFIBRIL ANGLE DEFINITION

The wall of an individual wood cell is comprised of two distinct sections, the primary cell wall and the secondary cell wall, both are comprised of microfibrils whose orientation within the primary wall and the 3 layers of the secondary wall differ. The primary cell wall contains microfibrils that are loosely and more or less randomly interwoven. The secondary cell wall is comprised of three distinct layers, referred to as the $S_1$, $S_2$, and $S_3$ layers. Each of the layers is comprised of microfibrils having different orientations (Figure 2.1). The $S_1$ layer is comprised of alternating lamellae of Z and S (predominately S) helical oriented microfibrils at an angle of 50 to 75 degrees relative to the fiber axis (Wilson and White 1986). Microfibrils in the thicker $S_2$ layer lie in Z helices, are steeply aligned, closely packed, and highly parallel to the fiber axis with a mean angle of 10 to 30 degrees relative to the fiber axis (Wilson and White 1986). A very

flat S helical orientation of microfibrils is found in the $S_3$ layer, with angles of 60 to 80 degrees relative to the cell axis (Wilson and White 1986). The $S_1$ and $S_3$ layers are approximately 0.1 to 0.2 μm thick, while the $S_2$ layer is approximately 0.6 μm thick (Panshin and De Zeeuw 1980). Properties of the predominately thicker $S_2$ layer consequently largely determine the properties of the cell wall (Megraw 1985). Owing to the thickness of the $S_2$ layer, microfibril angle (MFA) is quantified as the mean helical angle that the cellulose microfibrils of the $S_2$ layer of the cell wall make with the longitudinal axis of the cell (Megraw 1985).

MICROFIBRIL ANGLE AS A WOOD QUALITY INDICATOR

Meylan and Probine (1969) emphasized that the angle of the cellulose microfibrils in the $S_2$ layer of the tracheid cell wall is the only known physical characteristic capable of effecting large changes in the stiffness of wood. More recently, it has been acknowledged that MFA is one of the main determinants of stiffness (as the angle of the microfibrils decrease, values for stiffness increase) (Cave and Walker 1994). Differences in core (juvenile) and outer (mature) wood properties are moderately explained by differences in the angle of their wood microfibrils (Megraw 1985; Myzewski *et al*. 2004). Angles near the pith are large, decrease rapidly out to ten or more rings from the pith, and continue to drop but at a much slower rate until they eventually stabilize (Hiller 1964; Megraw 1985). The point at which this angle becomes stable varies depending on the growth rate of the tree (Hiller 1964). Microfibril angle varies considerably within the trunk of a tree with large angles common in the juvenile wood and small angles in the mature wood (Donaldson and Burdon 1995). There is also considerable variation in MFA within a growth ring as MFA varies between earlywood and latewood (Zobel and Jett 1995). Angles in the earlywood tend to be larger than those in the corresponding latewood (Pillow *et al*. 1953; Hiller 1964; Hiller and Brown 1967).

Density is frequently used as an indicator of wood quality because as mass increases, strength should increase as there is more cell wall material per unit volume. However, MFA is important as well as it quantifies the quality of mass by describing the orientation of the cellulose fibrils from which the cell walls are constructed. For example, wood with a high density and high MFA will not be as strong as wood having the same density with a low MFA.

Evans and Ilic (2001) demonstrated a greater correlation between MFA and wood stiffness than between density and stiffness. In their study, based on 20 mature alpine ash (*Eucalyptus delegatensis*, R.T. Baker) trees, they found that MFA variation alone accounted for 86% of the variation in stiffness while density only accounted for 70%. Addis *et al*. (1998) also found strong correlations with stiffness and MFA ($r = -0.913$). Accurate estimates of the correlation between MFA, specific gravity, and stiffness will be crucial to assign proper breeding selection weights to different genetic traits (Myszewski *et al*. 2004). Theory suggests that negative genetic correlations between MFA and core specific gravity would be favorable for a tree improvement program because it would imply that progeny with high specific gravity will tend to have low MFA, and that breeding for improvements in specific gravity, which is easier to measure and has a higher heritability than MFA, will indirectly lead to desirable changes in MFA (Myszewski *et al*. 2004).

SOMATIC EMBRYOGENESIS

Somatic embryogenesis is an advanced form of vegetative propagation used to mass produce high quality conifer seedlings. Somatic embryogenesis, for current conifer systems, is based on initiation and development of somatic embryos from zygotic embryos in an artificial environment and allows for production of virtually unlimited numbers of identical individuals from a single seed. Clonal propagation of high-value forest trees through somatic embryogenesis

has the potential to rapidly capture the benefits of breeding or genetic engineering programs, and to improve raw material uniformity and quality (Pullman *et al*. 2003). An understanding of the clonal variation in wood properties would provide the ability to select and deploy clonal lines with good growth traits and improved wood quality traits, offering tremendous potential for immediate crop improvement. This study will be the first to quantify wood properties in somatic loblolly pine clones.

NEAR INFRARED SPECTROSCOPY

Traditionally measurements of wood properties require destructive sampling, which does not allow for future observations if the tree is found to be superior for a specific trait. These traditional methods are also slow and cost-prohibitive when applied to the large number of samples needed for decision making in a breeding program (Jones *et al*. 2005). This creates an urgent need for rapid nondestructive methods to measure wood properties.

Estimation of wood properties by near infrared (NIR) spectroscopy relies on the provision of data that is used for the development of calibrations for specific properties (*e.g*. MFA in this study). SilviScan-2 (Evans 1997, 1999), an automatic wood microstructure analyzer, provides MFA estimates that have become the global standard for many species. Utilization of Partial Least Squares (PLS) regression (utilizing principle components to reduce data) in combination with the SilviScan-2 MFA data and the NIR spectral data permit the development of a MFA calibration. An MFA calibration by Schimleck and Evans (2002) was developed using seven factors and displayed an excellent relationship between SilviScan determined MFA and NIR fitted MFA ($R^2 = 0.95$). The performance of this calibration was tested on two intact radiata pine (*Pinus radiata*, D. Don) increment cores and it was found that NIR predicted MFA was in excellent agreement with MFA determined by SilviScan-2, with

prediction $R^2$ of 0.98 (core A) and 0.96 (core B). More recently, Jones *et al*. (2005) applied a MFA calibration based on 89 *Pinus taeda* L. radial strips from a wide range of sites in Georgia (USA) (9 sites from 3 physiographic regions) to a prediction set of 30 *P. taeda* radial strips (six different sites, but from the same geographic regions) and demonstrated that NIR spectroscopy could be successfully used to estimate MFA of radial strips from a wide range of sites not included in the calibration set (prediction $R^2$ ranged from 0.80 to 0.84). When properly calibrated, NIR spectroscopy should be capable of estimating wood properties in a fast, inexpensive, and nondestructive manner. These techniques will be used on the somatic clones to identify variation among and within the clones with respect to MFA.

TABLES AND FIGURES FOR CHAPTER 2

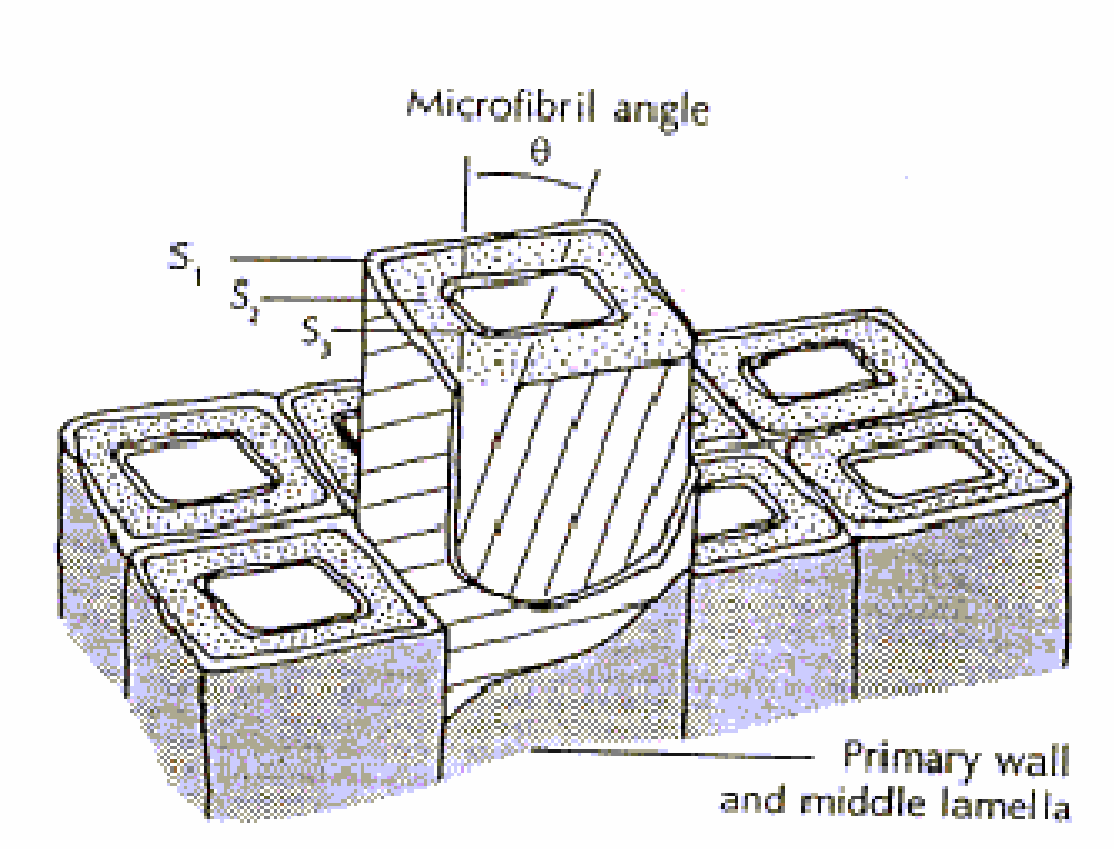


Figure 2.1. Secondary wall layers and microfibril angle of typical tracheid (adapted from Dickson and Walker (1997).

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# CHAPTER 3

# MATERIALS AND METHODS

## EXPERIMENTAL DESIGN

Four plantations of somatic loblolly pine clones were sampled after their fourth growing season for the estimation of wood properties. Two of the trial plantations were in Georgia (GA) and two were in Mississippi (MS). Locations (sites) of the plantations can be viewed in Figure 3.1 with associated coordinates (Table 3.1). The sites were selected to display a range of soil drainage characteristics. The site selection criteria aimed to acquire a wet and dry site in both Georgia and Mississippi. Table 3.2 shows drainage class and soil classification for each site sampled, as described by the Cooperative Research in Forest Fertilization (CRIFF) program at the University of Florida. A description of CRIFF soil groups can be viewed in Table 3.3. All four plantations were established in March 2000. The two sites in Georgia were sampled in January 2004 while the Mississippi sites were sampled in July 2005. To accommodate the variation in sampling dates, only data through the end of the fourth growing season were used in the analysis.

The study was designed in the form of an alpha lattice incomplete block. A subsample of the test was sampled. The subsample included ten distinct somatic clonal lines (SE) from each of three unrelated full-sib families (SE1, SE2, SE3) (Table 3.4). Lines were chosen to represent the range in performance for the family based on height growth. Also four lines from each site (two each from two additional families SE4 and SE5) were sampled to expand the potential range in variability (Table 3.4). Eight ramets were sampled from each line, within each family,

at each site, yielding 272 trees per site, or 1088 trees in the main sample established via somatic embryogenesis. In addition to the trees established via somatic embryogenesis, 12 zygotic full-sib (FS) trees, from each of three families (FS1, FS2, FS3) were sampled at each site for an additional 36 trees per site, or 144 trees in the main sample established via full-sib zygotic cross (Table 3.4). These three families (FS1, FS2, FS3) were directly related to SE1, SE2, and SE3. A line from each of three commercially utilized families established via open pollination was also sampled to examine variability under conditions of lesser genetic control. For these open pollinated families (OP), 12 trees per family were sampled at each site for an additional 36 trees per site, or 144 trees in the sample established via open pollination. A nested factorial design was sampled to include somatic embryogenic lines of interest nested within full-sib zygotic crosses along with full-sib zygotic crosses (controls), and half-sib zygotic (open pollinated controls) to provide information on variability under situations with varying levels of genetic control. The genetic families/lines, full-sib crosses, and half-sib trees sampled were the same for each site. While there was a target of 1,376 5mm (0.20 in) increment cores to be sampled in total, only 1,285 were obtained due to uncontrollable factors including mortality, fusiform rust infection, and poor form.

A subset of 12mm (0.47 in) cores was also collected at each site. These cores were collected for microfibril angle (MFA) determination by SilviScan-2 and subsequent NIR calibration. The subset was collected to include the range of variation among all families and types (method of establishment). The subset included 17 trees per site or 68 samples in total.

FIELD METHODS

Increment cores (5mm or 0.20 in) were collected after the 2004 growing season. Prior to collecting cores each sample tree was identified, flagged, measured for diameter at breast height

(DBH), and measured for total height with a Suunto hypsometer. A cordless drill was used in conjunction with a 5mm (0.20 in) increment bit to maximize efficiency of acquiring the large number of cores required. Sample trees were bored bark-to-bark through the pith to provide two samples (radii) for analysis. The target height level to collect cores was 2.5 feet (0.75 m) to insure all growth rings were included. Due to excessive branching of the young trees, a range of height levels was accepted for core collection (between 2 - 3 feet or 0.60 - 0.90 meters) to allow a range of possible drilling points. After removal of the core, 5mm (0.20 in) wooden dowels were used to plug the resultant holes in the tree to prevent exposure to insects or disease. Once obtained, each core was labeled and stored in a freezer awaiting drying and processing.

LAB METHODS

In the lab, cores were placed in an oven at 52$^{o}$C for 24 hours to reach a target moisture content of approximately 10%. One radius of each core was machined for X-ray densitometric and NIR spectroscopic analysis. Schimleck *et al*. (2003) found calibrations obtained from samples at 7% moisture content were superior to calibrations obtained from spectra collected from the same green samples before they were dried (green moisture content ranged from 100-150%). The machining process involved gluing an increment core radius between two grooved core holders, and using a twin-blade table saw to cut a thin strip from the core exposing the radial-longitudinal face. Prior methods for X-ray densitometry have oriented cores to expose the transverse face, but this was altered to accommodate the SilviScan-2 methodology which performs MFA analysis on the radial-longitudinal surface of the sample. Therefore, it was important to orient the clonal samples in the same manner as the SilviScan-2 sub-samples to assure the best possible calibration performance. Each strip includes the core itself, and is approximately 1.5 mm (0.06 in) thick in the tangential direction and 5mm (0.20 in) wide in the

longitudinal direction. The radial length of each sample was equal to that of the radius of the sampled tree. Two samples (radii) were useful for quality control as there is potential for orientation problems during the machining process.

The determination of MFA by SilviScan-2 was necessary to develop a MFA calibration that was used to predict the MFA's of the main sample set using NIR spectroscopy. NIR diffuse reflectance spectra were obtained using a FOSS NIRSystems Inc. Model 5000 scanning spectrometer (wavelength range 1100-2500 nm, 2 nm resolution). NIR spectra were obtained in 10mm (0.39 in) sections from the radial-longitudinal face of a strip cut from each core in a controlled environment (temperature = 20$^{o}$C, relative humidity = 40%) on the same strips analyzed by SilviScan-2. The resultant microfibril angle data provided by SilviScan-2 was averaged in to 10mm (0.39 in) sections. NIR spectra tend to be sensitive to the condition of the surface of the sample. Since the prediction set of cores were cut using the Forest Service twin-blade saw, an additional subset of 16 cores were selected and cut on the Forest Service twin-blade saw and sent to SilviScan-2 for measurement of MFA. Including the samples cut on the Forest Service twin-blade saw into the calibration accounted for noise created by difference in saw surface. A total of 413 spectra were collected from the 84 radial strips representing the calibration set, 5,813 spectra were collected from the 1,265 radial strips representing the prediction set. Partial Least Squares (PLS) regression (utilizing principle components to reduce data) was used to create a MFA model which was used to predict the MFA of all samples scanned with the NIR spectrometer.

Densitometry was performed on all strips. Using a specific gravity threshold of greater than 0.48 to delineate latewood, the ring width, density of earlywood, density of latewood, and

percent latewood were determined for each ring. These were averaged by each category through the fourth growth ring for each sample.

RANKING ANALYSIS

Initial analysis of the data obtained from field measurements and strips cut from cores, utilized SAS (SAS® 2005) to calculate averages with associated standard deviations by propagation method (type), family/line, and location planted (site). The averages with associated standard deviations were used to identify general trends by type and location as well as the ranking of samples. Variables examined included: diameter at breast height (DBH) (through the $4^{th}$ growing season), total height (THT), averaged weighted core specific gravity through the $4^{th}$ ring (WCSG), average early-wood specific gravity (EWSG), average late-wood specific gravity (LWSG), average percent latewood (LP), and stem oven-dried weight (ODWT) (lbs). WCSG was calculated by:

$$WCSG = \Sigma (RBA * RSG) / CBA$$

Where:

RBA = Individual Ring Basal Area;

RSG = Individual Ring Average Specific Gravity;

CBA = Core Basal Area through $4^{th}$ growth ring;

The method used to calculate stem ODWT incorporated growth and density data among samples. This was done to include multiple variables (THT, DBH, and WCSG) into a ranking classification. This methodology included the use of a lower coastal plain stem volume and taper equation (Harrison and Borders 1996) to calculate whole stem inside bark volume. The associated inside bark volume was then multiplied by the product of the corresponding sample

WCSG value and the value for the density of water (62.4 lb/ft$^3$) to achieve an estimate of ODWT. The method can be viewed as followed:

Inside bark stem volume = $b_0 * DBH^{b1} * H^{b2} – b_3 * (D_m^{b4} / DBH^{b4-2}) * (H – 4.5)$

Where:

DBH = Diameter at Breast Height (1.37 m);

H = Total Height;

$D_m$ = Merchantable top diameter (0.1 used for whole stem);

Lower Coastal Plain parameter estimates for inside bark stem volume and taper:

$b_o = 0.00071193$, $b_1 = 1.876991$, $b_2 = 1.321458$, $b_3 = 0.00217131$, $b_4 = 3.592491$;

Stem ODWT = Inside bark stem volume * ($DH_2O$ * WCSG)

Where:

$DH_2O$ = 62.4 (Density of water, lb/ft$^3$);

WCSG = sample average weighted core SG through 4$^{th}$ growth ring;

STATISTICAL ANALYSIS

A linear mixed-effects model was employed for estimating variance components and fixed-effects using the SAS System (SAS® 2005). The response variables of interest included: DBH, THT, WCSG, LP, and ODWT. The main fixed effect in the model was method of propagation (type) which illustrates varying degrees of genetic control. The sites selected for sampling represent a random sample of all sites in the corresponding region. Conversely, the families within a type represent a random sample of all families from the corresponding type. Here, sites and families represent random-effects, and their contribution to the variance of the response variables can be estimated. Also of interest is the variation among lines within the somatic embryogenesis derived clones, which can also be estimated. Let, $Y_{ijklmn}$ = response of

interest of the $n^{th}$ tree, in the $m^{th}$ line, of the $l^{th}$ family, of the $k^{th}$ type in the $j^{th}$ replicate of the $i^{th}$ site. Then the linear mixed-effects model can be written as:

$$Y_{ijklmn} = \mu + T_k + S_i + R(S)_{ij} + (SF)_{il} + F(T)_{kl} + I[L(T)_{km}] + I[(SL)_{im}] + e_{ijklmn} ,$$

where:

$\mu$ = the population mean;

$T_k$ = the fixed effect of the $k^{th}$ type;

$S_i$ = the random effect of the $i^{th}$ site, $\sim NID(0,\sigma^2_S)$;

$R(S)_{ij}$ = the random effect of the $j^{th}$ replicate within the $i^{th}$ site with, $R(S)_{ij} \sim NID(0,\sigma^2_{RS})$;

$(SF)_{il}$ = the random interaction effect of the $l^{th}$ family and $i^{th}$ site with, $(SF)_{il} \sim NID(0,\sigma^2_{SF})$;

$F(T)_{kl}$ = the random effect of the $l^{th}$ family within the $k^{th}$ type with, $F(T)_{kl} \sim NID(0,\sigma^2_{FT})$;

$I[L(T)_{km}]$ = the random effect of the $m^{th}$ line within the $k^{th}$ type with,

$I[L(T)_{km}] \sim NID(0,\sigma^2_{LT})$ and $I$ = 1 if type = SE, 0 otherwise;

$I[(SL)_{im}]$ = the random interaction effect of the $m^{th}$ line and $i^{th}$ location with,

$I[(SL)_{im}] \sim NID(0,\sigma^2_{SL})$.

$e_{ijklmn}$ = residual error, and $e_{ijklmn} \sim NID(0,\sigma^2 I_{ijklmn})$

Upon detection of a significant difference of the main fixed effect of type, a pairwise comparison was employed to identify which types illustrated significant differences among each other. Tukey's pairwise comparion test was employed to identify the contrasts among types.

BEST LINEAR UNBIASED PERDICTION (BLUP)

In order to make comparisons among random effects a procedure was utilized known as best linear unbiased prediction (BLUP). BLUP is a method which allows the random effects within a mixed model to be predicted. Since type FS and SE had overlapping families (FS 1, 2, 3 and SE 1, 2, 3), BLUP analysis was utilized to predict average family values for application of a

significant difference test. This was of interest to understand how the same families (common parents) grow with respect to method of propagation. Average values by region (Georgia and Mississippi) were also calculated and significant differences tested.

TABLES AND FIGURES FOR CHAPTER 3

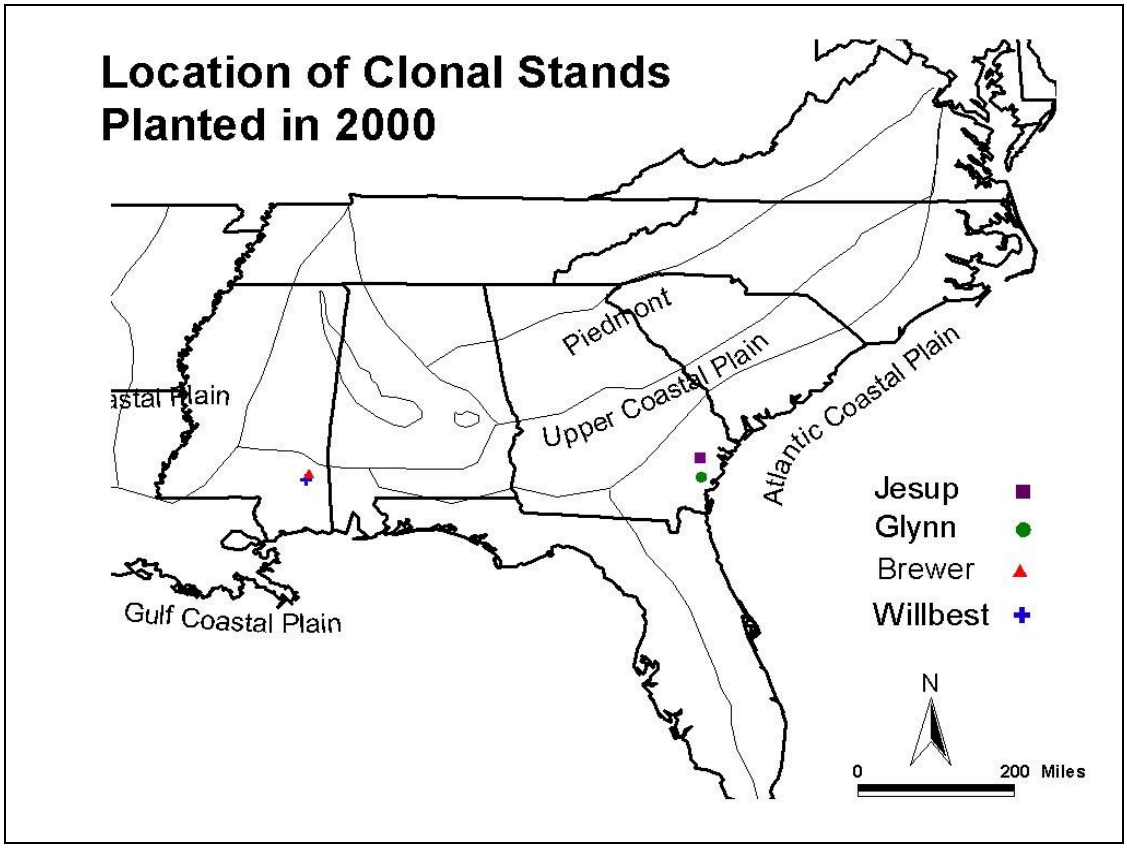


Figure 3.1. Stand location map for the 4 clonal loblolly pine plantations sampled.

Table 3.1. Coordinates (Latitude and Longitude) of 4 clonal loblolly pine plantations sampled.

| Location | Latitude (N) | Longitude (W) |
|---|---|---|
| Jesup, GA. | 31°73' | 81°82' |
| Glynn Co., GA. | 31°39' | 81°62' |
| Brewer, MS. | 31°36' | 88°85' |
| Will Best, MS. | 31°44' | 88°86' |

Table 3.2. CRIFF soil group with associated drainage class by site sampled.

| Location | CRIFF Soil Group | Drainage Class |
|---|---|---|
| Jesup, GA. | B | SWP / MW |
| Glynn Co., GA. | B | P / VP |
| Brewer, MS. | E | WD |
| Will Best, MS. | A | P |

Table 3.3. CRIFF soil group definitions (Jokela and Long 1999).

| CRIFF Soil Group | Major Land Area | Drainage | Important Features |
|---|---|---|---|
| A | Savannas | Very poor to somewhat poor | Sand to loamy sand surface layer less than 20 inches thick, with a finer textured soil horizon below. |
| B | Savannas | Very poor to somewhat poor | Sand to loamy sand surface layer greater than 20 inches thick, with a finer textured soil horizon below. |
| C | Flatwoods | Poor to somewhat poor | Spodic horizon below the surface layer. Sandy loam or finer textured soil horizon below the spodic horizon. |
| D | Flatwoods | Poor to somewhat poor | Spodic horizon below the surface layer. Sand to loamy sand soil horizon below the spodic horizon. |
| E | Uplands | Moderate to well | Sand to loamy sand surface layer less than 20 inches thick, with a finer textured soil horizon below. |
| F | Uplands | Moderate to well | Sand to loamy sand surface layer greater than 20 inches thick, with a finer textured soil horizon below. |
| G | Sandhills | Excessive | Sand to loamy sand surface layer at least 100 inches thick. |
| H | Depressions | Very poor | High in decomposing plant residues, often an organic soil. |

Table 3.4. Propagation and family information including weight sampled per location.

| **Type: Full Sib Somatic Clones** | | | | **Type: Full Sib Zygotes** | | **Type: Open Pollinated Zygotes** | |
|---|---|---|---|---|---|---|---|
| Family | Lines/Family | Ramets/Line | # Samples | Family | # Samples | Family | # Samples |
| SE1 | 10 | 8 | 80 | FS1 | 12 | OPI | 12 |
| SE2 | 10 | 8 | 80 | FS2 | 12 | OPII | 12 |
| SE3 | 10 | 8 | 80 | FS3 | 12 | OPIII | 12 |
| SE4 | 2 | 8 | 16 | | | | |
| SE5 | 2 | 8 | 16 | | | | |

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# CHAPTER 4

# GROWTH VARIATION

## MEANS COMPARSION – DIAMETER AT BREAST HEIGHT (DBH)

Illustration of superior growth and quality traits among somatic clones compared to controls (full and half-sib zygotes) would be beneficial for the future utilization of cloning techniques for the improvement of stock deployed by forest managers. An assessment of average growth (DBH through age 4) among all clones sampled compared to all controls by method of propagation (type) suggest clones were not achieving the growth rates of controls (Figure 4.1). This can be credited to the methodology applied to the selection of clonal lines sampled. Clonal lines were selected to include a range of growth rates (based on height data). Figure 4.2 shows the growth (DBH) performance of clones when the top three ranked clones were selected and compared to the controls. It can be seen that the top three ranked clones exceed the diameter growth of the controls at Jesup, Brewer, and Willbest. The average DBH for full-sib zygotics was greater than the top three ranked clones at Glynn by less than 0.10 of an inch.

## MEANS COMPARSION – TOTAL HEIGHT

The means comparison with respect to total height (THT) suggests all types were growing at equivalent rates (Figure 4.3). Inclusion of average THT values for the top three ranked clones with respect to DBH and THT did not change the comparison as they were comparable to the other lower ranked clonal lines. On average type SE, OP, and FS illustrated similar height patterns across locations. Unlike the means comparison with respect to DBH

where differences occurred across types and locations, THT remained relatively constant among types and locations.

ANALYSIS OF VARIANCE – DIAMETER AT BREAST HEIGHT

The analysis of variance for DBH including the main fixed effect of type with associated variance components of random effects can be viewed in Table 4.1, which shows that there was not a significant difference in DBH by type (P-value = 0.4426). Average DBH was estimated by the model for each type. Estimated mean DBH values for FS, OP, and SE types were 4.04, 3.91, and 3.74 inches respectively. The lower estimated mean value for type SE is likely due to the line selection criteria where lines were selected to illustrate a range of growth performance. Since a significant difference was not detected by type with respect to DBH pairwise difference tests were not performed. Variation was identified with respect to the random effect of site ($\sigma_S$ = 0.04700). The random effect of rep within site suggest microsite was also contributing to a considerable portion of the variation in DBH ($\sigma_{RS}$ = 0.03948). The largest estimated variance component was due to the random effect of family within type ($\sigma_{FT}$ = 0.08451), illustrating the existence of variation of family growth performance (DBH) within the respective type. The residual variance component for DBH was lowest for type SE ($\sigma_{SE}$ = 0.2413), followed by type OP ($\sigma_{OP}$ = 0.3117), and highest for the type FS ($\sigma_{FS}$ = 0.3561).

ANALYSIS OF VARIANCE – TOTAL HEIGHT

Analysis of variance results for THT along with estimates of the random-effects components of variance are presented in Table 4.2. THT was not found to differ significantly among the main fixed effect of type (P-value = 0.6053). Estimated mean THT values were found to be 20.04, 21.07, and 20.67 feet for OP, FS, and SE types, respectively. Variance component estimates for the random effects with respect to THT followed a very similar pattern

to that of the estimates for DBH. Location contributed a large portion of the variation in THT ($\sigma_S = 2.5518$) as well as the random effect of rep within site ($\sigma_{RS} = 0.9266$). The microsite (rep within site) influence on THT was also observed with DBH. Family within type was found to be significant as well ($\sigma_{FT} = 1.3570$). A random interaction effect was detected for family within site ($\sigma_{SF} = 0.09754$). The random effect of lines within type SE ($\sigma_{LT} = 0.85959$) was more significant than the variation associated with the random interaction effect of line and planting location ($\sigma_{SL} = 0.06562$). Residual variance estimates with respect to THT also followed the same pattern as observed for DBH with type FS having the largest estimate ($\sigma_{FS} = 5.8249$), followed by type OP ($\sigma_{OP} = 5.4221$), and type SE with the smallest estimate ($\sigma_{SE} = 3.8433$).

BLUP ANALYSIS – DIAMETER AT BREAST HEIGHT

Predicted DBH from BLUP analysis for families 1, 2, and 3 by method of propagation (FS and SE) can be viewed in Table 4.3. Predicted average family DBH values for the same families show FS achieved greater DBH growth. The predicted DBH growth for FS was significantly larger than SE at the $\alpha = 0.01$ level for families 1, 2, and 3 (P-value = 0.0016, 0.0014, and 0.0002 respectively). BLUP analysis also showed the average predicted DBH growth from the two sites in Georgia was significantly larger than that of Mississippi at the $\alpha = 0.01$ level (P-value = 0.0077).

BLUP ANALYSIS – TOTAL HEIGHT

Predicted THT from BLUP analysis for families 1, 2, and 3 by method of propagation (FS and SE) can be viewed in Table 4.4. FS trees had larger average predicted THT values than SE for all families (1, 2, and 3). The predicted THT growth for FS was significantly larger than SE at the $\alpha = 0.05$ level for families 1 and 2 (P-value = 0.0317 and 0.0153 respectively). FS family 3 was significantly larger than SE family 3 at the $\alpha = 0.10$ level (P-value = 0.0522).

Average predicted THT values for the two sites in Georgia were significantly taller than the average predicted THT values for the two sites in Mississippi at the $\alpha = 0.01$ level (P-value = <0.0001).

DISCUSSION

Increased growth rate in *P. taeda* is of great interest to forest managers as selection of trees which have fast growth has been shown to increase productivity of forest stands. The utilization of somatic embryogenesis to improve growth and quality traits (by increasing the level of genetic control) is of great interest to improving forest productivity. Results of this study suggest some SE lines are capable of achieving superior growth rates to previous OP and FS trees. Figure 4.2 shows that the top three clones ranked for DBH illustrated larger average values at Willbest, Brewer, and Jesup, and had average DBH at Glynn that was marginally lower (0.10 inches). There were not any recognizable differences in THT among the three types (Figure 4.3). This was further supported when the analysis of variance failed to detect a significant difference among the fixed effect of propagation type with respect to THT (Table 4.2). Although the analysis of variance did not detect significant differences in DBH or THT between the three types (Table 4.1 and 4.2), somatic clones have the ability to grow at equal or greater rates than full and half-sib zygotics.

Uniformity of superior growth characteristics among trees would also increase forest productivity. This study showed clones had lower residual variation than FS and OP with respect to DBH and THT (Table 4.1 and 4.2). This result shows that the increase in genetic control was effective at decreasing the sample residual variation, hence increasing uniformity. This would be useful to forest managers by maximizing the proportion of trees (per unit area) which show favorable growth characteristics.

The analysis of variance for both DBH and THT had large estimates of the variance component with respect to families within a particular type (Table 4.1 and 4.2). Therefore, family growth performance (DBH and THT) was not static within a particular type, but some families illustrated superior growth despite their method of propagation. Also a large contributor to the estimated variation in DBH and THT was the random effect of site (Table 4.1 and 4.2). Locations sampled had variation with respect to their associated CRIFF soil group which includes differences in soil characteristics and drainage class. The effect of water availability would be expected to have a profound effect on growth rates. Microsite (rep within site) was also responsible for variation in DBH and THT (Table 4.1 and 4.2). This would also be expected since opportunities for growth would not be static across a location, but dynamic as competition (above and below ground), and water availability can vary within a forest stand.

Contrast based on BLUP analysis for the same families by method of propagation (FS and SE), showed FS families 1, 2, and 3 achieved faster DBH growth than the same families propagated via SE. Although this difference was significant, predicted differences between FS 1, 2, and 3 versus SE 1, 2, and 3 were less than 0.5 inches at age four. Also, SE families 1, 2, and 3 grew slower than SE families 4 and 5 but since they were not directly related to FS 1, 2, and 3 they were not included in the contrast. Height growth was significantly larger for FS families 1, 2, and 3 than SE 1, 2, and 3 but at varying levels of significance (Table 4.4). The same trend was observed as with DBH, where SE families 4 and 5 had superior THT growth compared to SE 1, 2, and 3, but due to the lack of relation to families propagated via full-sib zygotic cross (1, 2, and 3) SE families 4 and 5 were excluded from the contrast.

Contrast, based on BLUP analysis, between the average of the two Georgia sites versus the average of the two Mississippi sites, with respect to DBH and THT, showed Georgia sites

significantly achieving superior growth rates compared to Mississippi sites (Table 4.3 and 4.4). Soil types were very similar between the sites, with the only major difference being depth of sandy top layer and/or drainage class. Brewer (CRIFF soil group E) was occupied on an upland version of the soil types found among Willbest, Glynn, and Jesup (CRIFF soil groups A and B). CRIFF soil group E has a relatively high clay content which allows it to maintain moisture and nutrients making it an excellent site for loblolly pine (Jokela and Long 1999). Since Brewer did not illustrate superior growth as compared to the other sites, despite its preferred soil group, climate is quite likely responsible for the increased growth rates observed in Georgia.

TABLES AND FIGURES FOR CHAPTER 4

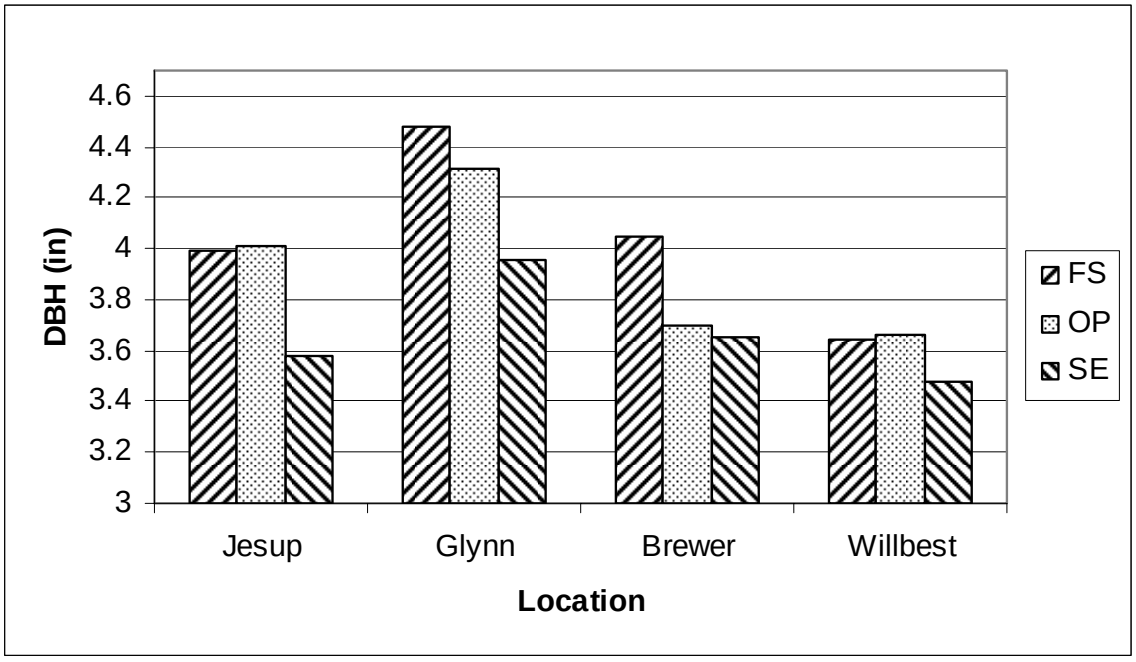


Figure 4.1. Average DBH through the 2004 growing season by type and location.

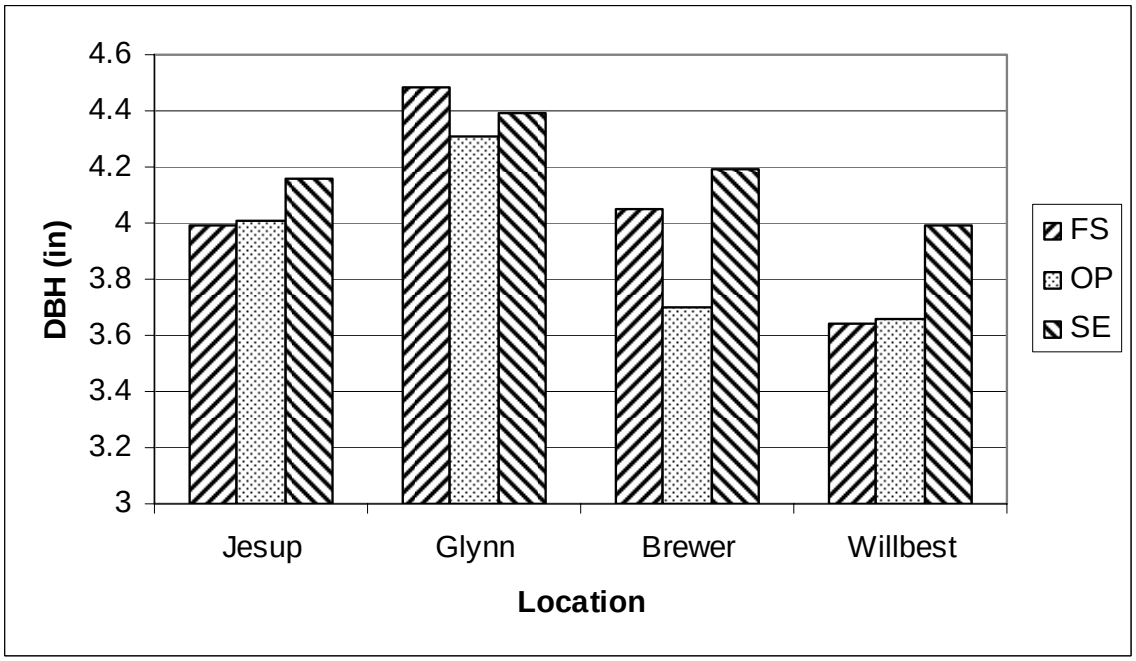


Figure 4.2. Average DBH through the 2004 growing season by type (including only the top 3 clones ranked for DBH) and location.


Figure 4.3. Average total height through the 2004 growing season by type and location.

Table 4.1. Analysis of Variance for the linear mixed effects model with respect to DBH.

| Variance Components | | | |
|---|---|---|---|
| Source[1] | Estimate | Standard Error | P-value |
| $\sigma^2_S$ | 0.047 | 0.04375 | 0.0702 |
| $\sigma^2_{RS}$ | 0.03948 | 0.01231 | 0.00035 |
| $\sigma^2_{FT}$ | 0.08451 | 0.04801 | 0.0196 |
| $\sigma^2_{SF}$ | 0.002361 | 0.003045 | 0.1095 |
| $\sigma^2_{SL}$ | 0.004315 | 0.005705 | 0.11235 |
| $\sigma^2_{LT}$ | 0.06909 | 0.02076 | 0.0002 |
| $\sigma^2_{OP}$ | 0.3117 | 0.03932 | < 0.0001 |
| $\sigma^2_{FS}$ | 0.3561 | 0.04419 | < 0.0001 |
| $\sigma^2_{SE}$ | 0.2413 | 0.01173 | < 0.0001 |
| Fixed Effects | | | |
| Source | Num. *d.f.* | Den. *d.f.* | F-value | P-value |
| $T_k$ | *2* | 7.77 | 0.91 | 0.4426 |

[1]Where:

$\sigma^2_S$ = random effect of site,
$\sigma^2_{RS}$ = random effect of replicate within site,
$\sigma^2_{FT}$ = random effect of family within type,
$\sigma^2_{SF}$ = random interaction effect of family and site,
$\sigma^2_{SL}$ = random interaction effect of line and site,
$\sigma^2_{LT}$ = random effect of line within type,
$\sigma^2_{OP}$ = half-sib (open pollinated) zygotic residual variation,
$\sigma^2_{FS}$ = full-sib zygotic residual variation,
$\sigma^2_{SE}$ = somatic embryogenesis clonal residual variation,
$T_k$ = fixed effect of type.

Table 4.2. Analysis of Variance for the linear mixed effects model with respect to total height.

| Variance Components | | | |
|---|---|---|---|
| Source[1] | Estimate | Standard Error | P-value |
| $\sigma^2_S$ | 2.5518 | 2.2055 | 0.0618 |
| $\sigma^2_{RS}$ | 0.9266 | 0.2756 | 0.0002 |
| $\sigma^2_{FT}$ | 1.357 | 0.7921 | 0.02165 |
| $\sigma^2_{SF}$ | 0.09754 | 0.06123 | 0.0278 |
| $\sigma^2_{SL}$ | 0.06562 | 0.09126 | 0.118 |
| $\sigma^2_{LT}$ | 0.8595 | 0.2648 | 0.0003 |
| $\sigma^2_{OP}$ | 5.4221 | 0.6738 | < 0.0001 |
| $\sigma^2_{FS}$ | 5.8249 | 0.722 | < 0.0001 |
| $\sigma^2_{SE}$ | 3.8433 | 0.1866 | < 0.0001 |
| Fixed Effects | | | |
| Source | Num. *d.f.* | Den. *d.f.* | F-value | P-value |
| $T_k$ | *2* | 7.4 | 0.54 | 0.6053 |

[1]Where:

$\sigma^2_S$ = random effect of site,
$\sigma^2_{RS}$ = random effect of replicate within site,
$\sigma^2_{FT}$ = random effect of family within type,
$\sigma^2_{SF}$ = random interaction effect of family and site,
$\sigma^2_{SL}$ = random interaction effect of line and site,
$\sigma^2_{LT}$ = random effect of line within type,
$\sigma^2_{OP}$ = half-sib (open pollinated) zygotic residual variation,
$\sigma^2_{FS}$ = full-sib zygotic residual variation,
$\sigma^2_{SE}$ = somatic embryogenesis clonal residual variation,
$T_k$ = fixed effect of type.

Table 4.3. DBH BLUP analysis with contrasts for families 1, 2, and 3 for type SE and FS and contrast for Mississippi (MS) vs Georgia (GA).

| Type | Family | Estimate | Standard Error | P-value |
|---|---|---|---|---|
| FS | 1 | 3.9498 | 0.1443 | - |
| FS | 2 | 4.0878 | 0.1478 | - |
| FS | 3 | 4.0741 | 0.1448 | - |
| SE | 1 | 3.5529 | 0.1454 | - |
| SE | 2 | 3.6792 | 0.144 | - |
| SE | 3 | 3.6015 | 0.1458 | - |
| | | Contrast | | |
| FS 1 vs SE 1 | | 0.3969 | 0.1219 | 0.0016 |
| FS 2 vs SE 2 | | 0.4087 | 0.1245 | 0.0014 |
| FS 3 vs SE 3 | | 0.4726 | 0.123 | 0.0002 |
| MS vs GA | | -0.117 | 0.04107 | 0.0077 |

Table 4.4. Total height BLUP analysis with contrasts for families 1, 2, and 3 for type SE and FS and contrast for Mississippi (MS) vs Georgia (GA).

| Type | Family | Estimate | Standard Error | P-value |
|---|---|---|---|---|
| FS | 1 | 20.7052 | 0.8995 | - |
| FS | 2 | 21.1517 | 0.9087 | - |
| FS | 3 | 21.3398 | 0.9009 | - |
| SE | 1 | 19.691 | 0.888 | - |
| SE | 2 | 19.9793 | 0.8854 | - |
| SE | 3 | 20.4166 | 0.889 | - |
| | | Contrast | | |
| FS 1 vs SE 1 | | 1.0142 | 0.4662 | 0.0317 |
| FS 2 vs SE 2 | | 1.1778 | 0.4791 | 0.0153 |
| FS 3 vs SE 3 | | 0.9233 | 0.4706 | 0.0522 |
| MS vs GA | | -0.9241 | 0.1961 | < 0.0001 |

# LITERATURE CITED

Jokela, E.J. and A.J. Long. 1999. Using Soils to guide fertilizer recommendations for southern pines. The University of Florida. Cooperative Extensions Service. 10pp.

# CHAPTER 5

# SPECIFIC GRAVITY VARIATION

## MEANS COMPARISION – SPECIFIC GRAVITY

Somatic clones illustrated higher average weighted core specific gravity (WCSG) compared to full-sib zygotic and open pollinated controls at all four locations (Figure 5.1). This is not surprising since Figure 5.1 includes all clones, which illustrated a variety of growth rates. Figure 5.2 shows how the WCSG values of the top three ranked clones for growth (DBH) compare to the controls and demonstrated that the fastest growing clones are still producing denser wood than the controls at all four locations. The inclusion of the top three clones ranked for WCSG illustrate much higher values than the controls (Figure 5.3). Therefore, clones which illustrate superior growth (DBH) when compared to that of the controls (Figure 5.2) will not have the highest WCSG's when compared to the top three ranked clones with respect to WCSG (Figure 5.3), but are almost directly comparable to the average WCSG values of all clones sampled (Figure 5.1). Average WCSG with associated standard deviations by type, rank, and location are listed in Table 5.1.

As previously mentioned, the increased production of denser latewood within the annual ring of a tree will have an additive effect on the overall SG of the stem. This relationship between the percent latewood (LP) within the annul ring and overall SG of the sample is often attributed to longer growing seasons in combination with late summer rainfall. In this study, it was observed that as genetic control increased (OP to FS to SE) the amount of latewood produced within the annual ring increased (Figure 5.4). Using LP values for the top three ranked

clones for growth (DBH) did not change this observation (Figure 5.5). This illustrates that the clones which showed superior growth, are not doing so at the expense of LP, and also showed higher density (greater LP) across locations.

MEANS COMPARISONS - LOCATION

Averages among all types, with associated standard deviations, for WCSG, earlywood SG, latewood SG, LP, and DBH by location can be observed in Table 5.2. The Georgia locations had higher average WCSG and LP compared to the Mississippi locations. Early and latewood SG was marginally higher for the Georgia locations when compared to the Mississippi locations. Since the difference in early and latewood SG is marginal, it is likely the higher LP found for the Georgia sites that contribute to higher WCSG in Georgia. Despite the larger SG averages found for the Georgia sites, variation is greater than observed for the Mississippi sites for all variables related to SG while variation among DBH is quite similar (Table 5.2).

MEANS COMPARISONS – OVEN-DRIED WEIGHT

The methodology used to calculate oven-dried weight (ODWT) incorporated multiple dimensions into a ranking analysis by including the growth capability of the trees sampled (THT and DBH) as well as the average WCSG. The calculated dry-weights were averaged by type (FS, OP, SE, line within SE) and location. The corresponding averages were ranked accordingly. Figure 5.6 shows averaged oven-dried weights by type and location and indicate that clones were not producing a combination of growth and density traits comparable to that of the controls, having the lowest oven-dried weights at all but one location. Figure 5.6 includes the average of all clones, some of which illustrated inferior growth and SG compared to the half and full-sib zygotic trees. Figure 5.7 makes the same comparison but instead of including all clones, only the average oven-dried weights for the top three ranked clones based on DBH

growth are included and shows that the top three ranked clones (based on DBH growth) nearly equal or exceed the controls for average oven dried weight at all locations.

ANALYSIS OF VARIANCE – SPECIFIC GRAVITY

The results of the linear mixed-effects model analysis of variance along with estimates of the random-effects components of variance with respect to WCSG are presented in Table 5.3. The main fixed effect of type was found to be significant at the $\alpha = 0.01$ level (P-value = 0.0034). Estimated mean values were found to be 0.406, 0.427, and 0.438 for OP, FS, and SE types respectively. Pairwise differences showed that both SE and FS had significantly larger WCSG estimates than OP at the $\alpha = 0.05$ level (P-value = 0.0027 and P-value = 0.0376, respectively). Also, SE and FS were not found to be significantly different from each other (P-value = 0.1505). The random effect of site ($\sigma_S = 0.000396$) contributed to a larger portion of the variation in WCSG than that of microsite or replicate within site ($\sigma_{RS} = 0.000146$). This was expected, since despite the existence of variation in microsite (soil characteristics, water availability, and competition), differences among planting location should be much more apparent. The random interaction effect of line within site was also evident ($\sigma_{SL} = 0.000162$) indicating line performance was influenced by planting location. Although variation existed, ($\sigma_{SF} = 0.000027$) the random interaction effect of family within site showed that families were not as sensitive to planting location as lines. The variation that existed among somatic clonal lines ($\sigma_{LT} = 0.000028$) could probably be credited to the sample selection methodology which incorporated selection of clones to illustrate a range of growth rates. Variation among families by type ($\sigma_{FT} = 0.000011$) contributed the least significant portion of variation, (*i.e.* families within a type illustrated minimal variation) even though the fixed effect of type is highly

significant. Also of interest, residual variance decreased as genetic control increased (OP = 0.000915, FS = 0.000747, SE = 0.000663).

ANALYSIS OF VARIANCE – LATEWOOD PERCENTAGE

Analysis of variance results with respect to LP along with estimates of the random effects components of variance are presented in Table 5.4. LP was statistically significant among the main fixed effect of type at the $\alpha = 0.05$ level (P-value = 0.011). Estimated mean LP values were found to be 25.39, 28.72, and 30.68 percent for OP, FS, and SE types, respectively. Pairwise comparisons showed type SE and FS significantly differed from OP, but at different levels of significance. FS had a higher LP than OP at the $\alpha = 0.10$ level (P-value = 0.0814), and SE had a higher LP than OP at the $\alpha = 0.01$ level (P-value = 0.0089). With respect to LP, type FS and SE were not found to be significantly different (P-value = 0.2922). The random effect of site contributed to a large portion of the variation for LP with an estimated variance component of $\sigma_S = 15.8737$. The random effect of replicate within site also varied significantly ($\sigma_{RS} = 6.4715$) illustrating variation due to microsite. This is expected since the same trend was observed for WCSG which is directly related to LP. It is highly likely that the same factors contributing to the variation in microsite for WCSG are responsible for the microsite variation in LP. The estimated variance component for the random effect of family within type ($\sigma_{FT} = 1.4064$) show that families within type capture a larger portion of the variation than the interaction effect associated with families and site ($\sigma_{SF} = 0.8907$). Therefore, there is more variation among families within a type than the interaction of the same family planted across varying locations. The interaction of the random effect of line and site ($\sigma_{SL} = 1.5364$) is slightly larger than that of family and site. This also corresponds to the variation observed with WCSG with respect to sensitivity of line performance among different planting locations. Line performance within type was observed to

be highly significant ($\sigma_{LT}$ = 8.0319) and is likely related to selection criteria of clonal lines as described with WCSG. Residual variance components decreased as levels of genetic control increased (OP = 39.4862, FS = 39.2042, SE = 38.4124).

ANALYSIS OF VARIANCE – OVEN-DRIED WEIGHT

The analysis of variance for ODWT including the main fixed effect of type and associated variance components of random effects can be viewed in Table 5.5 and shows there was not a significant difference in ODWT by type (P-value = 0.5545). Average stem ODWT as estimated by the model for each type FS, OP, and SE was 14.48, 12.95, and 12.21 lbs. respectively. Despite the lack of significant difference estimated by the model, type FS had the largest estimated stem weight. Once again, the variance component for the random effect of site was significant with respect to ODWT ($\sigma_S$ = 7.0218). The random effect of rep within site also contributed to the variation associated with stem ODWT ($\sigma_{RS}$ = 3.2342). There was also considerable variation among families within a type ($\sigma_{FT}$ = 6.8640). Minimal variation existed with respect to the random interaction effects of family and site ($\sigma_{FS}$ = 0.3003) as well as the interaction of line and site ($\sigma_{SL}$ = 0.3113). Line performance within a family also showed considerable variation ($\sigma_{LT}$ = 3.2864), which is expected since this trend has been observed for growth and density, in collaboration with the clonal line selection criteria previously discussed.

BLUP ANALYSIS – SPECIFIC GRAVITY

Predicted WCSG from BLUP analysis for families 1, 2, and 3 by method of propagation (FS and SE) can be viewed in Table 5.6. Predicted average family WCSG values for the same families show SE estimates were larger than FS estimates for all families. Despite the larger predicted WCSG for SE families, the difference was not significant for families 1 and 3, but family 2 SE had significantly larger WCSG than family 2 FS at the $\alpha$ = 0.01 level (P-value =

0.0069). Predicted WCSG was significantly larger for the two Georgia sites than the two Mississippi sites (P-value = <0.0001).

BLUP ANALYSIS – LATEWOOD PERCENTAGE

The BLUP analysis with respect to LP followed the same trend observed with WCSG (Table 5.7). Average predicted LP values were larger for the same families established via SE than FS. Significance was not present for families 1 or 3, while SE family 2 had significantly more LP than FS family 2 at the $\alpha = 0.05$ level (P-value = 0.0176). Predicted average LP for the two Georgia sites was significantly larger than the average predicted LP for the two Mississippi sites at the $\alpha = 0.01$ level (P-value = <0.0001), which was also observed with WCSG.

BLUP ANALYSIS – OVEN-DRIED WEIGHT

The average predicted ODWT based on BLUP analysis for families 1, 2, and 3 for type FS and SE can be viewed in Table 5.8 and shows that FS predicted ODWT was considerably larger than type SE. This difference was highly significant ($\alpha = 0.01$) in favor of all FS families (families 1, 2, and 3; P-value = 0.0022, 0.0017, and 0.0003 respectively). This is not surprising since growth for FS families was significantly larger than SE families and WCSG was not (except family 2) and ODWT calculations incorporate both growth and WCSG. Also, average family ODWT for the two Georgia sites was significantly larger than the two Mississippi sites at the $\alpha = 0.01$ level (P-value = <0.0001).

DISCUSSION

The potential for increased genetic control to improve growth rates, as discussed previously, is important but other factors should be considered. Fast growth due to deployment of intensive genetic and silvicultural practices has the potential to decrease the wood quality of southern pine plantations if the growth increase is manifested in the spring. Trees which grow

faster in the spring are expected to have lower average SG owing to a lower proportion of latewood which has a negative effect on average WCSG. But trees which illustrate a combination of rapid growth and good wood properties would be very useful for increasing southern pine plantation productivity.

Here we found that there are clonal lines which demonstrated superior growth and could produce wood of higher density compared to FS and OP families (the controls). The mean WCSG values of controls compared to all clones and the top 3 ranked clones for WCSG (Figures 5.1 and 5.3 respectively) showed the top 3 ranked clones for WCSG had far superior average WCSG values to that of the controls. Although, this shows some clones are capable of producing wood with superior average SG as compared to the controls, it does not provide any insight on how they would compare to the controls with respect to growth rate, which is of prime importance when making selection and deployment decisions. The average WCSG for the top three ranked clones for DBH compared to controls showed that the same clones which had superior growth also have higher average WCSG values than controls at all four locations (Figure 5.2). The ability to select clones which illustrate favorable growth (DBH) and quality (SG) characteristics indicate that somatic embryogenesis has real potential for achieving gains in southern pine plantations. Similar observations were made for LP. All clones and the top 3 ranked clones for DBH both had higher LP than controls, which likely had a large contribution to the synonymous observations made with WCSG (Figure 5.4 and 5.5). Recognition of clones which grow at equal or greater rates than controls but produce more latewood within an annual ring and hence produce higher WCSG, are beneficial for promoting the use of SE technology in increasing the productivity and quality of southern pine plantations.

The inclusion of average ODWT by type and location allowed for a ranking system which would incorporate average growth and density values. It was shown that clones were inferior with respect to ODWT (Figure 5.6). The selection criteria for clonal lines (based on THT data) which included clones with a range of growth potential, is likely responsible for reducing the average ODWT's estimated among all clones. Average ODWT's of the top 3 ranked clones for DBH have higher estimates of ODWT than controls at three of the four locations (Figure 5.7). The combination of growth and SG estimated among top DBH ranked clones contributed heavily to their calculated ODWT's. Clones which illustrate a combination of these qualities would most likely be selected for deployment.

An examination of the averaged data also suggests differences among locations, with estimates of WCSG and LP being higher for Georgia than Mississippi (Table 5.2). When comparing averages among Georgia and Mississippi, with respect to early and latewood, differences are marginal, while differences are more evident when comparing WCSG and LP. The larger average LP's observed for Georgia sites were responsible for the higher average WCSG's that were reported for Georgia locations. The larger LP's are probably caused by a longer growing season related to late summer precipitation. Observed variation was lower for Mississippi sites than in Georgia sites (Table 5.2).

There was a significant difference among the fixed effect of type with respect to WCSG and LP. Significant differences were detected at the $\alpha = 0.01$ level for WCSG and $\alpha = 0.05$ level for LP (Table 5.3 and 5.4). Pairwise differences showed that both SE and FS had significantly larger estimates of WCSG than OP. SE was significantly larger than OP at the $\alpha = 0.01$ level while FS was significantly larger than OP at the $\alpha = 0.05$ level showing the level of confidence for improving important wood quality characteristics (SG) is increasing with increased levels of

genetic control. Although the estimates for SE were greater than that of FS, they were not found to be significantly different from each other. Pairwise comparisons for LP showed results similar to those for WCSG, as SE was found to have significantly higher LP than OP at the $\alpha = 0.01$ level while FS also had statistically higher LP than OP at the $\alpha = 0.10$ level. FS and SE were not found to be significantly different (with respect to LP) despite the larger estimate for SE. Both WCSG and LP, characteristics related to wood quality, increased as the level of genetic control increased (OP to FS to SE).

To improve the wood quality of southern pine plantations by deploying clonal stock, it would be advantageous for clones to illustrate improvements in uniformity of favorable traits (SG) as compared to controls. This study found estimates of residual variation (with respect to WCSG and LP by method of propagation) decreased as genetic control increased (Table 5.3 and 5.4). This result suggests that clones express less variation than controls (with respect to WCSG and LP), and potentially will improve the overall wood quality of southern pine plantations by maximizing the proportion of trees which demonstrate superior quality traits for which they were selected.

The variance component estimates, with respect to both WCSG and LP, showed a large portion of the variation was due to the random effect of site as well as the random interaction effect of rep within site (Table 5.3 and 5.4). This is expected as locations sampled had variation with respect to their associated CRIFF soil group which includes differences in soil characteristics and drainage class. As described in Chapter 4, the effect of water availability would be expected to have a profound effect on growth rates which have an influence on SG. Also, as characteristics that influence growth change within a stand (microsite), opportunities for fast growth change, influencing the mechanical properties (SG) of the wood being produced.

The analysis of variance for ODWT, found no significant difference for the fixed effect of type (Table 5.5). This is likely a product of the lack of significant difference detected with DBH and THT since they are a major contributor to the estimates of ODWT. The residual variance associated with SE was considerably lower than that of FS and OP (Table 5.5).

Prediction of the same families, 1, 2, and 3, for WCSG and LP by method of propagation (SE and FS), based on BLUP, showed clones had higher average family WCSG and LP, but not all families were significant (Table 5.6 and 5.7). For both WCSG and LP, SE family 2 was significantly larger than FS family 2. Predicted ODWT for families 1, 2, and 3 showed FS zygotics were significantly heavier than clones (Table 5.8). The significance of FS having larger estimates of ODWT is not surprising since FS families illustrated significantly larger average values for DBH and THT, which are major contributors to ODWT as calculated in this study. Also of importance, Georgia sites significantly outperformed Mississippi sites with respect to WCSG, LP, and ODWT (Table 5.6, 5.7, and 5.8).

TABLES AND FIGURES FOR CHAPTER 5


Figure 5.1. Average weighted core specific gravity by type and location.


Figure 5.2. Average weighted core specific gravity by type (including only the top 3 clones ranked for DBH) and location.


Figure 5.3. Average weighted core specific gravity by type (including only the top 3 clones ranked for SG) and location.

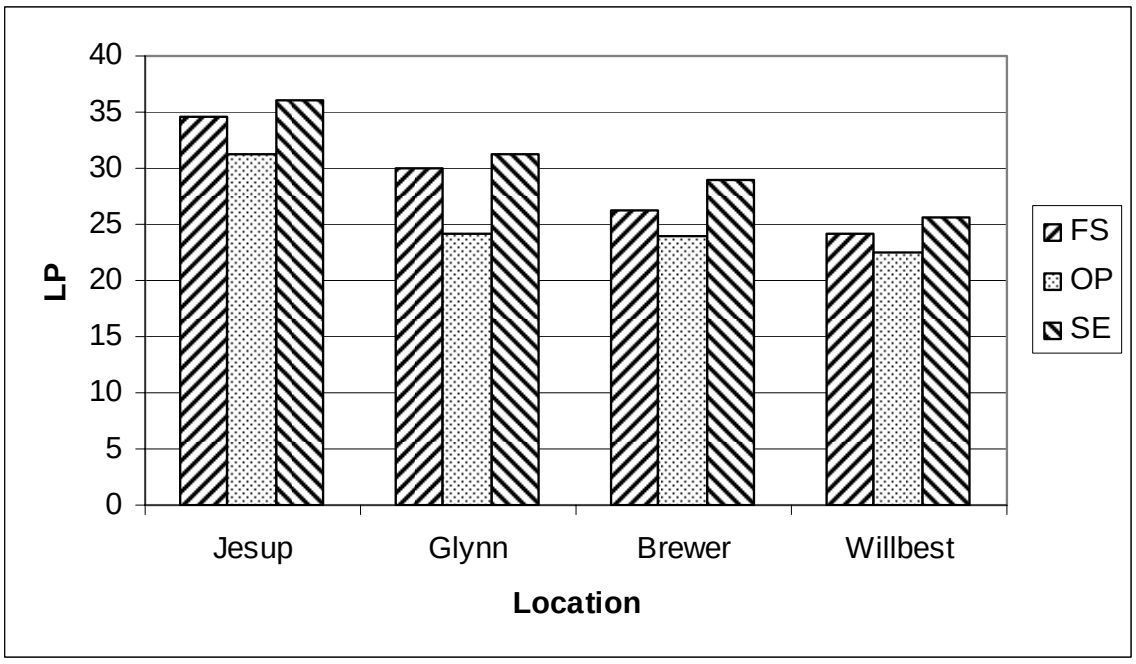


Figure 5.4. Average latewood percentage (through 4$^{th}$ growth ring) by type and location.


Figure 5.5. Average latewood percentage (through 4th growth ring) by type (including only the top 3 clones ranked for DBH) and location.

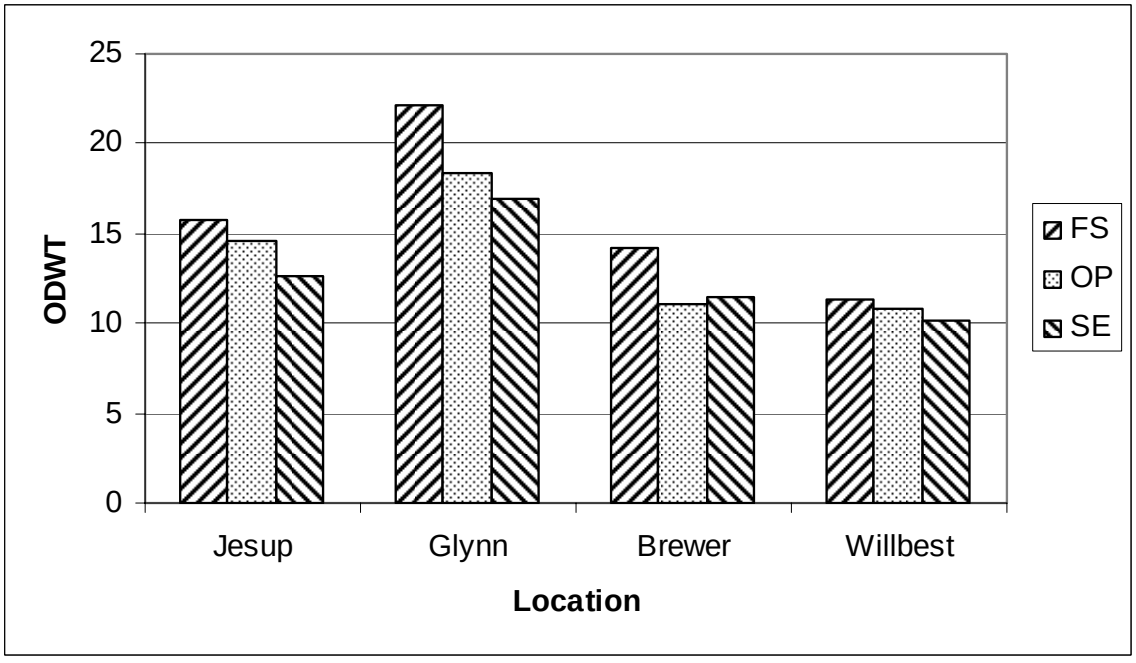


Figure 5.6. Average stem dry weight by type and location.

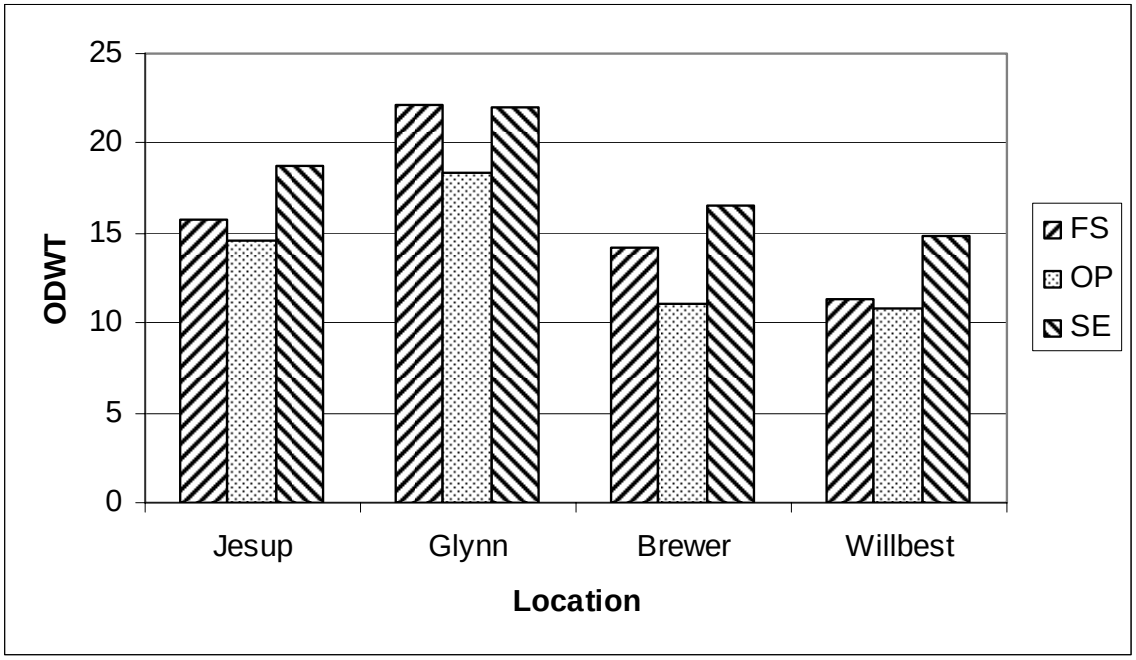


Figure 5.7. Average stem dry weight by type (including only the top three clones ranked for DBH) and location.

Table 5.1. Average weighted core specific gravity with standard deviation by type, rank, and location.

| Type | Location | | | |
|---|---|---|---|---|
| | Jesup | Glynn | Brewer | Willbest |
| Avg. SG Full-Sib | 0.454 | 0.435 | 0.408 | 0.409 |
| | 0.039 | 0.031 | 0.024 | 0.023 |
| Avg. SG Half-Sib | 0.432 | 0.412 | 0.396 | 0.396 |
| | 0.043 | 0.029 | 0.027 | 0.026 |
| Avg. SG All Clones | 0.463 | 0.446 | 0.423 | 0.416 |
| | 0.037 | 0.038 | 0.022 | 0.025 |
| Avg. SG Top 3 Clones for Growth | 0.463 | 0.449 | 0.431 | 0.43 |
| | 0.027 | 0.031 | 0.018 | 0.026 |
| Avg. SG Top 3 Clones for WCSG | 0.494 | 0.488 | 0.445 | 0.451 |
| | 0.035 | 0.038 | 0.013 | 0.023 |

Table 5.2. All type (FS, OP, SE) averages with standard deviations for weighted core specific gravity, earlywood specific gravity, latewood specific gravity, latewood percentage, and DBH by location.

| Location | Core SG | EW SG | LW SG | LW % | DBH (in.) |
|---|---|---|---|---|---|
| Jesup, GA. | 0.459 | 0.358 | 0.644 | 35 | 3.7 |
| | 0.039 | 0.028 | 0.031 | 8.90 | 0.67 |
| Glynn Co., GA. | 0.441 | 0.358 | 0.633 | 30 | 4.1 |
| | 0.038 | 0.027 | 0.030 | 8.49 | 0.56 |
| Brewer, MS | 0.419 | 0.34 | 0.618 | 28 | 3.7 |
| | 0.024 | 0.016 | 0.025 | 6.16 | 0.66 |
| Willbest, MS | 0.413 | 0.338 | 0.633 | 25 | 3.5 |
| | 0.025 | 0.017 | 0.027 | 5.53 | 0.60 |

Table 5.3. Analysis of Variance for the linear mixed effects model with respect to weighted core specific gravity.

| Variance Components | | | |
|---|---|---|---|
| Source[1] | Estimate | Standard Error | P-value |
| $\sigma^2_S$ | 0.000396 | 0.000343 | 0.062 |
| $\sigma^2_{RS}$ | 0.000146 | 0.000044 | 0.0002 |
| $\sigma^2_{FT}$ | 0.000011 | 0.000013 | 0.1012 |
| $\sigma^2_{SF}$ | 0.000027 | 0.000026 | 0.073 |
| $\sigma^2_{SL}$ | 0.000162 | 0.000049 | 0.0002 |
| $\sigma^2_{LT}$ | 0.000028 | 0.000019 | 0.0338 |
| $\sigma^2_{OP}$ | 0.000915 | 0.000115 | < 0.0001 |
| $\sigma^2_{FS}$ | 0.000747 | 0.000093 | < 0.0001 |
| $\sigma^2_{SE}$ | 0.000663 | 0.000032 | < 0.0001 |
| Fixed Effects | | | |
| Source | Num. *d.f.* | Den. *d.f.* | F-value | P-value |
| $T_k$ | 2 | 7.13 | 13.99 | 0.0034 |

[1]Where:

$\sigma^2_S$ = random effect of site,
$\sigma^2_{RS}$ = random effect of replicate within site,
$\sigma^2_{FT}$ = random effect of family within type,
$\sigma^2_{SF}$ = random interaction effect of family and site,
$\sigma^2_{SL}$ = random interaction effect of line and site,
$\sigma^2_{LT}$ = random effect of line within type,
$\sigma^2_{OP}$ = half-sib (open pollinated) zygotic residual variation,
$\sigma^2_{FS}$ = full-sib zygotic residual variation,
$\sigma^2_{SE}$ = somatic embryogenesis clonal residual variation,
$T_k$ = fixed effect of type

Table 5.4. Analysis of Variance for the linear mixed effects model with respect to latewood percentage.

| Variance Components | | | |
|---|---|---|---|
| Source[1] | Estimate | Standard Error | P-value |
| $\sigma^2_S$ | 15.8737 | 13.8994 | 0.06335 |
| $\sigma^2_{RS}$ | 6.4715 | 1.9986 | 0.0003 |
| $\sigma^2_{FT}$ | 1.4064 | 1.3404 | 0.07035 |
| $\sigma^2_{SF}$ | 0.8907 | 1.045 | 0.0985 |
| $\sigma^2_{SL}$ | 1.5364 | 1.1109 | 0.04165 |
| $\sigma^2_{LT}$ | 8.0319 | 2.4459 | 0.00025 |
| $\sigma^2_{OP}$ | 39.4862 | 5.0052 | < 0.0001 |
| $\sigma^2_{FS}$ | 39.2042 | 4.8895 | < 0.0001 |
| $\sigma^2_{SE}$ | 38.4124 | 1.8678 | < 0.0001 |
| Fixed Effects | | | |
| Source | Num. *d.f.* | Den. *d.f.* | F-value | P-value |
| $T_k$ | *2* | 7.09 | 9.1 | 0.011 |

[1]Where:

$\sigma^2_S$ = random effect of site,
$\sigma^2_{RS}$ = random effect of replicate within site,
$\sigma^2_{FT}$ = random effect of family within type,
$\sigma^2_{SF}$ = random interaction effect of family and site,
$\sigma^2_{SL}$ = random interaction effect of line and site,
$\sigma^2_{LT}$ = random effect of line within type,
$\sigma^2_{OP}$ = half-sib (open pollinated) zygotic residual variation,
$\sigma^2_{FS}$ = full-sib zygotic residual variation,
$\sigma^2_{SE}$ = somatic embryogenesis clonal residual variation,
$T_k$ = fixed effect of type

Table 5.5. Analysis of Variance for the linear mixed effects model with respect to stem dry weight.

| Variance Components | | | |
|---|---|---|---|
| Source | Estimate | Standard Error | P-value |
| $\sigma^2_S$ | 7.0218 | 6.1642 | 0.06365 |
| $\sigma^2_{RS}$ | 3.2342 | 0.9719 | 0.0002 |
| $\sigma^2_{FT}$ | 6.864 | 3.8688 | 0.019 |
| $\sigma^2_{SF}$ | 0.3003 | 0.2485 | 0.05675 |
| $\sigma^2_{SL}$ | 0.3113 | 0.3472 | 0.0925 |
| $\sigma^2_{LT}$ | 3.2864 | 1.0197 | 0.0003 |
| $\sigma^2_{OP}$ | 26.4774 | 3.3214 | < 0.0001 |
| $\sigma^2_{FS}$ | 26.9254 | 3.3382 | < 0.0001 |
| $\sigma^2_{SE}$ | 14.2871 | 0.6926 | < 0.0001 |
| Fixed Effects | | | |
| Source | Num. *d.f.* | Den. *d.f.* | F-value | P-value |
| $T_k$ | *2* | 7.67 | 0.64 | 0.5545 |

[1]Where:

$\sigma^2_S$ = random effect of site,
$\sigma^2_{RS}$ = random effect of replicate within site,
$\sigma^2_{FT}$ = random effect of family within type,
$\sigma^2_{SF}$ = random interaction effect of family and site,
$\sigma^2_{SL}$ = random interaction effect of line and site,
$\sigma^2_{LT}$ = random effect of line within type,
$\sigma^2_{OP}$ = half-sib (open pollinated) zygotic residual variation,
$\sigma^2_{FS}$ = full-sib zygotic residual variation,
$\sigma^2_{SE}$ = somatic embryogenesis clonal residual variation,
$T_k$ = fixed effect of type

Table 5.6. Weighted core specific gravity BLUP analysis with contrasts for families 1, 2, and 3 for type SE and FS and contrast for Mississippi (MS) vs Georgia (GA).

| Type | Family | Estimate | Standard Error | P-value |
|---|---|---|---|---|
| FS | 1 | 0.4283 | 0.01098 | - |
| FS | 2 | 0.4232 | 0.0115 | - |
| FS | 3 | 0.4289 | 0.01099 | - |
| SE | 1 | 0.4376 | 0.01106 | - |
| SE | 2 | 0.4396 | 0.01104 | - |
| SE | 3 | 0.4352 | 0.01107 | - |
| | | Contrast | | |
| FS 1 vs SE 1 | | -0.00923 | 0.005615 | 0.1092 |
| FS 2 vs SE 2 | | -0.01643 | 0.005717 | 0.0069 |
| FS 3 vs SE 3 | | -0.00629 | 0.005658 | 0.2742 |
| MS vs GA | | -0.01572 | 0.002464 | < 0.0001 |

Table 5.7. Latewood percentage BLUP analysis with contrasts for families 1, 2, and 3 for type SE and FS and contrast for Mississippi (MS) vs Georgia (GA).

| Type | Family | Estimate | Standard Error | P-value |
|---|---|---|---|---|
| FS | 1 | 29.3296 | 2.2621 | - |
| FS | 2 | 27.6823 | 2.2804 | - |
| FS | 3 | 29.1605 | 2.265 | - |
| SE | 1 | 30.3053 | 2.277 | - |
| SE | 2 | 30.9233 | 2.2711 | - |
| SE | 3 | 30.0316 | 2.2795 | - |
| | | Contrast | | |
| FS 1 vs SE 1 | | -0.9757 | 1.2755 | 0.4493 |
| FS 2 vs SE 2 | | -3.241 | 1.3002 | 0.0176 |
| FS 3 vs SE 3 | | -0.8711 | 1.2859 | 0.5026 |
| MS vs GA | | -2.7951 | 0.5451 | < 0.0001 |

Table 5.8. Stem dry weight BLUP analysis with contrasts for families 1, 2, and 3 for type SE and FS and contrast for Mississippi (MS) vs Georgia (GA).

| Type | Family | Estimate | Standard Error | P-value |
|---|---|---|---|---|
| FS | 1 | 14.8601 | 1.8131 | - |
| FS | 2 | 16.1977 | 1.8389 | - |
| FS | 3 | 16.5535 | 1.817 | - |
| SE | 1 | 11.5059 | 1.7569 | - |
| SE | 2 | 12.6604 | 1.7507 | - |
| SE | 3 | 12.4919 | 1.7593 | - |
| | | Contrast | | |
| FS 1 vs SE 1 | | 3.3541 | 1.0709 | 0.0022 |
| FS 2 vs SE 2 | | 3.5373 | 1.1045 | 0.0017 |
| FS 3 vs SE 3 | | 4.0617 | 1.0812 | 0.0003 |
| MS vs GA | | -2.112 | 0.397 | < 0.0001 |

# CHAPTER 6

# MICROFIBRIL ANGLE (MFA) VARIATION

## DEVELOPMENT OF A CALIBRATION FOR MFA

The calibration for MFA was developed using the Partial Least Square (PLS) regression option within the Unscrambler (version 8.0) software package (Camo AS, Norway). A second derivative math treatment was applied to the raw NIR spectra (using the Savtizky-Golay approach, with left and right gaps of 8 nm) to reduce noise present within NIR data (Næs *et al*. 2002). Calibrations were developed with 4 cross-validation segments. Calibration performance was assessed using the Standard Error of Calibration (SEC) (determined from the residuals of the final calibration), the Standard Error of Cross Validation (SECV) (determined from the residuals of each cross validation phase), and the coefficient of determination ($R^2$).

A total of 413 spectra were collected from the 84 radial strips selected for calibration purposes. The resultant MFA calibration had 10 factors recommended and gave a SEC = 2.21 degrees, SECV = 2.21 degrees, and $R^2$ = 0.69 (Figure 6.1). Outliers were examined to investigate if any samples were not well fitted by the model. The plot of residual sample X-variance (Figure 6.2) showed two large spikes (> 0.2 x $10^9$) for samples 60 and 378) indicating that these samples have spectral variation unlike the other calibration spectra and could be considered outliers. The plot of residual sample Y-variance (variation related to the MFA data) (Figure 6.3) also showed the presence of two spikes (> 150 for samples 381 and 382) indicating that these samples could be considered outliers as well with respect to their MFA data. Figure 6.4 shows a three dimensional plot with respect to the X (spectral data) and Y-axis (MFA data),

here the four identified spectra (samples 60, 378, 381, and 382) can be seen to fall well outside the range of all other spectra. Since these spectra were considered outliers, based on their large X or Y-variance, it was decided to remove them from the calibration set in an attempt to improve the MFA calibration. The 4 spectra were removed and a new calibration created based on 409 spectra from the 84 radial strips. The new MFA calibration (Figure 6.5) illustrated improved statistics, 10 factors were again recommended, but the standard errors were reduced (SEC = 1.97, SECV = 1.97), and the $R^2$ improved ($R^2$ = 0.74). Plots of residual sample variance for the new model are shown in Figure 6.6 (X-axis) and Figure 6.7 (Y-axis), a plot showing both X (spectral data) and Y (MFA data) is given in Figure 6.8. A few samples still have X or Y variance that is greater than the majority of the samples, but the level of variation is far lower than observed for the original calibration. The second MFA calibration was applied to the 5,813 spectra collected from the 1,265 radial strips representing the prediction set.

MEANS COMPARISION – MFA

The MFA data from each 10mm (0.394 in) section for each sample was averaged from pith to bark and weighted accordingly. Each section was averaged in accordance with its associated basal area from the pith in a very similar manner as the WCSG data except instead of data averaged and weighted by each ring it was averaged and weighted by each 10mm (0.394 in) section. The resultant weighted core MFA was then averaged by type and location for initial analysis to identify trends (Figure 6.9). From the initial analysis it can be seen that average MFA among types within each location was very similar (within 1 degree). Glynn and Willbest both had higher average MFA values for all types.

ANALYSIS OF VARIANCE – MFA

The results of the linear mixed-effects model analysis of variance along with estimates of the random-effects components of variance with respect to MFA are presented in Table 6.1. There was no significant difference between the main fixed effect of type (P-value = 0.8208). Estimated mean values were found to be 31.44, 31.48, and 31.10 degrees for OP, FS, and SE types respectively. The random effect of site contributed to variation in MFA ($\sigma_S = 0.5811$), but the random effect of replicate within site was more significant ($\sigma_{RS} = 1.2015$). The estimated variance component for family within type was also relatively large ($\sigma_{FT} = 0.6993$). The estimated variance components suggest much of the variation in MFA was due to families within the respective type and to replicates within a location. The estimated variance due to the random interaction effect of family within site was small ($\sigma_{SF} = 0.0371$). The random interaction effect of line within site was more significant ($\sigma_{SL} = 0.1921$). This suggests that within a specific family MFA was not significantly affected by planting location.

BLUP ANALYSIS – MFA

The average predicted MFA based on BLUP analysis for families 1, 2, and 3 for type FS and SE can be viewed in Table 6.2. Predicted MFA among families 1, 2, and 3 for both SE and FS are very similar. However, the slight variation present is not recognizable by type but among families (family 1 – FS = 31.57, SE = 31.21; family 2 – FS = 32.03, SE = 32.33; and family 3, FS = 30.83, SE = 30.49). This observation was supported by the contrast between the same family by type FS and SE. No significant difference was detected among the same family by type (P-value = 0.4758, 0.5935, and 0.5144 for families 1, 2, and 3 respectively). No significant difference was detected with respect to MFA between Georgia and Mississippi (P-value = 0.9721).

DISCUSSION

The MFA calibration performed well, with the range of predicted MFA's agreeing with the range of MFA's measured by SilviScan-2, and allowed predictions to be made for the large sample set (N = 1,265). However, the limited range of MFA represented in the calibration set likely contributed to the narrow range of NIR predicted MFA. All trees sampled were only 4 years old, composed of juvenile wood, and presumably some compression wood, resulting in a limited range of MFA's as low angles were not present at such a young age. As the trees age and lower angles are produced, calibration statistics and hence performance would be expected to improve since the range of MFA's would be greater. For example, Jones *et al*. (2005) achieved a superior MFA calibration (729 spectra from 89 radial strips, 7 factors, and $R^2 = 0.90$) for loblolly pine aged 21 to 26 years. Another factor that weakens the statistics of the MFA calibration is the observation that the precision of SilviScan-2 MFA measurements decrease as MFA increases, *i.e*. for high MFA's measurement error is greater. Schimleck et al. (2005) report that the reduction in precision associated with high MFA contributes to the weakness in statistics for calibrations based on samples having high MFA, *i.e*. a calibration based on samples having MFA's ranging from 30 to 45° will give weaker statistics than a MFA calibration based on samples with MFA's ranging from 15 to 30°. Calibrations created in this study were base on juvenile wood samples which had relatively low densities and high MFA's.

Results of this study suggest that MFA is not stable enough at age 4 to decipher differences among individuals. This is supported by Figure 6.10, as well as the lack of significant differences detected by the analysis of variance. Had the same analysis been performed at a later age, when mature wood is present, it is hypothesized that with the increased range in MFA represented in the sample, differences among individuals would be more evident

and therefore detectable. Figure 6.11 illustrates how MFA decreases (the MFA range increases) as distance from the pith increases (*e.g*. presence of mature wood).

The methodology tested in this study with respect to the use of NIR spectroscopy as a rapid, non-destructive tool for accessing the wood properties of large sample sets is promising; however, care needs to be taken to ensure that sample preparation methods are consistent between calibration and prediction sets. In this study it was found that NIR spectra are sensitive to the condition of the surface of a sample, *i.e*. samples cut using one twin-blade saw (SilviScan-2) gave slightly different spectra to those cut using a different saw (Forest Service). Therefore, it is important for future studies to maintain a consistent methodology when acquiring radial strips from increment cores that form the calibration and prediction sets. The easiest way to achieve this would be to machine all samples (cut radial strip from increment core) using the same saw (*e.g*. Forest Service twin-blade saw) and send these radial strips to SilviScan-2 for MFA measurement, rather than having a subset of cores cut on the SilviScan-2 twin-blade saw. Also, samples sent to SilviScan-2 for MFA measurement should not be acetone extracted unless all samples included in the prediction set are going to have extractives removed as well (current SilviScan-2 practice is to extract all cores prior to analysis).

Another limitation found in this study is the use of 5mm (0.20 in) increment cores as opposed to 12mm (0.47 in) increment cores. The use of a smaller core (5mm or 0.20 in) increases the difficulty of orienting the cores during the gluing process to ensure that the radial face of the sample is consistently exposed after they are cut with the twin-blade saw. In this study we found that the orientation of many cores was slightly off-set, which also influences the NIR spectra obtained. Fortunately spare cores (the opposite radius) were available and cut. The

small core also increases the difficulty of cutting a radial strip as there is no room for error associated with making sure the twin-blade saw passes through the center of the increment core.

TABLES AND FIGURES FOR CHAPTER 6

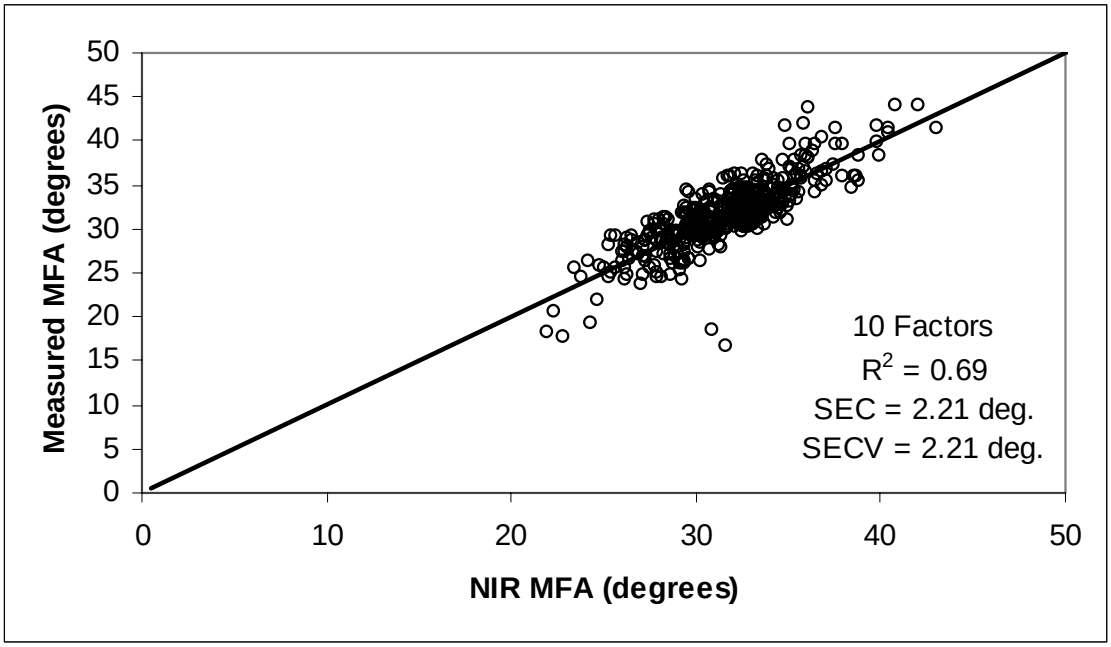


Figure 6.1. Microfibril angle calibration with number of Factors, $R^2$, SEC, and SECV (outliers included).

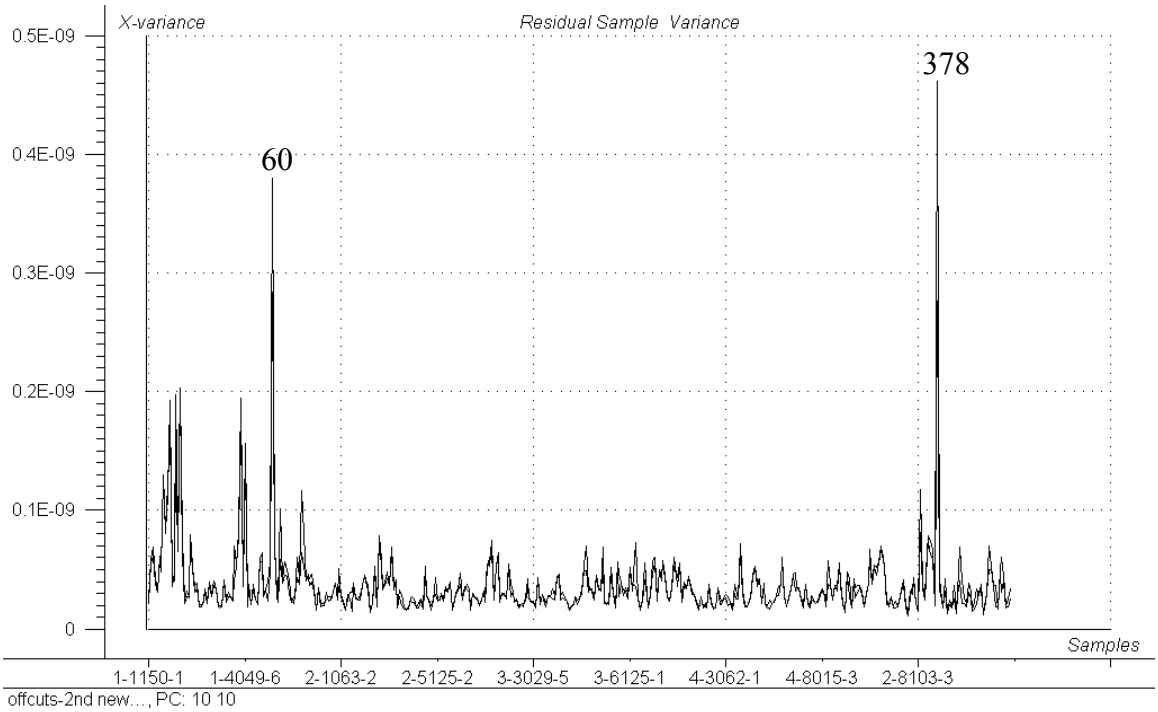


Figure 6.2. Residual sample variance plot to identify outliers with respect to X-variance (outliers included).

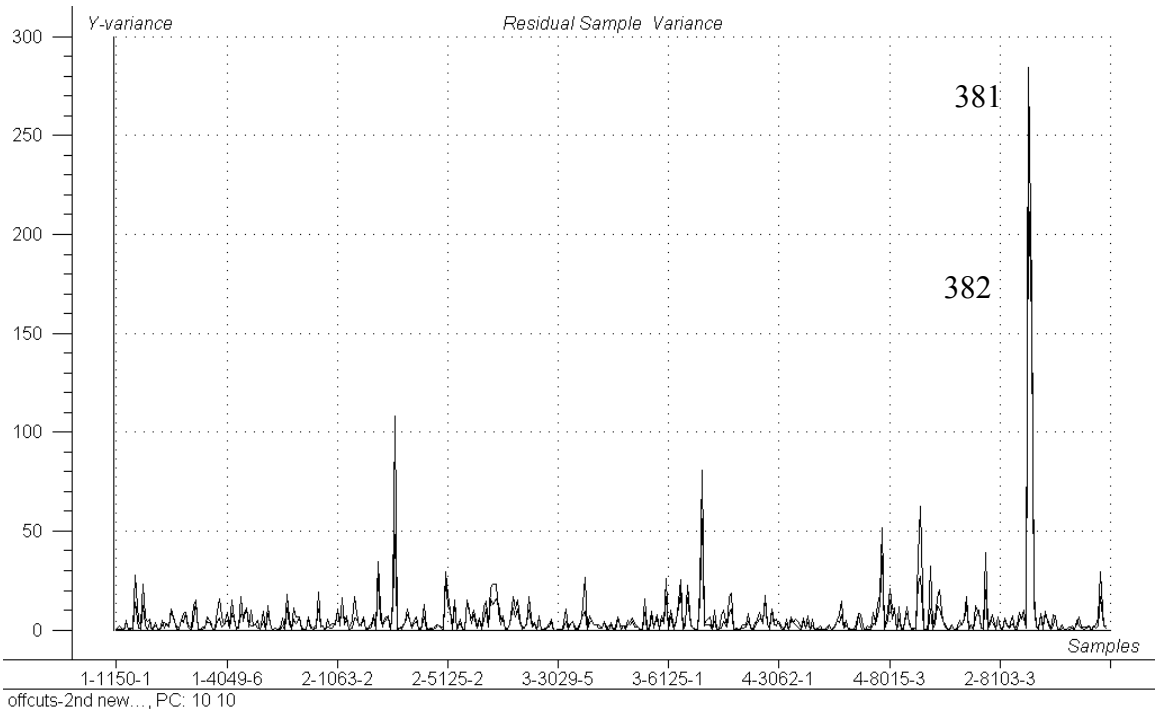


Figure 6.3. Residual sample variance plot to identify outliers with respect to Y-variance (outliers included)

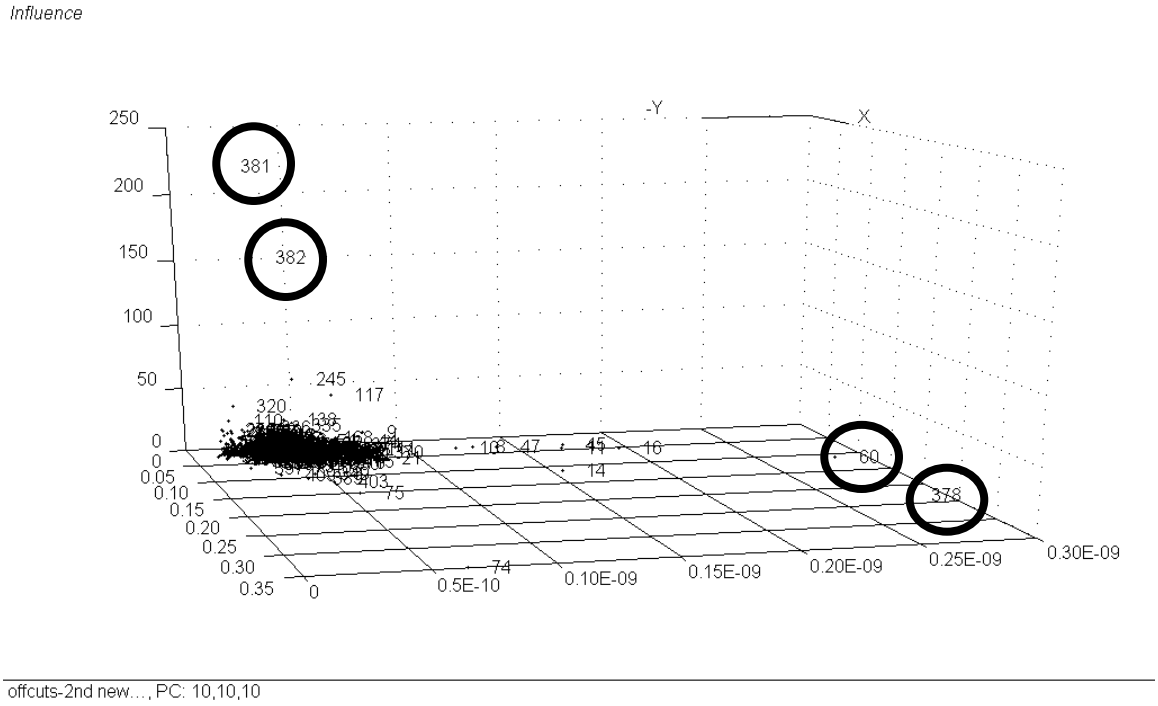


Figure 6.4. Residual sample variance plot to identify outliers with respect to X and Y- variance (outliers included).

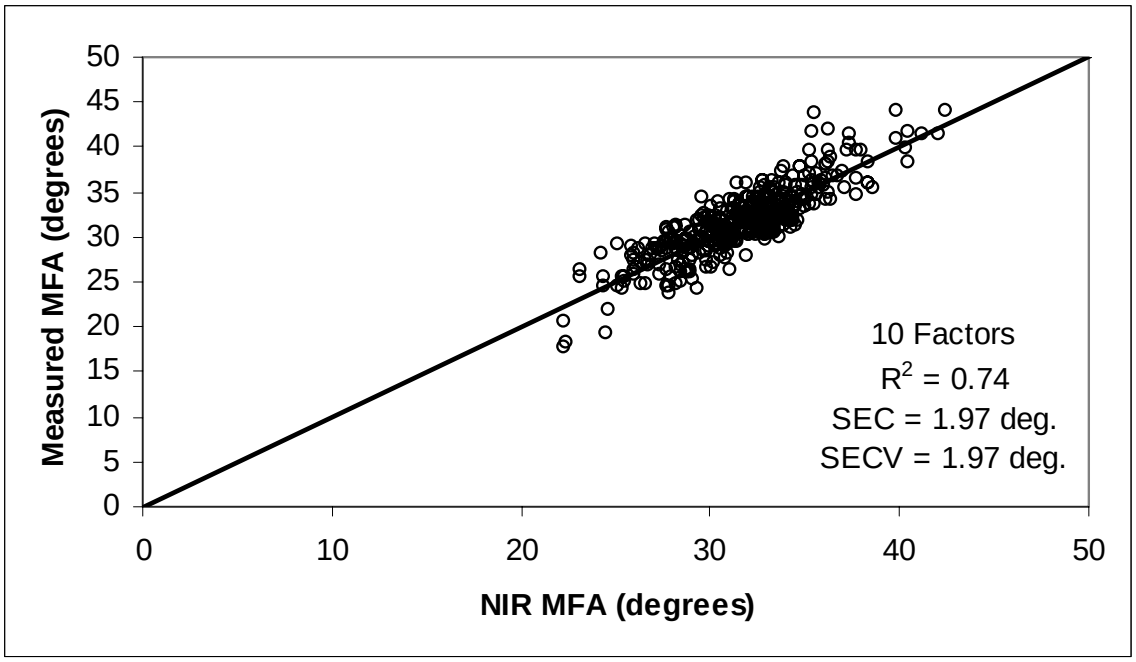


Figure 6.5. Microfibril angle calibration with number of Factors, $R^2$, SEC, and SECV (outliers removed).

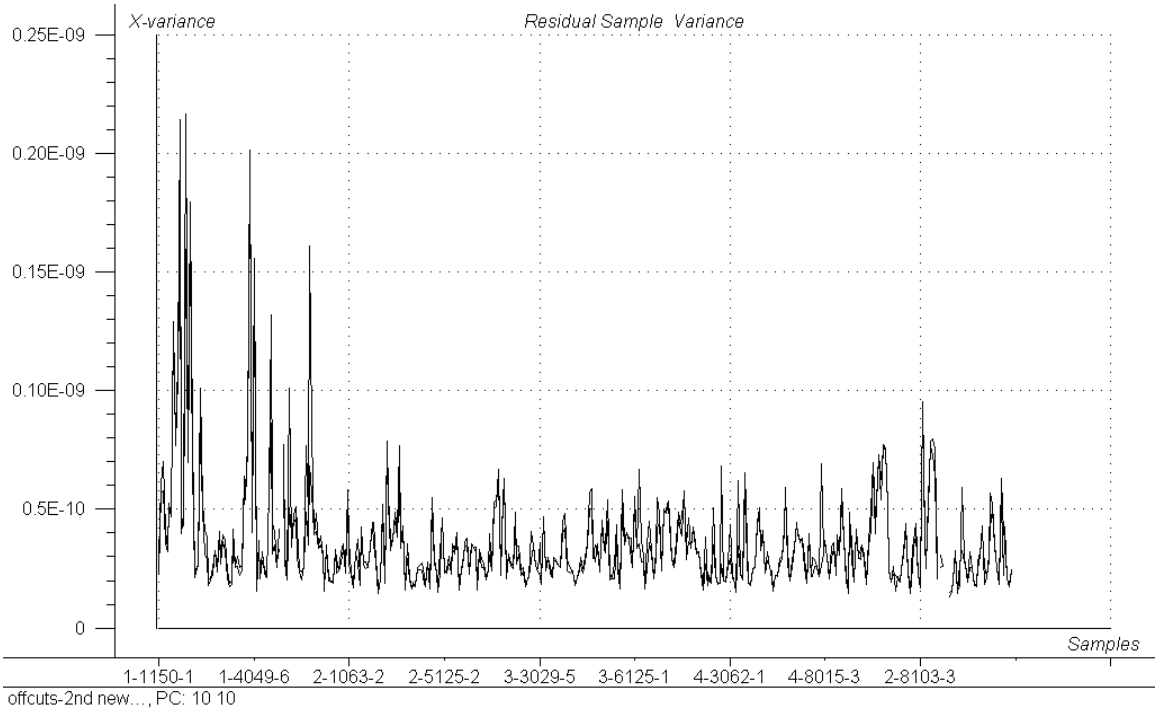


Figure 6.6. Residual sample variance plot to identify outliers with respect to X-variance (outliers removed).

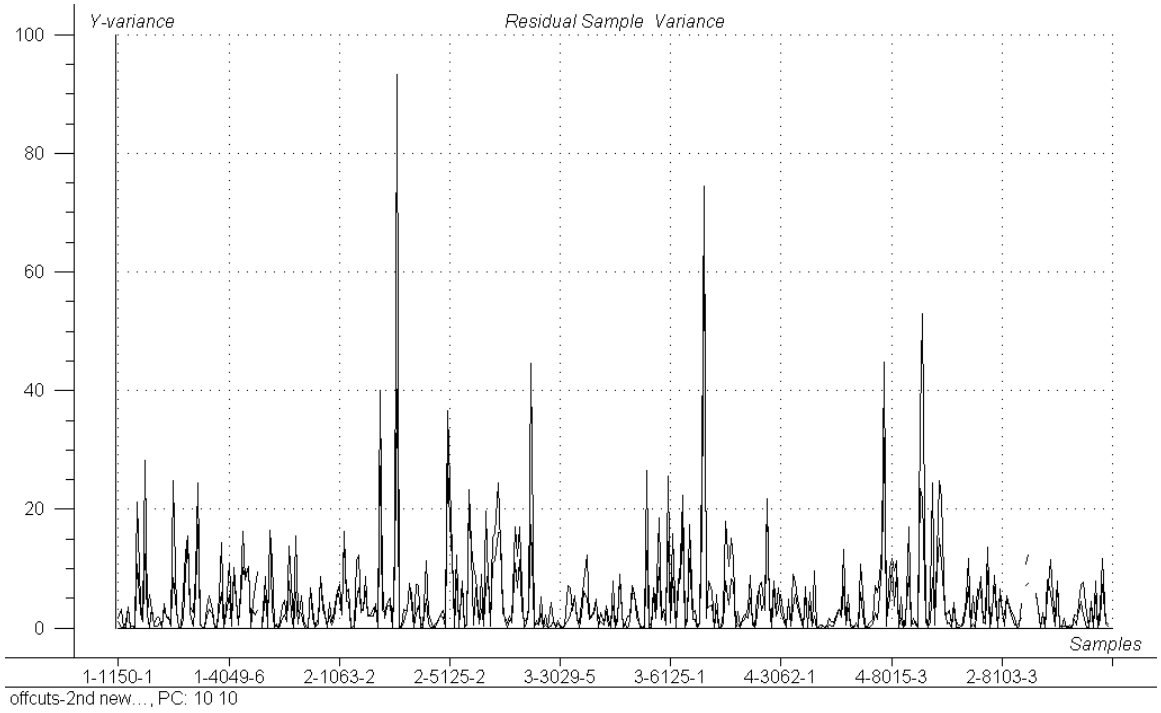


Figure 6.7. Residual sample variance plot to identify outliers with respect to Y-variance (outliers removed).

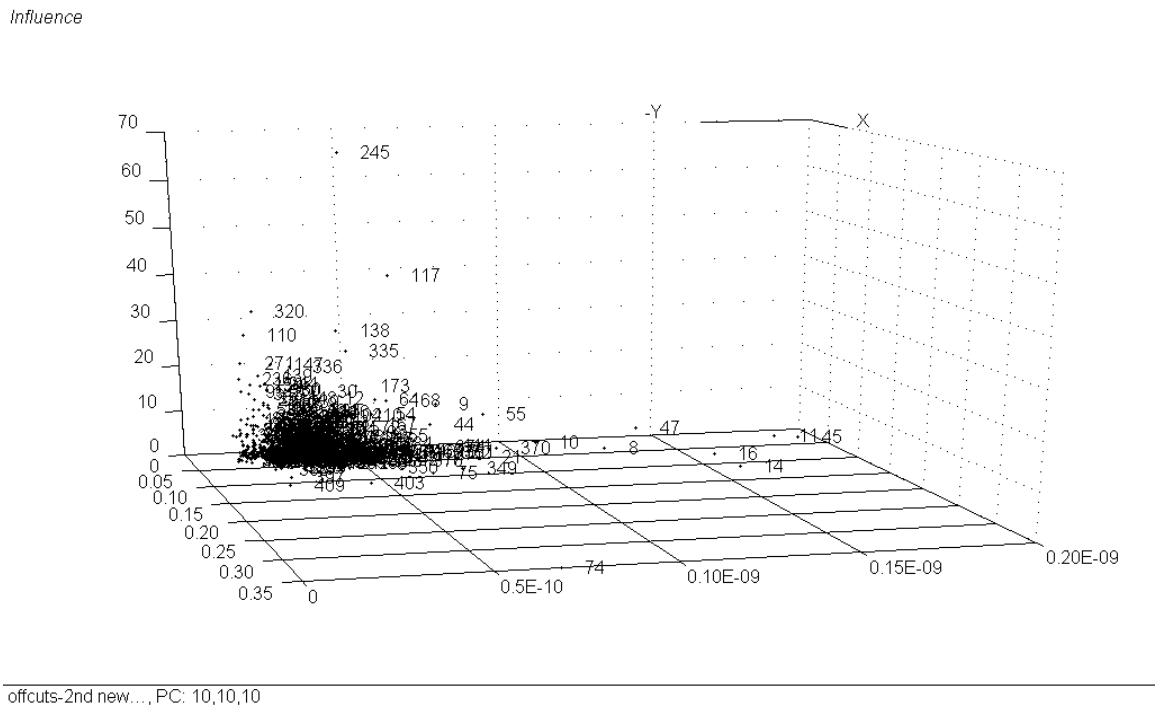


Figure 6.8. Residual sample variance plot to identify outliers with respect to X and Y- variance (outliers removed).

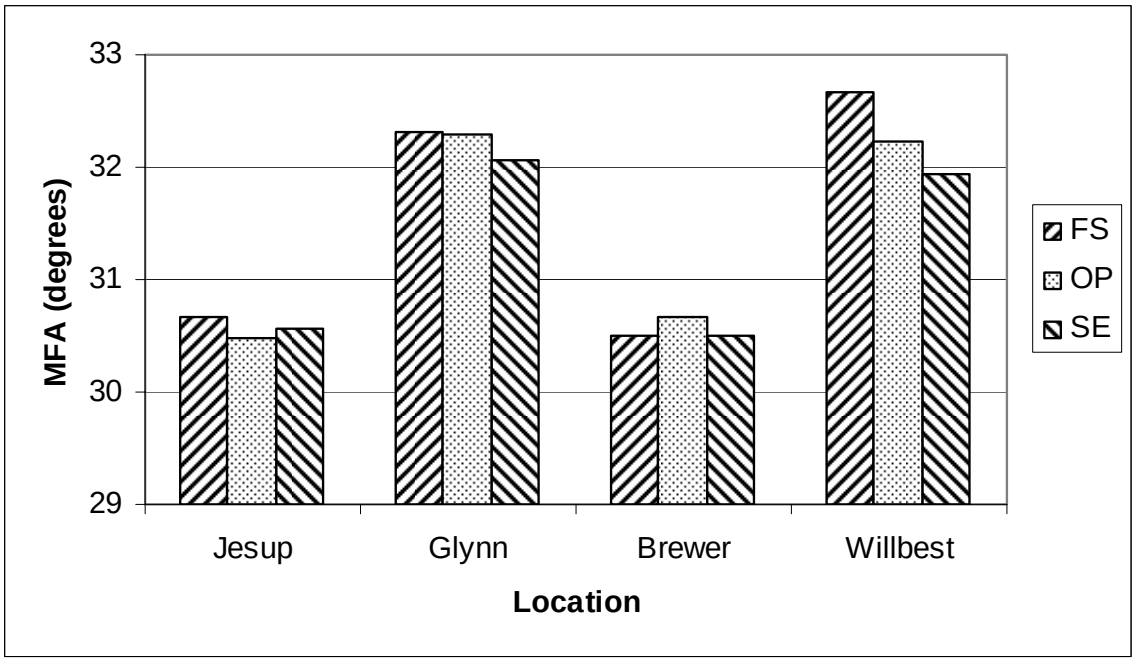


Figure 6.9. Average microfibril angle by type and location.

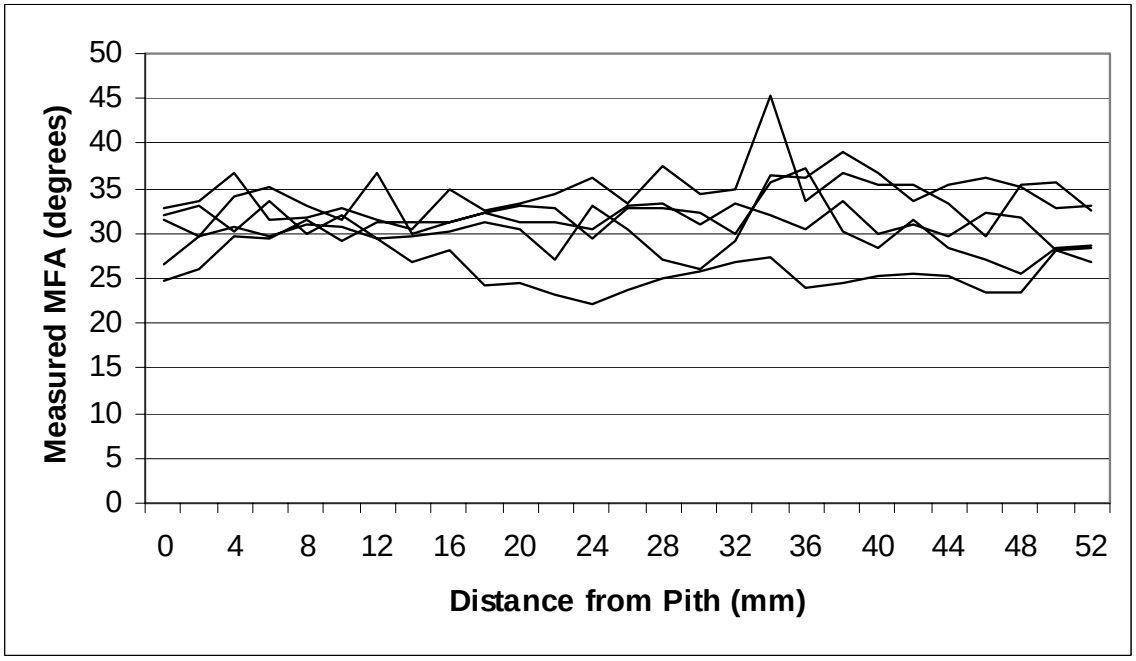


Figure 6.10. SilviScan-2 measured micorfibril angle for 5 samples included in the calibration set (2 mm resolution).

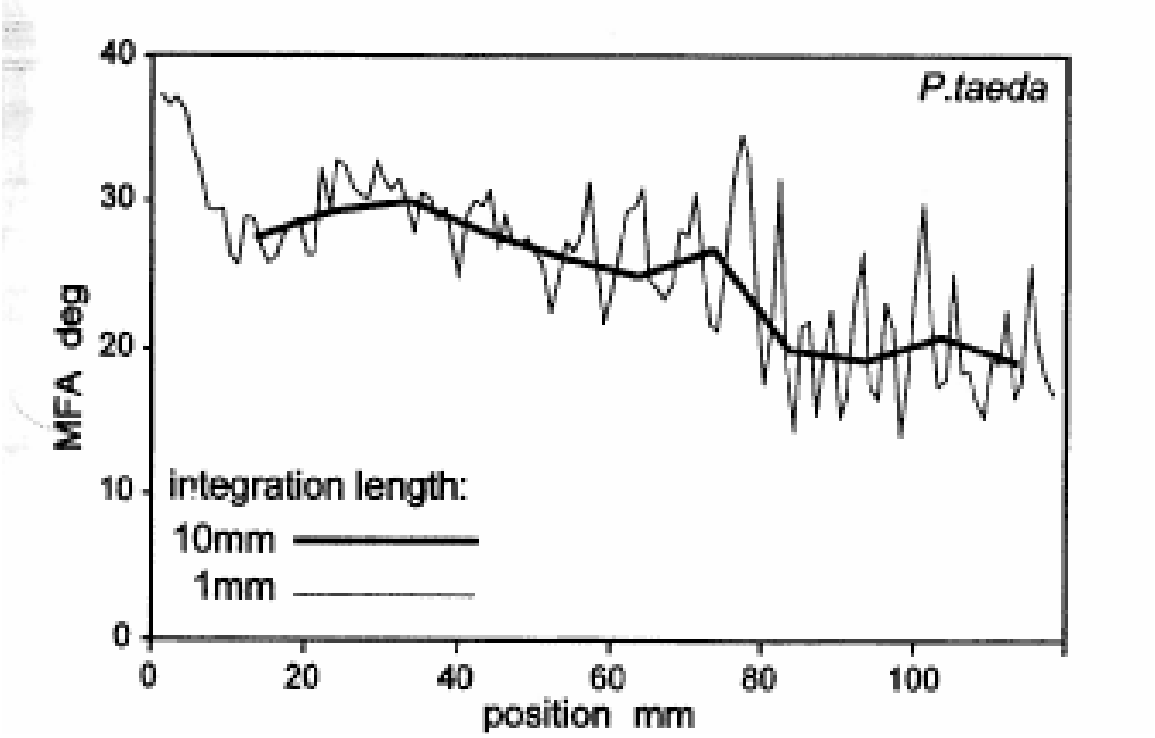


Figure 6.11. Measured microfibril angle from pith to bark at 1 and 10 mm resolution (adapted from Evans 1997).

Table 6.1. Analysis of Variance for the linear mixed effects model with respect to microfibril angle.

| Variance Components | | | |
|---|---|---|---|
| Source[1] | Estimate | Standard Error | P-value |
| $\sigma^2_S$ | 0.5811 | 0.6284 | 0.0888 |
| $\sigma^2_{RS}$ | 1.2015 | 0.3756 | 0.00035 |
| $\sigma^2_{FT}$ | 0.6993 | 0.4325 | 0.02645 |
| $\sigma^2_{SF}$ | 0.0371 | 0.08144 | 0.1765 |
| $\sigma^2_{SL}$ | 0.1921 | 0.1949 | 0.0811 |
| $\sigma^2_{LT}$ | 0.3875 | 0.1843 | 0.0089 |
| $\sigma^2_{OP}$ | 7.981 | 0.9866 | < 0.0001 |
| $\sigma^2_{FS}$ | 10.1534 | 1.2928 | < 0.0001 |
| $\sigma^2_{SE}$ | 7.6071 | 0.3719 | < 0.0001 |

| Fixed Effects | | | | |
|---|---|---|---|---|
| Source | Num. *d.f.* | Den. *d.f.* | F-value | P-value |
| $T_k$ | 2 | 8.53 | 0.2 | 0.8208 |

[1]Where:

$\sigma^2_S$ = random effect of site,
$\sigma^2_{RS}$ = random effect of replicate within site,
$\sigma^2_{FT}$ = random effect of family within type,
$\sigma^2_{SF}$ = random interaction effect of family and site,
$\sigma^2_{SL}$ = random interaction effect of line and site,
$\sigma^2_{LT}$ = random effect of line within type,
$\sigma^2_{OP}$ = half-sib (open pollinated) zygotic residual variation,
$\sigma^2_{FS}$ = full-sib zygotic residual variation,
$\sigma^2_{SE}$ = somatic embryogenesis clonal residual variation,
$T_k$ = fixed effect of type

Table 6.2. Microfibril angle BLUP analysis with contrasts for families 1, 2, and 3 for type SE and FS and contrast for Mississippi (MS) vs Georgia (GA).

| Type | Family | Estimate | Standard Error | P-value |
|---|---|---|---|---|
| FS | 1 | 31.5733 | 0.6235 | - |
| FS | 2 | 32.0339 | 0.6477 | - |
| FS | 3 | 30.8328 | 0.6334 | - |
| SE | 1 | 31.2048 | 0.5083 | - |
| SE | 2 | 32.3253 | 0.5084 | - |
| SE | 3 | 30.4863 | 0.5114 | - |
| | | Contrast | | |
| FS 1 vs SE 1 | | 0.3685 | 0.5151 | 0.4758 |
| FS 2 vs SE 2 | | -0.2914 | 0.5446 | 0.5935 |
| FS 3 vs SE 3 | | 0.3465 | 0.5299 | 0.5144 |
| MS vs GA | | -0.0089 | 0.2285 | 0.9721 |

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# CHAPTER 7

# CONCLUSIONS

The application of somatic embryogenesis for the improvement of growth and quality traits of loblolly pine is of great interest as it has the potential to provide large numbers of superior stock and hence increase the productivity of southern pine plantations. This study investigated loblolly pine outplanted at four locations. Sample trees were the result of 3 methods of propagation representing three different levels of genetic control from half-sib (open pollinated) zygotic seedings, full-sib zygotic seedlings, and clones established via somatic embryogenesis. This study illustrates that the use of advanced cloning techniques can improve the quantity and quality of merchantable wood produced by forest plantations as somatic clones demonstrated equivalent to improved growth rates, increased uniformity, and improved wood quality characteristics.

The analysis of variance results did not indicate significant differences among types (method of propagation) with respect to diameter at breast height (DBH) or total height (THT). Despite this, ranking analysis indicates that certain somatic embryogenic lines within a family are capable of superior growth as compared to controls. These are the lines that would most likely be selected for large-scale deployment. Lack of significance detected for the growth traits may be attributed to the inclusion of several clonal lines that showed inferior growth. Therefore, these lines reduced clonal family growth estimates since the analysis performed here examined growth based on family averages. Although clones did not illustrate significant improvements in

growth as compared to controls, residual variation was lower for clones (with respect to DBH and THT), which suggests an increase in uniformity with the increase in genetic control.

Clones illustrated significantly superior wood quality characteristics with respect to weighted core specific gravity (WCSG) and latewood percentage (LP) as compared to half-sib open pollinated trees. Clones were not significantly different than full-sib zygotics, but the estimates of WCSG and LP were higher. Although no significant differences were detected among types with respect to dry stem weight (ODWT), the clonal lines which illustrated superior growth as compared to controls, also illustrated superior wood quality characteristics with respect to WCSG and LP, which is advantageous for future deployment of somatic clones. Estimated residual variation was also found to be lower in clones than that of controls with respect to WCSG, LP, and ODWT, indicating an increase in uniformity, which is beneficial for utilizing somatic embryogeneic techniques to increase forest productivity.

This study indicated that MFA was not stable at age 4, with SilviScan-2 measured MFA varying considerably from pith to bark; hence differences could not be detected. Despite the lack of differences detected among types, this study indicates that increases in growth in some clonal lines were not to the detriment of MFA. We also showed that NIR spectroscopy can be utilized to predict wood properties of a large sample set where only a small percentage of the samples were actually analyzed. The MFA calibration was based on juvenile wood samples, which inherently had high MFA's, and as a consequence the range of MFA's represented in the calibration set was limited. In addition, there is greater error associated with the measurement of high MFA by SilviScan-2, which increases calibration error, especially if the set is limited to samples with high angles. Despite these constraints, a reasonable MFA calibration was attained allowing prediction on all samples. It is inferred, that inclusion of older samples (which contain

mature wood, and have low MFA's) would allow development of a stronger calibration, which would provide more precise predictions.

There were several limitations recognized in this study. The unstable nature of MFA in juvenile wood makes it an unlikely candidate for accessing wood quality in early clonal screening while specific gravity (SG) was more variable among the clonal lines allowing for detection of statistical differences, and making it a more likely candidate. A consistent radial strip surface should be obtained for both calibration and prediction sets, *i.e.* all strips should be cut using the same saw. The use of 5 mm (0.20 in) cores increases the difficulty related to achieving the correct orientation during the gluing process and it also increases the difficulty of ensuring that the twin-blade saw is lined up in the center of the increment core prior to cutting the radial strip.

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