

ABSTRACT

ECKARD, JONATHAN TYLER. Rapid Screening for Solid Wood Quality Traits in Clones of Loblolly Pine (*Pinus Taeda* L.) by Indirect Measurements. (Under the direction of Dr. Fikret Isik and Dr. Bronson Bullock.)

Clonal forestry has the potential to greatly improve the quality and uniformity of wood derived from pine plantations. However, conventional field sampling and laboratory analysis of wood samples are prohibitively time consuming and expensive to facilitate the necessary mass screening of wood quality in clones. New methods need to be assessed for providing indirect non-destructive measurements of wood quality that can be rapidly and reliably utilized in young clonal tests. This study assessed a drill resistance tool called the Resistograph and a time-of-flight acoustic tool called the TreeSonic for their efficiency at screening young clones of loblolly pine for three economically important solid wood properties: wood density, modulus of elasticity (MOE), and modulus of rupture (MOR).

A single clonal trial containing clones from three elite full-sib families of loblolly pine was used to assess the efficiency of indirect selection for wood quality. Increment cores and static bending samples were collected along with growth measurements from the clones at age 8. Basic wood density was measured at breast height and for the whole-stem using x-ray densitometry on the increment cores and weight/volume measurements on clear wood bending samples. MOE and MOR were determined from static bending tests on clear wood samples. Clone means for solid wood properties were moderately to highly repeatable. Intense clonal selection resulted in genetic gains over the overall mean ranging from 11.1 % for wood density to 19.9 % for MOE.

After adjustment for effects of friction, clone means for Resistograph amplitude values had moderately strong correlations with wood density at both breast-height (0.75) and for the entire stem (0.72). Genetic correlations between amplitude and density were quite strong (0.92–1.00). Amplitude was weakly correlated with MOE and MOR at the phenotypic level but was moderately correlated at the genetic level. Clonal variation explained only 20 % of the phenotypic variance for amplitude, such that disparities between the genetic and phenotypic correlations were due to the low repeatability of clone means for amplitude. The efficiency of the Resistograph was high for screening clones for wood density (0.78–0.89) and moderate for MOR (0.67) and MOE (0.42).

TreeSonic stress wave speed measurements (SWS) were highly repeatable (0.85) and had moderate and highly significant clone mean correlations with mechanical wood properties. SWS was largely uncorrelated with wood density. Thus, SWS was highly efficient at selecting clones for MOE (0.81), moderate for MOR (0.59), and poor for density (0.03); reverse of the results for amplitude. SWS and amplitude were uncorrelated and provided independent information regarding the variation in mechanical wood properties, such that combining them into a single index for selection increased selection efficiencies.

Simultaneous gain for growth and solid wood quality could be achieved using clonal selection indices based on indirect wood quality measurements. Substantial gain for both growth and wood quality required that moderate proportional economic weights be used. Simultaneous selection was complicated by negative genetic correlations between MOE and growth traits, as well as by relatively lower variation among clone means for MOR and

density compared to volume. Obtaining considerable gains for wood traits from selection within these families resulted in large reductions from optimal volume gains.

Drill resistance and acoustic methodologies can be reliably applied for screening and selecting clones for wood quality. These methods were shown to be rapid, inexpensive, and effective at providing desirable genetic gains. Reliability and selection efficiencies are expected to improve, especially for the Resistograph, as the sources contributing to extraneous environmental error in the measurements are identified and corrected.

Rapid Screening for Solid Wood Quality Traits in Clones of Loblolly Pine (*Pinus Taeda L.*)

by Indirect Measurements

by

Jonathan Tyler Eckard

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APPROVED BY:

Dr. Fikret Isik
Co-Chair of Advisory Committee

Dr. Bronson Bullock
Co-Chair of Advisory Committee

Dr. Bailian Li

Dr. Marcia Gumpertz

DEDICATION

This thesis is dedicated to my mother, Karen Bowman, who gave me the endless strength and encouragement needed to make it this far.

BIOGRAPHY

The author is a native of Raleigh, North Carolina born in 1981. Prior to his graduate work, he obtained his Bachelor's degree from the Forest Management program at NCSU in 2005, graduating Summa Cum Laude and Valedictorian. In recognition of his academic success, he was the recipient of several honors including the Forest Service Award for Excellence in Science and Mathematics, Maki-Gemmer-Johnson Academic Scholarship, James L. Goodwin Academic Scholarship, and induction into both Phi Kappa Phi and Xi Sigma Pi honor societies. After graduation he gained experience in forest research in the areas of silviculture and tree improvement at the Rayonier Southeast Forest Research Center. His involvement and growing interest in tree improvement research led him back to NCSU to pursue a Master's of Science in Forestry with the NCSU Cooperative Tree Improvement Program. As a research assistant, he focused on wood quality screening research in loblolly pine and was also involved in a number of other departmental research endeavors. He is currently employed as a forest geneticist with Smurfit-Stone Container Corporation based in Fernandina Beach, Florida. This document is the compilation of his graduate research.

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General Introduction

Clonal Forestry

The selection of superior individuals from within families for mass production via vegetative propagation and subsequent deployment is referred to as clonal forestry. There is currently a great deal of interest in the utilization of clonal forestry in the southeastern U.S. due to large potential genetic gains and increased uniformity to be realized in intensively managed pine plantations. Genetic gains for productivity, stem form, and wood quality would result from capturing the total genetic value (additive and non-additive) of superior selections in the clonal deployment populations. The greater uniformity resulting from clonal deployment is the subject of the research note that constitutes Chapter 4.

Interest in clonal forestry and advancement in associated technology have sparked the rapid development and improvement of vegetative propagation and tissue culture techniques as well as the establishment of rooted cutting operations for loblolly pine by forest companies. Methodological refinements for somatic embryogenesis in pine species have not only provided an efficient means of clonal replication, but have also facilitated the long term preservation of genotypes such that selections remain available for multiplication and deployment after field testing. Such advancements have made possible the efficient mass production of tested and selected clones on a scale necessary for operational deployment.

Efficient selection of superior clones for deployment will require the use of large clonally replicated field tests to mass screen clones from elite crosses. Such tests have been established for loblolly pine and used to screen a large numbers of clones for economically

important traits such as growth, form, disease resistance, and survival; the result has been promising estimates of genetic gain for these traits. Unfortunately, such large scale clonal screening has not been performed for important solid wood traits such as density, stiffness, and strength.

Importance of Solid Wood Properties

The need for consideration of solid wood properties in southern pines has been amplified by an increasing focus on intensive forest management for rapid sawtimber production. Genetically improved planting stock combined with intensive silvicultural inputs provides an economic incentive for growing sawtimber on short rotations (20-25 years). Much of the wood harvested from these young plantations is produced by the juvenile cambium and is typically referred to as juvenile wood. Juvenile wood is characterized by low density, high microfibril angles, and short tracheids. These underlying physical properties of the juvenile wood combine to result in reduced strength, stiffness, and structural stability of sawn boards, and thereby reduced value and utility compared to mature wood. As these wood properties are usually associated with a considerable genetic control, the selection and deployment of superior clones should result in sizable gains and increased uniformity for wood quality in pine plantations which would allow manufacturers to more consistently produce higher value products from the resulting wood resource.

Three important solid wood properties were assessed in a clonal trial of loblolly pine for this research: Modulus of elasticity (MOE), modulus of rupture (MOR), and wood density. MOE is a measure of wood stiffness that reflects the degree of recoverable deformation

under low stress loads during bending. MOR is a measure of wood strength that reflects the maximum load carrying capacity of a member in bending. These two mechanical properties are used indicate suitability of the wood for particular structural applications and are therefore direct determinants of product grade and value, especially when mechanical stress grading is employed. Wood density is a complex function of cell size, cell wall thickness, and percentage of latewood versus earlywood cells. This property is related not only to wood strength and stiffness, but also to the yield and quality of wood fiber. For this reason, wood density has been the most widely considered wood property for the southern pines.

Clonal Screening for Wood Quality

Despite the increasing importance of solid wood properties, mass screening of clones to identify genotypes with superior wood quality for deployment has been prohibitively expensive due to the time consuming and destructive nature associated with directly measuring wood traits. Direct measurement of mechanical wood properties requires bending tests of either sawn boards or clear wood samples cut from the stem. Direct measurement of breast height wood density requires the removal and laboratory analysis of increment cores. Additionally, these properties are highly variable within the stem, both radially from the pith to the bark and vertically from the stump to the crown, and obtaining average values over the entire merchantable stem requires felling and sectioning of trees. If wood quality is to be considered for clonal selection, then new methods need to be explored for providing non-destructive indirect measurements that are well correlated with target wood traits at both breast height and whole-stem levels and that can be rapidly and effectively used in young clonal tests.

A new tool called the Resistograph may hold potential for rapid clonal screening for wood density and mechanical wood properties. This tool is a portable drilling device that measures the amplitude of power consumption as it drills through wood material. The Resistograph has been tested in progeny trials of loblolly pine and it has been determined that the amplitude of drill resistance is positively related to the wood density of standing trees. Chapter 1 builds upon this previous research to examine the efficiency of the Resistograph at screening a clonal test for breast-height and whole-stem wood density. The relationships between Resistograph amplitude measurements and mechanical wood properties are examined in Chapter 2.

Time-of-flight acoustic tools that measure the velocity of acoustic waves traveling across a fixed distance may provide another method for rapidly measuring mechanical wood properties in standing trees. The premise is that acoustic waves travel faster through stiffer wood, such that acoustic velocity provides a measurement of stiffness called the dynamic MOE. This property of acoustic waves has been successfully applied to developing resonance based acoustic tools for log sorting in *Pinus radiata* and *Eucalyptus spp.* More recently, the technology has been adapted to develop “time-of-flight” based acoustic tools for measuring wood stiffness in standing trees. Chapter 2 examines the utility of a time-of-flight tool called the TreeSonic for screening clones for mechanical wood properties.

Simultaneous Selection of Multiple Traits

While it is desirable to select and deploy clones with superior wood quality, other economically important traits such as volume, disease resistance, and stem form must be

considered for clonal selection. Selection index methodology can therefore be employed to provide an efficient means of simultaneous selection for multiple traits. This involves combining information on economic values, repeatabilities, and variances for each trait, as well as relationships among all traits to derive a single index on which to base selection. The objective of Chapter 3 was to use genetic parameters estimated for total height, breast height diameter, volume, MOE, MOR, and wood density to assess the potential for simultaneous improvement of growth and wood quality through the use of clonal selection indices. While genetic parameters (repeatabilities, variances, and correlations) can all be estimated from genetic testing, economic values are difficult to determine and often vary by organization and over time. Thus, clonal selection indices were assessed for a range of economic weights for growth and wood quality in Chapter 3.

Chapter 1

Rapid Screening for Wood Density in Clones using the Resistograph

1.1 Introduction

Wood density is perhaps the most widely recognized property affecting the quality of wood derived from pine plantations. It has a large effect on both the structural properties of the solid wood (Yang and Evans 2003, Megraw 1985) and the yield and quality of pulp products (Blair et al. 1975, Kibblewhite and Lloyd 1983, Whiteman et al. 1996, Wimmer 2002). Consideration of wood density is further amplified by increasing quantities of juvenile wood harvested from young, intensively managed plantations (Kennedy 1995). This wood is comprised mostly of earlywood with large thin-walled cells, resulting in a low-density wood that is less desirable or insufficient for certain applications (Zobel and Van Buijtenen 1989). Fortunately, sufficient genetic variation exists to change juvenile wood density in young plantations by selection and deployment of superior genotypes (Zobel et al. 1978).

Selection of superior clones within families for deployment (i.e. clonal forestry) based on wood density has the potential to greatly improve the quality and uniformity of wood derived from young pine plantations (Libby and Rauter 1984). Efficient employment of clonal forestry to improve wood density would require the use of large clonally replicated field tests to mass screen clones from elite crosses (Isik et al. 2005). While these clonal tests have been established for loblolly pine (*Pinus taeda*) and used to screen clones for growth and disease resistance traits (Isik et al. 2005), such large scale clonal screening of wood density has yet to be employed. The major limitation has been the cost associated with measuring a large sample of trees for wood density.

Wood density is most accurately represented by a weighted mean of several samples taken

along the stem. This whole-stem measure accounts for the variability in density found throughout the stem of pine species (Megraw 1985, Burdon et al. 2003). However, wood density measured from a single increment core removed at breast height has been found to be a cost efficient and less destructive alternative to whole-stem sampling (Raymond et al. 1998). Unfortunately, both methods require subsequent laboratory processing and analysis of the wood samples using a volumetric method (ASTM 1985) or X-ray densitometry (Gartner et al. 2002). These laboratory procedures are prohibitively time consuming and expensive for large scale clonal screening. A rapid, reliable, and cost efficient method for obtaining a measurement of wood density in the field is necessary to facilitate clonal screening for wood density.

Instruments that have been assessed for producing rapid field measurements of wood density include the Torsiometer and Pilodyn Wood Tester (Cown 1978, Taylor 1981, Sprague et al. 1983). The Torsiometer method uses a torque measuring device mounted on an increment borer to record the force required to turn the borer. Although this method reduces subsequent laboratory work, it requires manual removal of increment cores (Sprague et al. 1983). The Pilodyn Wood Tester is a small tool that propels a spring loaded needle into the tree to measure the depth of penetration. High clone mean correlations have been found between the depth of needle penetration and density of the outer rings (Cown 1978). However, this technique is confined to measuring the outerwood and is not recommended for through-the-bark measurements (Cown 1978, Kock and Fins 2000). Variable relative efficiencies of 9.5% to 83% were reported by Sprague et al. (1983) when using Pilodyn penetration for indirect selection of wood density.

A new device produced by IML (Instrumenta Mechanik Labor) called the Resistograph may hold greater potential for rapid assessment of wood density. The Resistograph is a portable drilling device designed for structural examination of timbers, poles, and standing trees (Winistorfer, 1995). The tool measures the resistance in terms of power consumption (amplitude) as a 3 mm drilling needle is advanced into wood material at a constant speed. By measuring amplitude relative to drilling depth, the tool produces a profile of resistance through the wood material. An onboard electronic unit records the drilling profile data and can be used to automatically output mean values of the bark-to-bark resistance profiles after drilling each tree. Compared to alternative methods for measuring wood density, the Resistograph measures a larger proportion of the wood, from bark-to-bark, and is less destructive (Isik and Li, 2003).

Several studies have examined the relationships between Resistograph measurements and wood density. Mean values of resistance profiles from the Resistograph have been shown to be highly correlated ($R^2 > 0.8$) with gross density of dry wood (Rinn et al. 1996). Chantre and Rozenberg (1997) found a moderate correlation between mean values for radial x-ray densitometry and Resistograph profiles obtained from standing trees in a 25-year-old Douglas-fir stand. Isik and Li (2003) found a strong family mean correlation (0.92) between amplitude and wood density measured at breast height in a loblolly pine progeny trial. The same study also found a strong additive genetic correlation between these two traits resulting in high indirect selection efficiencies for family means using the Resistograph. However, the effectiveness of the tool at screening clones for density in large field tests remains to be assessed.

This research evaluates the utility of the Resistograph for rapidly screening clones of loblolly pine within elite full-sib families for wood density in a young clonally replicated field test. The specific objectives were to 1) examine the relationships between Resistograph amplitude values and both breast height and whole-stem wood density measured directly from increment cores, 2) estimate the relative contribution of genetic and environmental factors to the variation observed in these traits, and 3) to estimate the efficiency of clonal selection for wood density based on amplitude relative to selection based on breast height increment cores. If clone mean amplitude values are highly repeatable and have a strong genetic correlation with direct laboratory measurements of wood density, then the Resistograph may have potential for large scale clonal screening for wood density. In this case, the Resistograph would be a valuable tool to facilitate the future improvement of wood quality in intensively managed pine plantations.

1.2 Materials and Methods

1.2.1 Material and Data Collection

Three partially related full-sib families of loblolly pine were selected from the North Carolina State University – Industry Cooperative Tree Improvement Program on the basis of superior volume production and rust resistance. Progeny from these three families were clonally replicated by means of hedging and subsequent vegetative propagation to produce rooted cuttings. The rooted cuttings were used for establishment of a clonal test in Berkley County, South Carolina in December 1997. The field design was a randomized complete block with families planted as block plots within each replication. Clones within these

families were planted in single tree plots, such that each clone was represented with a single ramet in each block.

Ninety clones, consisting of 30 from each full-sib family, were randomly selected from the test at age-8 for measurement of breast height wood density. Six ramets of each clone were sampled at breast height (1.4 m) for 12 mm bark-to-bark increment cores using gas powered drills. Increment cores were taken in the same direction for all trees and any cores containing major defects such as branches, compression wood, or resin pockets were discarded and re-sampled. Diameters at breast height were measured for all cored trees. After completion of field sampling, cores were placed in a forced air oven for drying. The dried cores were then split at the pith and one half was selected for processing into 2 mm radial strips. These 2 mm strips were conditioned to a moisture content of 8% prior to analysis of wood density.

Wood density (kg/m^3) was measured on the 2 mm radial strips by x-ray densitometry using a QMS Model QTRS-01X Tree Ring Analyzer. The x-ray attenuation measured by the densitometer was related to density by $\mu_l = \mu_m \times \rho$, where μ_l is the measured attenuation of the x-ray beam passed through the sample, μ_m is the sample mass attenuation coefficient, and ρ is density. Therefore, calculating density required the mass attenuation coefficient (cm^2/g) of the wood to be known. Calibration to the appropriate mass attenuation coefficient was conducted using a set of 24 radial strips from loblolly pine cores with densities previously determined by standard volumetric methods. The 24 mass attenuation coefficients were averaged to provide the final value to be used for calculating wood density.

All trees sampled for breast height increment cores were also drilled with the Resistograph model F-400 to provide paired measurements of density and amplitude (Figure 1.1). Drilling was conducted in the same direction as the core hole and within 5 cm above or below the core hole when possible. Each tree was drilled once from bark to bark at a forward speed of 25 cm/min. Drilling files for each tree were stored on the onboard electronic unit. These files contained amplitude profile data such as tree identification and percent amplitude measurements at each 0.1 mm increment of drilling depth. The means of the amplitude profiles (\overline{AMP}) were automatically computed by the electronic unit after drilling each tree. The drilling files were later exported and converted to text files using the included F-Tools Pro software for further manipulation in SAS (SAS Institute Inc. 1999).

From the original 90 clones, a sub-sample of 36 clones consisting of 12 from each full-sib family was used for analysis of whole-stem density. These 36 clones were randomly selected from the quartiles of the distribution of clone mean density to provide a representative sample. The selected clones were felled in 3 blocks, resulting in 3 ramets sampled for each clone. From each felled tree, six 12 mm bark-to-bark increment cores were taken at fixed heights from the stump (0.2 m) to the base of the live crown (6.2 m) at 1.2 m intervals for determination of wood density. Diameters outside bark at each of the six sampling heights were measured to allow for calculation of sectional volumes between each pair of samples.

1.2.2 Calculating Whole-Stem Density

Sectional volumes between each pair of sample heights were calculated using Smalian's

volume equation (Avery and Burkhardt 2002) such that $V_{ij} = 1.2 \times (B_i + B_j) / 2$, where V_{ij} is the sectional volume and B is the basal area with subscripts referring to the top and bottom of the stem sections. Smalian's formula yielded accurate bolt volumes since differences between diameters at consecutive sampling heights did not exceed 30% (Ministry of Forestry 1999). The mean density of each section was determined by weighing the density at the top and bottom of the section by the basal area at the corresponding sampling heights such that $\rho_{ij} = (\rho_i B_i + \rho_j B_j) / (B_i + B_j)$, where ρ_{ij} is the sectional density and ρ_i and ρ_j are the densities measured from increment core samples at the top and bottom of the stem section. Whole-stem wood density was then calculated as an average of the mean sectional densities weighted by the sectional volumes. A summary of the data used to calculate whole-stem density shown Figure 1.2 indicates the typical longitudinal trend for wood density in loblolly pine (Megraw 1985).

1.2.3 Processing Amplitude Profiles

Amplitude profiles produced by the Resistograph exhibited a strong increasing trend in percent amplitude with drilling depth due to increasing friction along the drilling needle (Figure 1.3). These increasing trends were often non-linear and were confounded with the effects of ring density, such that no single parameter could be defined to adjust the profiles for all trees. Centered moving averages and centered moving minimums were therefore applied to the exported amplitude profile data as suggested by Isik and Li (2003) to reduce the profile irregularities and potentially improve the relationship between mean amplitude and wood density (Figure 1.3). Centered moving averages were calculated using a 1 mm

window while centered moving minimums were calculated using a 10 mm window. Subtracting the centered moving minimums from the centered moving averages for each 0.1 mm of drilling depth yielded an adjusted amplitude profile that eliminated the increasing trend in the profiles (Figure 1.4). The mean of the centered moving minimums from the first half of the profiles were added back to the adjusted values to restore the appropriate scale and yield the final adjusted amplitude profile.

A SAS macro (see Appendix) was used with the EXPAND and MEANS procedures to apply this profile adjustment to all measured trees (SAS Institute Inc. 1999). The macro read the drilling file for each tree, performed the indicated adjustments, and output mean values of the adjusted profiles. Adjusted mean amplitude (\overline{AMP}_{ADJ}) was then compared to unadjusted mean amplitude (\overline{AMP}) based on efficiency for selecting clones for wood density.

1.2.4 Statistical Analysis

Phenotypic relationships between wood density, amplitude, and diameter traits were assessed by fitting linear regression models. As the objective of this study was to assess within-family selection of clones, it was not desired to include the intertrait covariance caused by the full-sib families in these calculations. The differences among the three families could result in large grouping of data points that would create misleading correlations. Family effects were therefore removed by including a family class variable as a covariate in the linear models using PROC GLM (SAS Institute Inc. 1999). T-tests were performed at the 95% confidence level to assess the significance of these intertrait phenotypic correlations.

The phenotypic variance for each trait was partitioned into its genetic and environmental components according to the following general linear mixed model:

[Eq. 1.1]
$$y_{ijk} = \mu + b_i + f_j + bf_{ij} + c(f)_{k(j)} + \varepsilon_{ijk}$$

where y_{ijk} is the individual phenotypic observation, μ is the grand mean, b_i is the fixed effect of the i th block, f_j is the fixed effect of the j th family, bf_{ij} is the fixed interaction effect between the i th block and j th family, $c(f)_{k(j)}$ is the random effect of the k th clone within the j th family, and ε_{ijk} is the random environmental error. Based on mixed model assumptions, the random clone within family effects were assumed to be $\sim \text{NID}(0, \sigma_{C(F)}^2)$, while the random residual environmental effects were assumed to be $\sim \text{NID}(0, \sigma_E^2)$. Clone within family ($\sigma_{C(F)}^2$) and residual (σ_E^2) variance components were estimated using Restricted Expected Maximum Likelihood methods by fitting the mixed model in ASReml (Gilmour et al. 2002).

By fixing the family effect (f_j) in the mixed model, it was assumed that there was no variance associated with full-sib families. Thus, the estimated individual tree phenotypic variance within families ($\hat{\sigma}_{C(F)}^2 + \hat{\sigma}_E^2$) was interpreted as the total phenotypic variance. Correspondingly, the phenotypic variance of the clone means was calculated as $\hat{\sigma}_{C(F)}^2 + \hat{\sigma}_E^2 / b$, where b is the number of ramets per clone and is equivalent to the number of blocks.

Variance components estimated from fitting the mixed models were used to calculate the phenotypic coefficient of variance for clone means ($CV_{\bar{C}(F)}$), percent of the phenotypic variance explained by clonal variance ($\% \sigma_{C(F)}^2$), and repeatability of clone means ($H_{\bar{C}(F)}^2$) for each trait. These parameters were calculated as shown in Equations 2 through 4 below:

$$[\text{Eq. 1.2}] \quad CV_{\bar{C}(F)} = \frac{\sqrt{\sigma_{C(F)}^2 + \sigma_E^2 / b}}{\bar{X}} \times 100$$

$$[\text{Eq. 1.3}] \quad \% \sigma_{C(F)}^2 = \frac{\sigma_{C(F)}^2}{\sigma_{C(F)}^2 + \sigma_E^2} \times 100$$

$$[\text{Eq. 1.4}] \quad H_{\bar{C}(F)}^2 = \frac{\sigma_{C(F)}^2}{\sigma_{C(F)}^2 + \sigma_E^2 / b}$$

where $\hat{\sigma}_{C(F)}^2 + \hat{\sigma}_E^2$ is the total phenotypic variance, $\sigma_{C(F)}^2 + \sigma_E^2 / b$ is the phenotypic variance of clone means, and \bar{X} is the grand mean. Genetic correlations ($r_{G(x,y)}$) and environmental correlations ($r_{E(x,y)}$) between pairs of traits were estimated by fitting bivariate models in ASReml (Gilmour et al. 2002) for each pair of traits to satisfy the following formulas:

$$[\text{Eq. 1.5}] \quad r_{G(x,y)} = \frac{\sigma_{C(F)xy}}{\sqrt{\sigma_{C(F)x}^2 \sigma_{C(F)y}^2}}$$

[Eq. 1.6]
$$r_{E(x,y)} = \frac{\sigma_{Exy}}{\sqrt{\sigma_{Ex}^2 \sigma_{Ey}^2}}$$

where $\sigma_{C(F)xy}$ is the clone within family covariance for traits x and y , $\sigma_{C(F)x}^2$ is the clone within family variance for trait x , $\sigma_{C(F)y}^2$ is the clone within family variance for trait y , σ_{Exy} is the environmental covariance for traits x and y , σ_{Ex}^2 is the environmental variance for trait x , and σ_{Ey}^2 is the environmental variance for trait y . Standard errors for repeatabilities and genetic correlations were estimated using the delta method (Lynch and Walsh 1998).

Percent genetic gains from within family clonal selection were calculated according to Curnel et al. (2003) and O'Neill et al. (2005). Percent genetic gain for whole-stem wood density (trait y) resulting from direct selection on whole-stem wood density was estimated as shown in Equation 7. Percent genetic gains for whole-stem wood density resulting from indirect selection on either breast height density or amplitude (trait x) were estimated according to Equation 8:

[Eq. 1.7]
$$\% \Delta G_{y(y)} = i_y H_{\bar{C}(F)y}^2 CV_{\bar{C}(F)y}$$

[Eq. 1.8]
$$\% \Delta G_{y(x)} = i_x H_{\bar{C}(F)x} H_{\bar{C}(F)y} r_{G(x,y)} CV_{\bar{C}(F)y}$$

where $H_{\bar{C}(F)y}^2$ is the clone mean repeatability for whole-stem density, $H_{\bar{C}(F)x}$ is the square root of the clone mean repeatability for the indirect trait, $CV_{\bar{C}(F)y}$ is the clone mean phenotypic coefficient of variance for density, $r_{G(x,y)}$ is the genetic correlation, and i_x and i_y are the standardized selection differentials. The relative selection efficiencies (Q) of the were calculated as a ratio of the estimated genetic gains for whole-stem wood density as shown in Equation 9:

$$[\text{Eq. 1.9}] \quad Q = \frac{i_x H_{\bar{C}(F)x} H_{\bar{C}(F)y} r_{G(x,y)} CV_{\bar{C}(F)y}}{i_y H_{\bar{C}(F)y}^2 CV_{\bar{C}(F)y}} = \frac{H_{\bar{C}(F)x} r_{G(x,y)}}{H_{\bar{C}(F)y}}$$

1.2.5 Cost Analysis

Breast height increment coring is currently the standard method used for large scale measurement of wood density, as it is generally provides an accurate and cost effective alternative to whole-stem sampling (Raymond et al. 1998). Therefore, it was desired to specifically compare the Resistograph to breast height increment coring in terms of efficiency at screening clones for whole-stem wood density. However, a simple assessment of Q (relative efficiency) as calculated in Equation 9 would be misleading since the Resistograph should reduce the total cost of clonal screening relative to using wood cores. To assess the magnitude of the cost differential, the costs per tree (c) for measuring density using the Resistograph and increment cores were estimated. These costs included both field sampling and laboratory analysis and were estimated based on the number of trees or samples

that could be measured per day as determined by prior experience. The following assumptions were made when estimating costs:

- Average DBH of 16.5 cm (average diameter in this study)
- Forward speed of 25 cm / minute with the Resistograph
- Two person field crew working an 8 hour day at \$15 / hour
- One person for lab analysis working an 8 hour day at \$15 / hour

Using the cost estimates, several combinations of the number of clones measured with the Resistograph (n_x) and the number of ramets per clone measured with the Resistograph (b_x) were identified that satisfied the following equality:

$$[\text{Eq. 1.10}] \quad n_x b_x c_x = n_y b_y c_y \Rightarrow n_x b_x = 540 \frac{c_y}{c_x}$$

where the product of $n_x b_x c_x$ is the total cost of sampling with the Resistograph and the product of $n_y b_y c_y$ is the total cost of measuring density with breast height increment cores. Values of $n_y = 90$ and $b_y = 6$ were held constant during the analysis, such that the sample size for increment coring equaled the 540 trees measured in this study. Thus, Equation 13 can be expressed such that the combinations of n_x and b_x are constrained by the per tree cost ratio of direct to indirect measurement of breast height density (c_y/c_x). These combinations of

n_x (which determines i_x) and b_x were then substituted into Equation 9 to solve for a cost standardized relative efficiency for a range of sampling scenarios with the Resistograph.

1.3 Results

1.3.1 Descriptive Statistics

Descriptive statistics for all measured traits in this study are shown in Table 1.1. The overall mean wood density of these 8-year-old trees was 400 kg/m³ and 379 kg/m³ for breast height and whole-stem density, respectively. A fixed effects ANOVA showed that, while there were no significant differences among full-sib families for either breast-height or whole-stem density, there were highly significant clonal differences within families (results not shown). These differences are indicated by the range of clone means for wood density traits (Table 1.1). Overall mean amplitude was 41.3% and 27.6% for unadjusted mean amplitude (\overline{AMP}) and adjusted mean amplitude (\overline{AMP}_{ADJ}), respectively. \overline{AMP}_{ADJ} had a lower mean relative to \overline{AMP} as a result of removing the increasing trend from the amplitude profiles. Amplitude had a much larger clone mean coefficient of variance (10.6-11.9%) than wood density (3.9-4.0%), such that amplitude provided good separation of clone means for screening.

1.3.2 Relationship between amplitude and density

Positive linear phenotypic relationships were found between wood density and amplitude as indicated by the fitted regression models in Figure 1.5. At the individual tree level, amplitude (\overline{AMP}) was not a strong predictor of wood density. Individual tree \overline{AMP} values

explained only 23 and 26 percent of the within-family variation for whole-stem and breast height density, respectively (Figure 1.5). Thus, $\overline{\text{AMP}}$ had a moderate individual tree phenotypic correlation with both breast height (0.51) and whole-stem (0.45) density. A moderate phenotypic relationship was also found between $\overline{\text{AMP}}$ and DBH at the individual tree level (Figure 1.6).

The strength of the phenotypic relationship between $\overline{\text{AMP}}$ and density improved at the clone mean level, with increased clone mean correlations of 0.72 for breast height density and 0.59 for whole-stem density (Table 1.2). The prediction intervals around the regressions in Figure 1.7 show that, on average, the Resistograph could estimate whole-stem clone mean density to $\pm 25 \text{ kg/m}^3$ and breast height clonal density to $\pm 22 \text{ kg/m}^3$ at the 95% confidence level. Figure 1.8 shows that breast height increment cores provided a more accurate prediction of whole-stem density as indicated by the high clone mean correlation (0.91). On average, breast height increment cores were capable of predicting clone mean whole-stem density to $\pm 13 \text{ kg/m}^3$.

Given the strong increasing trend in percent amplitude with drilling depth (Figure 1.3), it was not surprising that $\overline{\text{AMP}}$ was found to be positively correlated with DBH. The strength of this phenotypic correlation at the individual tree level (0.54) was comparable to the strength of the individual tree correlation between $\overline{\text{AMP}}$ and breast height density. The correlation between $\overline{\text{AMP}}$ and DBH remained comparably strong at the clone mean level (Table 1.2). Despite the strong relationship between $\overline{\text{AMP}}$ and DBH, there were no significant

correlations between wood density and DBH at the clone mean level (Table 1.2). For this reason, DBH was considered a contaminant variable causing unwanted variability in the amplitude measurements.

Adjusting the amplitude profiles using centered moving minimums and centered moving averages was successful at removing the increasing trend with drilling depth (Figure 1.4). Using $\overline{\text{AMP}}_{\text{ADJ}}$ reduced the clone mean correlation with DBH to 0.20, such that it was no longer significant at the 95% confidence level (Table 1.2). Adjustments to the amplitude profiles were not successful at improving individual tree phenotypic correlations between amplitude and density. However, the adjustments did slightly improve the clone mean correlation between amplitude and breast height density. Using $\overline{\text{AMP}}_{\text{ADJ}}$ also considerably strengthened the relationship between amplitude and whole-stem density at the clone mean level, increasing this correlation from 0.59 to 0.72 (Figure 1.7). The prediction intervals around the fitted regressions in Figure 7 show that, on average, the adjusted Resistograph values could estimate whole-stem clone mean density to $\pm 22 \text{ kg/m}^3$ and breast height clonal density to $\pm 21 \text{ kg/m}^3$ at the 95% confidence level. Using DBH as a covariate in the linear models without adjustment of the amplitude profiles yielded slightly inferior improvements.

Partitioning the phenotypic covariance revealed a strong genetic correlation between breast height wood density and both $\overline{\text{AMP}}$ (0.83) and $\overline{\text{AMP}}_{\text{ADJ}}$ (0.92) as shown in Table 1.3. The genetic correlations between amplitude and breast height density had small standard errors (Table 1.3). The genetic relationships between amplitude and whole-stem density were even stronger than that found for breast height density, but were associated with larger standard

errors. Environmental factors tended to cause a positive covariance between density and amplitude (Table 1.3). The relationship between $\overline{\text{AMP}}$ and DBH was undesirably strong at the genetic level ($r_G = 0.43$). However, this genetic relationship was eliminated by using $\overline{\text{AMP}}_{\text{ADJ}}$ as indicated in Table 1.3. No significant genetic correlations were found between wood density traits and DBH in these clones (Table 1.3).

1.3.3 Genetic Gain and Relative Efficiency

Parameter estimates from fitting the linear mixed model are shown in Table 1.4. Genetic differences among clones within families explained only 20% of the within family phenotypic variance for amplitude. Genetic control of wood density was much greater, as clonal variance accounted for 45% and 72% of the total within family phenotypic variance for breast height and whole-stem density, respectively. Therefore, clone means were highly repeatable for breast height ($H^2_{\bar{C}(F)} = 0.83$) and whole-stem ($H^2_{\bar{C}(F)} = 0.89$), while clone means for amplitude were less repeatable ($H^2_{\bar{C}(F)} = 0.60$).

Genetic gain for whole-stem wood density was 7.4 % when selection was based directly on this trait. This genetic gain for whole-stem density decreased to 6.9 % and 5.5 % when selection was based on breast height wood density and adjusted amplitude, respectively. Thus, clonal selection was highly efficient when based on breast height density ($Q = 0.95$), but slightly less efficient when based on adjusted amplitude from the Resistograph ($Q = 0.75$). The efficiency of the Resistograph was improved slightly when the target trait for clonal selection is considered to be breast height wood density (Table 1.5).

Results from the cost analysis of each method are shown in Table 1.6. It should be noted that the cost estimates for laboratory analysis of increment cores was a conservative estimate based on volumetric measurements rather than the more time consuming densitometry procedures used in this study. It can be seen in this cost analysis that the main advantage to the Resistograph lies in the elimination of laboratory processing of wood samples. The cost of sampling and measuring breast height cores for density was estimated to be 4 times the cost of screening an equal number of clones with the Resistograph in this analysis. Therefore, 4 times as many trees sampled for breast height increment cores could be sampled with the Resistograph for the same cost. When values for i_x and b_x were adjusted to account for this cost differential, relative efficiencies of slightly greater than one were found for the Resistograph compared to breast height increment coring (Figure 1.10).

1.4 Discussion

The main factor limiting large scale wood density assessment in clonal trials is the cost associated with field sampling and laboratory analysis of wood samples. The strong correlations between breast height and whole-stem density found in this study as well as others (Igartua et al. 2003, Evans et al. 1997, Raymond and Muneri 2001, Raymond et al. 1998) suggests that measuring density from breast height increment cores is more cost efficient than whole-stem sampling. This is confirmed by the high relative efficiency for selection based on increment cores (Table 1.5) and the large cost differential between breast height coring and whole-stem sampling (Table 1.6). However, sampling increment cores from breast height does not eliminate the need for costly laboratory procedures. A superior

alternative to breast height increment coring must therefore reduce the cost associated with these laboratory procedures, while still providing a quality phenotypic criterion for selection. Experience with the Resistograph in this study has verified that the tool is capable of greatly reducing the cost of obtaining measurements of wood density (Table 1.6). The tool may only be slightly more rapid than using gas powered increment borers in the field, but it provides a bark-to-bark measurement of wood density without the need for any wood samples or laboratory procedures.

It is therefore left to discern whether the Resistograph provides a quality phenotypic criterion for clonal selection. To provide such a selection criteria, amplitude must produce clone means that are repeatable and have a strong genetic correlation with wood density. Fortunately, the current research estimated a strong genetic correlation between amplitude and breast height density (0.92). This strong genetic correlation is in agreement with Isik and Li (2003), who found an additive genetic correlation between amplitude and density of 0.95 in a multi-site progeny trial of loblolly pine. Such strong genetic correlations suggest that the mean values of amplitude and x-ray densitometry profiles are two traits that are influenced by essentially the same genes.

The absence of comparably strong phenotypic correlations between amplitude and density in this study despite the underlying genetic relationship also corresponds with previous research. Weak to moderate individual tree correlations ranging from 0.29 to 0.65 were found by Isik and Li (2003). Chantre and Rozenberg (1997) were also only able to detect a moderate correlation of 0.65 between mean values for radial x-ray densitometry and

Resistograph profiles from a 25-year-old Douglas-fir stand. While stronger phenotypic correlations with wood density are often reported for the Pilodyn, these are typically based on multiple penetration measurements per stem (Cown, 1978, Sprague et al. 1983, Taylor 1981, Raymond et al. 1998). As an example, a strong clone mean phenotypic correlation of -0.96 was found between Pilodyn and wood density measurements by Cown (1978). However, repeated measures were obtained per ramet and wood density assessment was based only on the outer three rings in the region of Pilodyn penetration.

The disparity between the phenotypic and genetic correlations for the between amplitude and density is explained by the fact that the phenotypic covariance can be partitioned into genetic and environmental factors (Falconer and Mackay 1996). Specifically, the phenotypic correlation between amplitude and density is a function of the genetic correlation, environmental correlation, and the *repeatability* of both traits. As the repeatability of the traits decreases, the environmental correlation (see Table 1.3) has a greater influence on the phenotypic correlation (Falconer and Mackay, 1996).

This study detected a relatively low repeatability for clone mean amplitude measurements (Table 1.4). Only 20 percent of the within family clonal variance for amplitude was explained by genetics compared to 45 percent for wood density determined from increment cores. Correspondingly, Isik and Li (2003) found that 16 and 35 percent of the within-family variance was due to additive genetic variance for amplitude and increment core density, respectively. The estimates in the current study are slightly higher since the variance due to clones is composed of both additive and non-additive genetic variances (Table 1.1). Thus,

the absence of strong phenotypic correlations between amplitude and wood density appears to be due to the large amount of residual environmental variation affecting the amplitude measurements.

The increasing trends in the amplitude profiles and resulting correlations with DBH were found to be one source of error for amplitude measurements in this study. However, density and DBH together only explained 45% of the variation in amplitude. Numerous other uncontrolled environmental factors must have resulted in the low repeatability of the amplitude measurements. Some potential sources of error are listed below:

- Increment cores and Resistograph amplitude measurements were not taken at the same location on the stem. Although all efforts were made to have increment cores and Resistograph measurement taken in close proximity (within 5 cm), branching or other defects sometimes made this impossible.
- When drilling a tree with the Resistograph, it is not always obvious whether the drilling needle has intercepted the pith of the tree. Missing the pith would have result non-perpendicular piercing of tree ring boundaries, which were shown by Rinn et al. (1996) to have a considerable effect on Resistograph amplitude profiles. This source of variability was partially controlled with increment cores, as cores missing the pith were discarded and re-sampled.
- Defects such as branches, resin pockets, or compression wood could have also effected

the Resistograph measurements without being noticed. Once again, this potential source of variability was at least partially controlled with increment cores by discarding and retaking cores that contained these defects or by only using the half of the core that contained clear wood.

- In addition to increasing trends in the amplitude profiles, other authors cited further profile irregularities including indistinct ring boundaries, distortions of the drilling profiles, and meandering contours as possible sources of error (Isik and Li, 2003, Rinn et al. 1996, Chantre and Rozenberg 1997).
- There is some evidence that the Resistograph measurements could be affected by moisture content of the wood (Rinn et al. 1996). If this is true, it would undoubtedly affect the relationship between amplitude and wood density since amplitude was measured in live trees with variable moisture contents while density was measured from oven dried increment cores equilibrated to 8% moisture content.

Despite these sources of error, the Resistograph provides a fairly high efficiency (0.78) relative to clonal selection based on breast height increment cores. Additionally, the lower cost required to screen clones with the Resistograph means that greater selection intensity can be achieved and/or a greater number of ramets can be measured, resulting in essentially equivalent genetic gain ($Q \approx 1$) compared to increment coring. If the sources of error reducing the strength of the relationship between clone means phenotypes for amplitude and wood density can be identified and resolved, then it appears that the Resistograph would

provide a more cost effective method of clonal screening than sampling breast height increment cores. Profile irregularities may be addressed by hardware or software upgrades and/or by improving measurement methodology. It is possible that other confounding factors, such as moisture content, could be controlled statistically by including them as covariates in the analysis of amplitude (such research is ongoing).

An additional value to the Resistograph is that it is much less destructive than increment coring. The 3 mm drilling needle leaves a hardly identifiable hole that remains filled with the residual wood material, thereby preserving the genetic material and minimally affecting future test performance. This non-destructive nature of the Resistograph may suggest that the tool has utility for screening even younger clonal trials (< 4 years-old) for wood density. The value of screening clonal trials at such a young age results from the ability to reduce deployment time of the superior clones as well as the generation time if the selections will be used for subsequent breeding. While growth and form traits can be assessed in these young tests, it is difficult and perhaps infeasible to take wood samples for traditional assessment of wood density from such small trees. The Resistograph could be used to rapidly drill these small trees and provide an indirect assessment of wood density. However, the performance of the Resistograph in this context would depend on both inter-trait and age-age correlations.

1.5 Conclusions

Results of this study show that the Resistograph provides a non-destructive and low cost alternative for measuring wood density that can be used to achieve genetic gains in wood

density from clonal selection. The tool provides an indirect measurement of wood density that has a strong genetic relationship with breast height and whole-stem density determined from increment cores. Moderate correlations between clone means for amplitude and density and the low repeatability of amplitude measurements suggest that there are a number of factors affecting amplitude other than wood density. Improvements to the tool and its onboard software, measurement methodology, and statistical analysis could help to generate amplitude measurements that are less affected by these extraneous factors. Further studies are needed to isolate what factors are effecting amplitude measurements, and to determine whether amplitude can provide a better prediction of clone mean density when the factors are controlled. If these factors can be identified and controlled, the Resistograph will likely prove to be a more cost efficient alternative to traditional breast height increment coring. Additionally, the relationship between amplitude measurements on young trees (< 4 years old) and wood density in older trees needs to be studied to determine the efficiency of the Resistograph for screening in younger clonal trials.

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Figure 1.1. The Resistograph system being employed to measure amplitude in a young clonal trial as an indirect measurement of wood density.

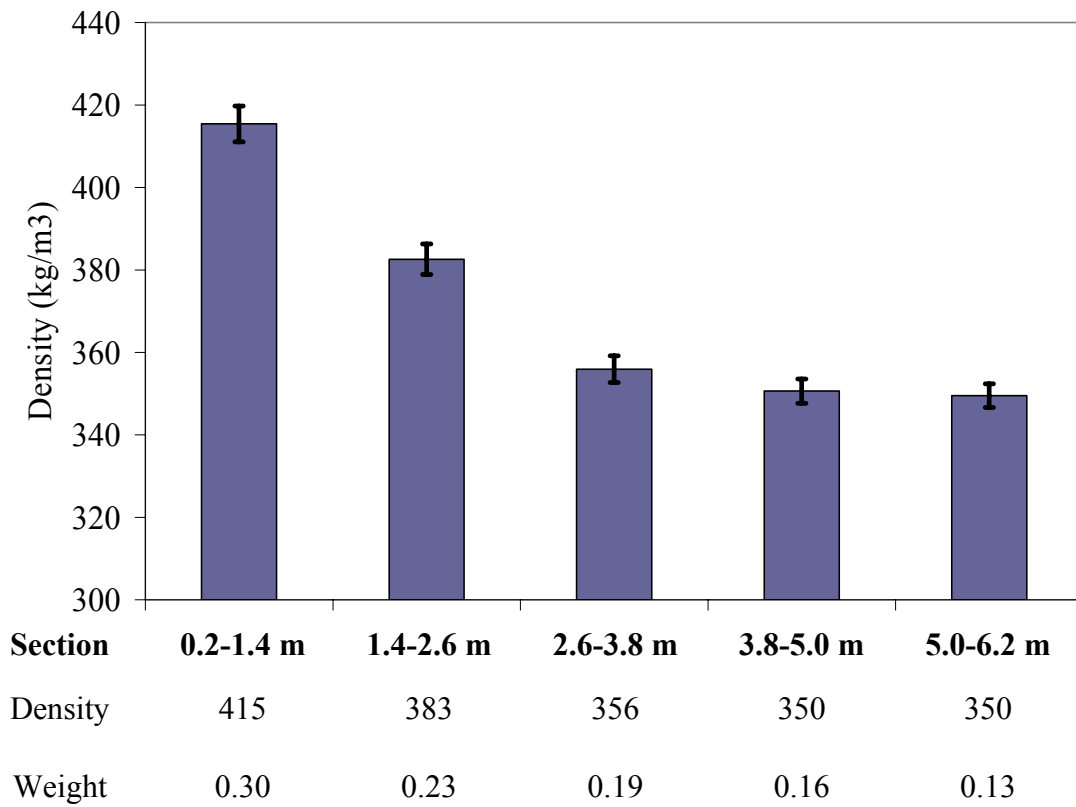


Figure 1.2. Summary of the results of whole-stem sampling for wood density showing the mean of the sectional densities and proportional volume weighting factors used to calculate whole-stem densities. Error bands show 95% confidence intervals for sectional densities.

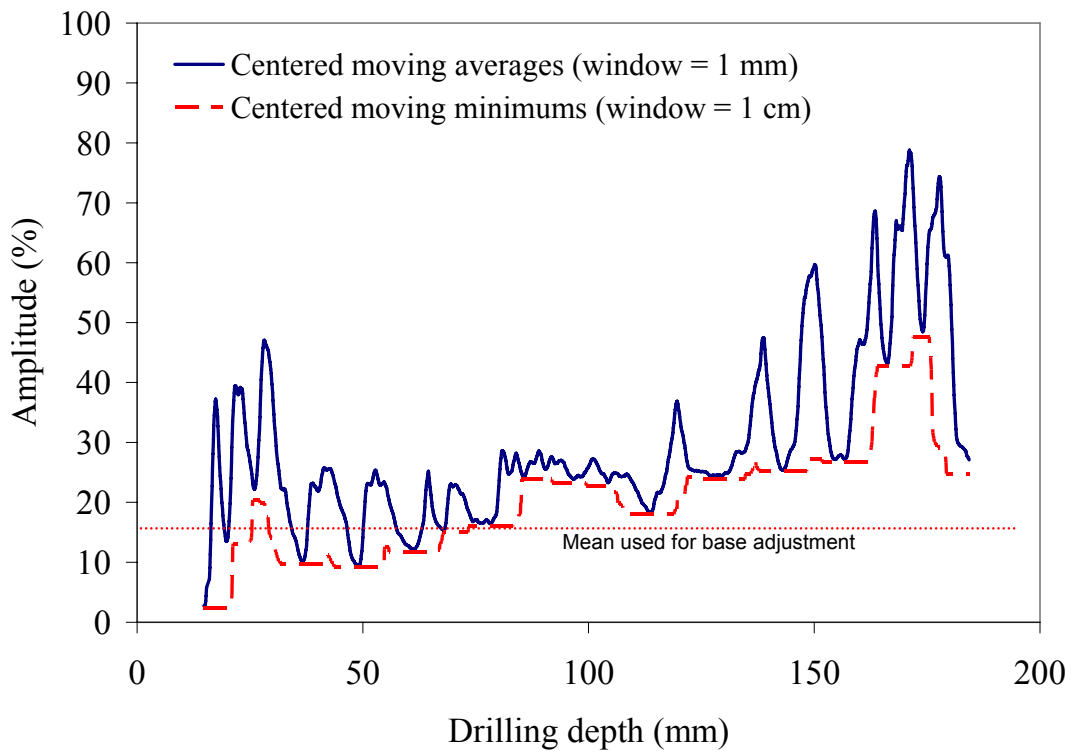
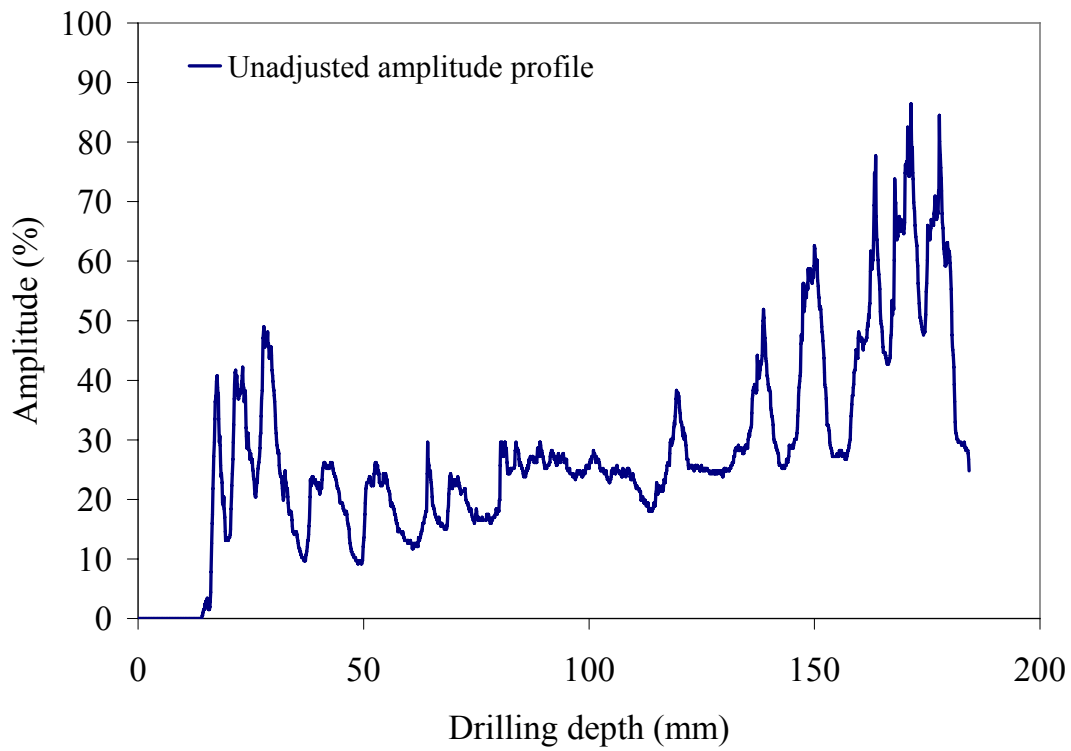


Figure 1.3. Example of a raw amplitude profile exported from the Resistograph (top) and the centered moving averages and minimums (bottom) used to adjust the raw amplitude profile.

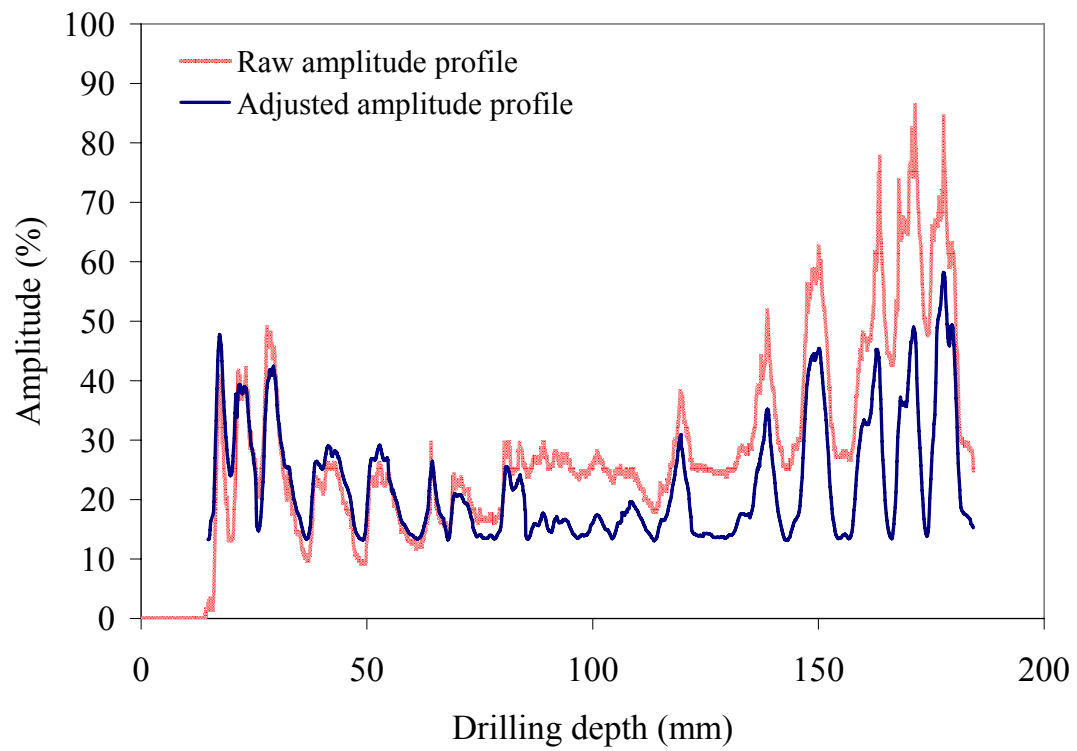


Figure 1.4. Example of an amplitude profile after adjustment for profile irregularities compared to the raw amplitude profile exported from the Resistograph.

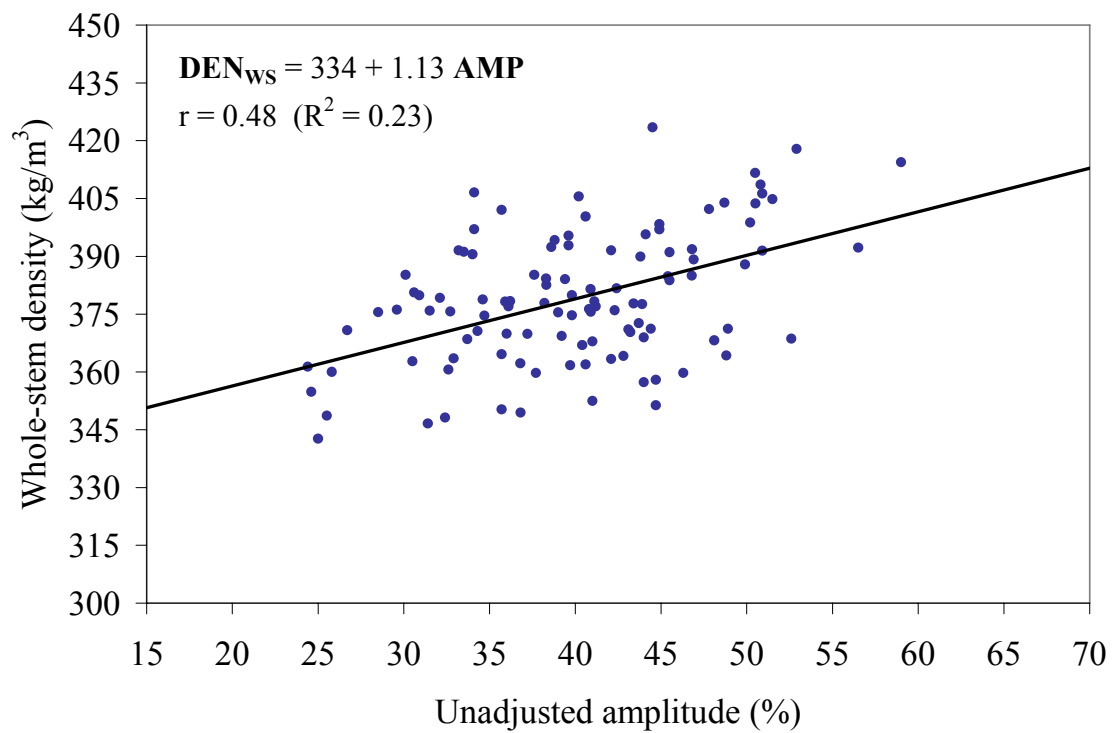
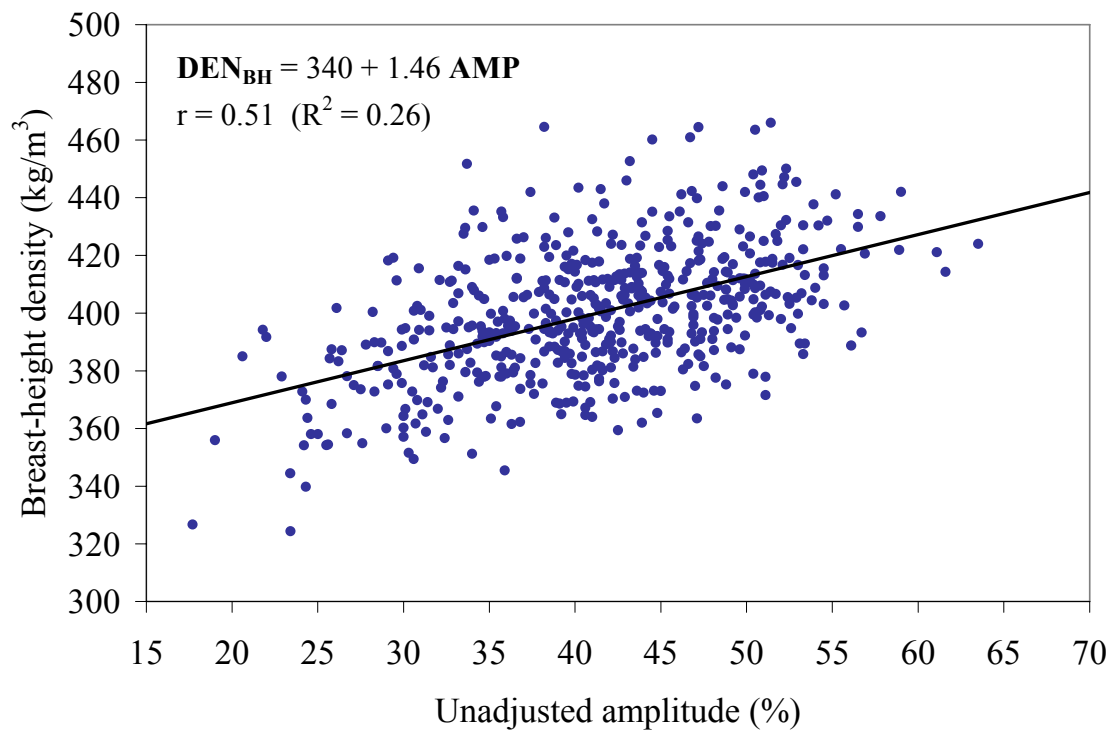


Figure 1.5. Individual tree phenotypic relationships between unadjusted amplitude (AMP) and both breast-height density (*top*) and whole-stem density (*bottom*) with fitted regression.

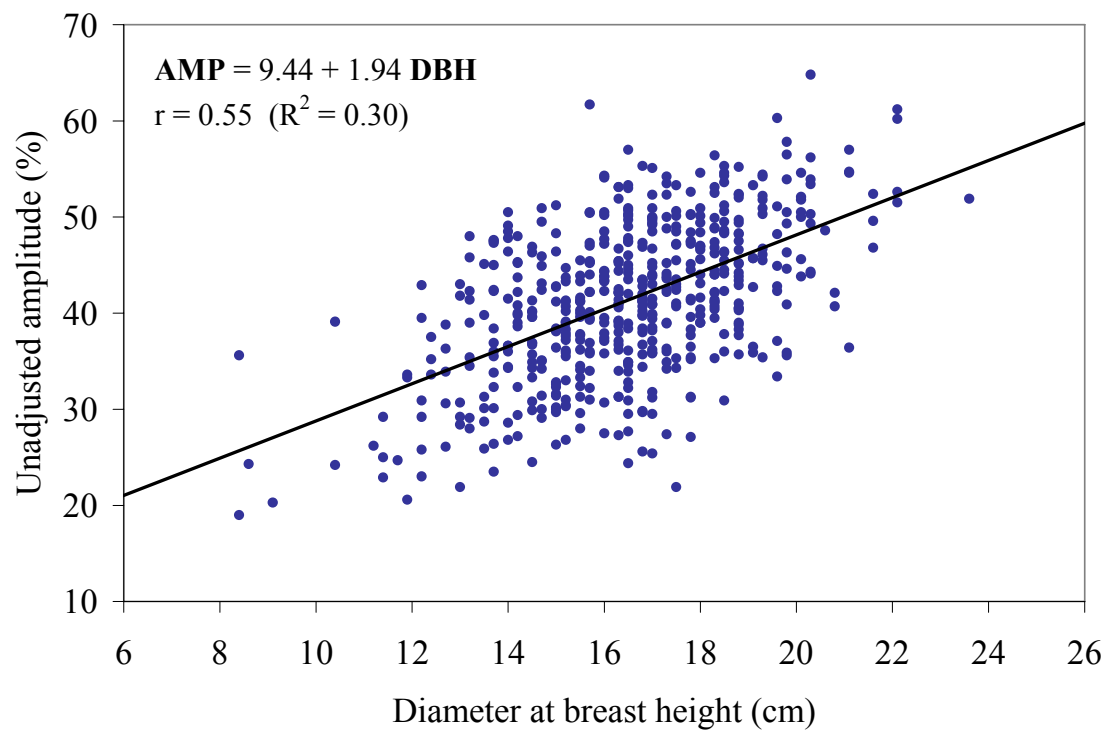


Figure 1.6. Individual tree phenotypic relationship between unadjusted amplitude (AMP) and diameter at breast height (DBH) with fitted regression.

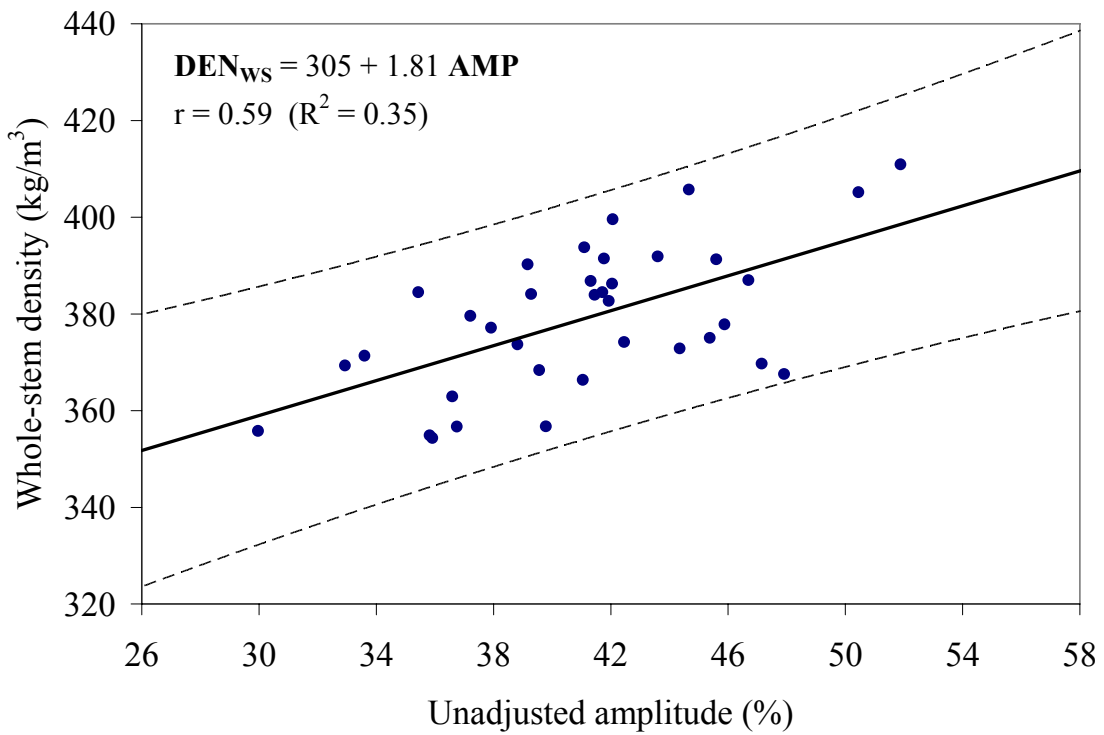
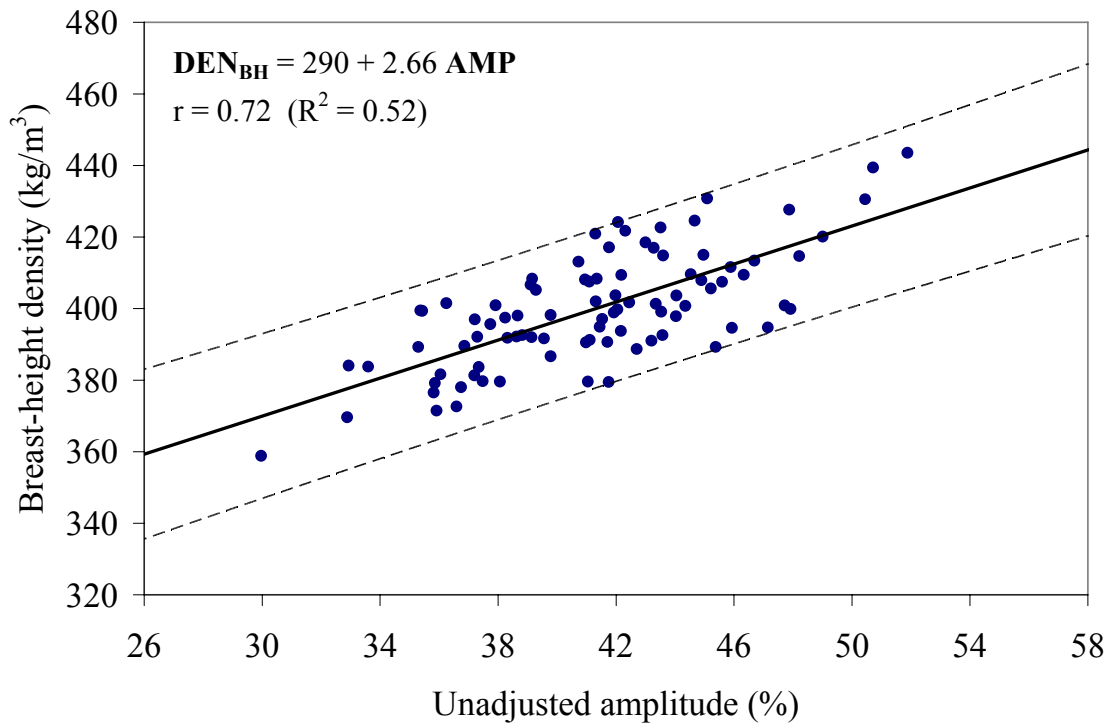


Figure 1.7. Fitted clone mean regressions of breast-height (*top*) and whole-stem (*bottom*) density on unadjusted Resistograph amplitude. Dashed lines indicate 95% prediction interval.

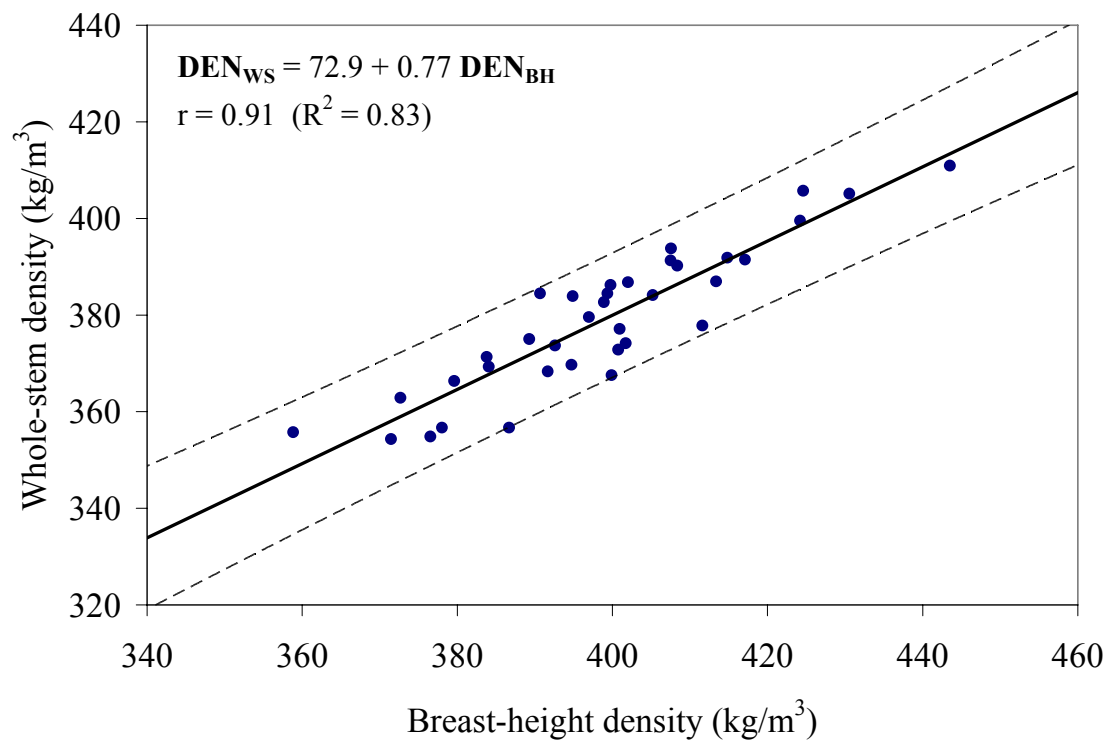


Figure 1.8. Fitted clone mean regression of whole-stem density (DEN_{WS}) on breast-height density (DEN_{BH}) of increment cores. Dashed lines indicate 95% prediction interval.

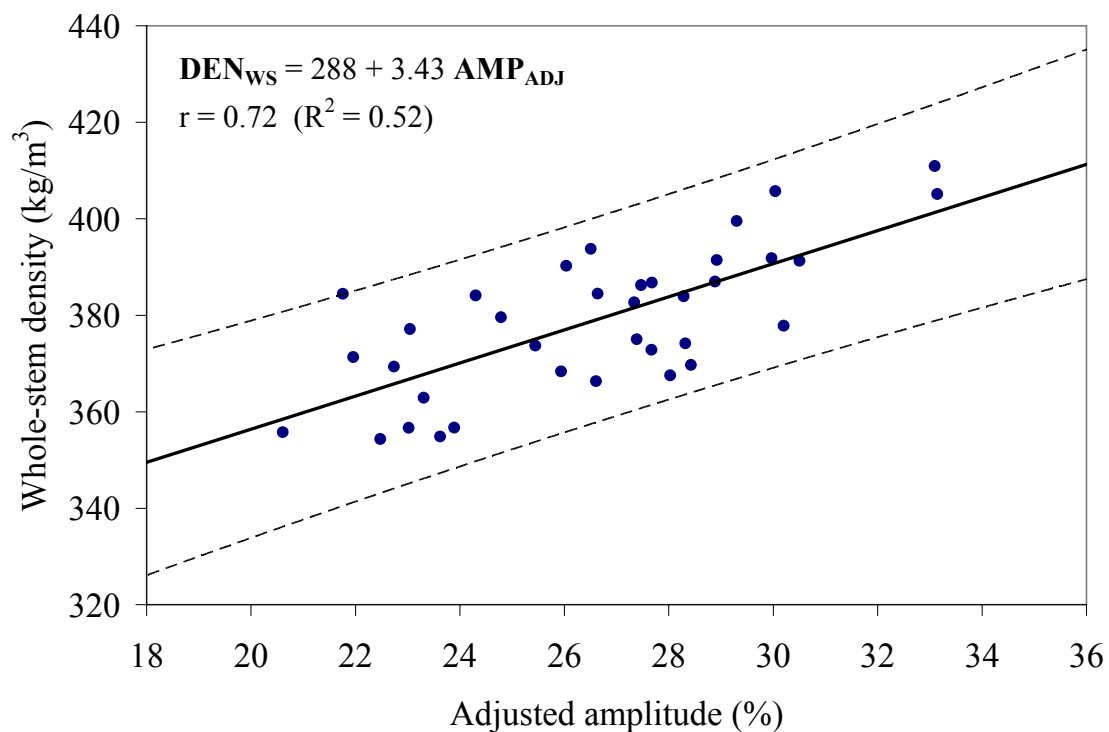
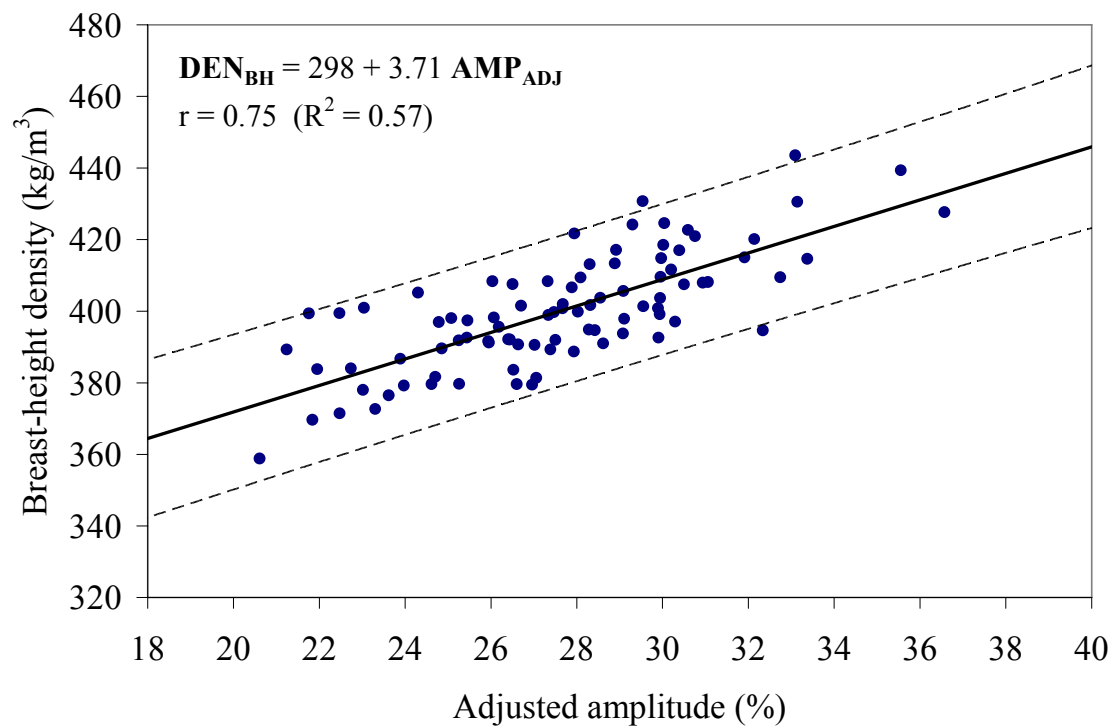


Figure 1.9. Fitted clone mean regressions of breast-height (*top*) and whole-stem (*bottom*) density on adjusted Resistograph amplitude. Dashed lines indicate 95% prediction interval.

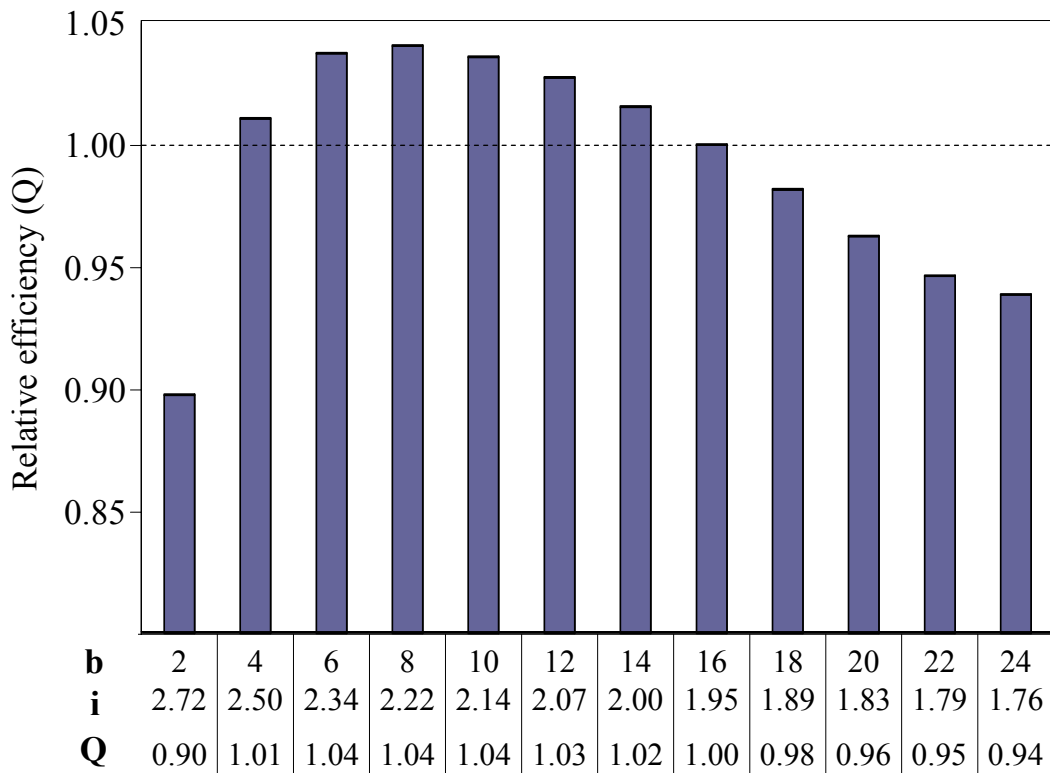


Figure 1.10. Relative efficiencies for using the Resistograph to select for breast-height wood density assuming that sampling with the Resistograph is 4 times less expensive than sampling and processing increment cores.

Note: b = number of ramets per clone, i = selection intensity (held at 1.76 for increment cores). Each combination of b and i results in a sampling intensity that is 4 times that for coring in this study. Efficiency increases initially as the number of ramets measured with the Resistograph increases but eventually decreases due to loss in selection intensity.

Table 1.1. Descriptive statistics for density, amplitude, and diameter traits including the grand mean, range of clone means, and phenotypic coefficients of variance for clone means.

Trait	Mean	Min	Max	CV_{$\bar{e}(f)$}
Breast height density (kg/m ³)	400	357	442	4.01
Whole-stem density (kg/m ³)	379	349	405	3.98
Unadjusted amplitude (%)	41.3	31.3	53.2	10.56
Adjusted amplitude (%)	27.6	21.7	36.4	11.86
Breast height diameter (cm)	16.5	13.1	20.4	8.09

Table 1.2. Clone mean phenotypic correlations for density, amplitude, and diameter traits. Correlations followed by an asterisk are significant at the 0.05 level. DEN_{WS} = whole-stem density, DEN_{BH} = breast-height density, AMP = unadjusted amplitude, AMP_{ADJ} = adjusted amplitude, and DBH = diameter at breast height.

	DEN _{BH}	AMP	AMP _{ADJ}	DBH
DEN _{WS}	0.91*	0.59*	0.72*	0.18
DEN _{BH}		0.72*	0.75*	0.15
AMP _{RAW}			0.89*	0.47*
AMP _{ADJ}				0.20

Table 1.3. Genetic (below diagonal) and environmental (above diagonal) correlations for density, amplitude, and diameter traits. Correlations are followed by their approximate standard errors. DEN_{WS} = whole-stem density, DEN_{BH} = breast-height density, AMP = unadjusted amplitude, AMP_{ADJ} = adjusted amplitude, and DBH = diameter at breast height.

	DEN _{WS}	DEN _{BH}	AMP	AMP _{ADJ}	DBH
DEN _{WS}	-	0.65	0.46	0.30	0.10
DEN _{BH}	0.98 (0.03)	-	0.51	0.42	0.46
AMP _{RAW}	0.95 (0.27)	0.83 (0.07)	-	0.79	0.56
AMP _{ADJ}	0.97 (0.23)	0.92 (0.06)	0.95 (0.03)	-	0.41
DBH	0.13 (0.31)	0.05 (0.14)	0.43 (0.14)	0.09 (0.17)	-

Table 1.4. Parameter estimated from fitting the linear mixed model quantifying the environmental and genetic variance for wood density and amplitude variables.

Parameter Estimate	Breast-height Density (kg/m ³)	Whole-stem Density (kg/m ³)	Unadjusted Amplitude (%)	Adjusted Amplitude (%)
$\sigma_{C(F)}^2$	213 (39.2)	202 (57.4)	11.5 (2.93)	6.4 (1.65)
σ_E^2	265 (18.2)	76 (14.8)	45.3 (3.08)	26.1 (1.77)
$\sigma_{\bar{Y}}^2$	258 (39.1)	227 (57.4) ¹	19.0 (2.88)	10.7 (1.62)
% $\sigma_{C(F)}^2$	45%	72%	20%	20%
$H_{\bar{C}(F)}^2$	0.83 (0.03)	0.89 (0.04) ¹	0.60 (0.07)	0.60 (0.07)

Note: estimates are followed by their standard errors. Parameter estimates are the clone within family variance ($\sigma_{C(F)}^2$), environmental variance (σ_E^2), clone mean phenotypic variance ($\sigma_{\bar{Y}}^2$), percent of phenotypic variance explained by clonal variance (% $\sigma_{C(F)}^2$), and clone mean repeatabilities ($H_{\bar{C}(F)}^2$).

¹clone mean variance and repeatability for whole-stem density were based on 3 ramets per clone while the other traits were based on 6 ramets per clone.

Table 1.5. Estimates of percent genetic gains and selection efficiencies for whole-stem wood density based on direct and indirect selection.

Target Trait	Selection Trait	% ΔG ¹	Q
DEN _{WS}	DEN _{WS}	7.4%	1.00
DEN _{WS}	DEN _{BH}	6.9%	0.95
DEN _{WS}	AMP _{ADJ}	5.5%	0.75
DEN _{WS}	AMP	5.1%	0.70

¹Percent gain estimates are based on a selection intensity of 2.063 (selection of 1% of the clones).

Table 1.6. Estimated costs for obtaining measurements of wood density by Resistograph, breast height increment cores, and whole-stem sampling.

	Sampling Method		
	Resistograph	Increment Core	Whole-stem
Field Sampling			
Trees per day	350	250	25
Time per tree	1.4 minutes	2.0 minutes	20 minutes
Cost per tree	\$0.70	\$1.00	\$10.00
Lab Analysis			
Samples per day	0	60	60
Samples per tree	0	1	6
Time per tree	0 minutes	8 minutes	48 minutes
Cost per tree	\$0.00	\$2.00	\$12.00
Cost per Tree	\$0.70 / tree	\$ 3.00 / tree	\$22.00 / tree
Cost ratio	1	4	30

Note: See Section 1.2.4 “Cost Analysis” for a list of assumptions.

¹Cost ratio indicates the approximate ratio of the per tree cost of each method to the per tree cost of sampling with the Resistograph.

Chapter 2

Rapid Screening of Clones for Mechanical Wood Traits using Acoustic and Drill Resistance Methods

2.1 Introduction

Mechanical properties relating to the strength and stiffness of wood, such as the modulus of rupture (MOR) and longitudinal modulus of elasticity (MOE), are direct determinants of the utility and value of wood products for structural application. For this reason, the increasing furnish of wood from young, intensively managed pine plantations creates a problem for manufacturers of solid wood products. A large proportion of the wood harvested from these fast grown pine plantations is obtained from the physiologically juvenile core, and is characterized by physical properties that greatly reduce its strength and stiffness compared to the mature wood (Kretschmann and Bendtsen 1992, Larson et al. 2001). Negative impacts on costs and revenues can therefore be expected as industry becomes increasingly dependent on this fast grown wood resource (Kretschmann and Bendtsen 1992).

Considerable variation exists among trees within these short rotation pine plantations for corewood properties. The extent of this variation can be seen by the large range in stiffness values found by Grabianowski et al. (2006) in an 8-year-old radiata pine (*Pinus radiata*) stand. As a result, the corewood for a proportion of trees in a stand may be adequate in terms of mechanical properties, while it is undoubtedly inadequate in others (Walker and Nakada 1999). Such variability in mechanical wood properties has presented a challenge to manufacturers as it has impeded them from consistently producing high grade structural wood products. Thus, considerable effort has been focused on developing and assessing methods for segregating logs based on strength and stiffness; particularly resonance based acoustic methods (Dickson et al. 2003, Matheson et al. 2002, Tsehaye et al. 2000b).

A more long-term objective compared to log based sorting methods would be to exploit the variation endemic to these young trees in a selection program to genetically improve the mechanical properties of the juvenile wood furnish. Particularly, the selection and deployment of superior clones (i.e. clonal forestry) could provide substantial gains in both the quality and uniformity of the wood derived from plantations (Libby and Rauter 1984). Clonal selection could be most directly applied if based on MOE and MOR, as opposed to underlying physical properties affecting wood strength and stiffness, since it is the mechanical properties that are being valued (Tsehaye et al. 2000a, Lindstrom 2002).

Efficient employment of clonal forestry to improve MOE and MOR would require mass screening of clones from elite crosses within large clonally replicated field tests (Isik et al. 2005). While such tests have been established for loblolly pine and used to screen clones for growth and disease resistance traits (Isik et al. 2005), such large scale clonal screening of MOE and MOR has yet to be employed. The main limitation has been the cost associated with measuring these traits on a large scale. Traditional laboratory assessment of MOE and MOR requires static bending tests of standardized clearwood samples. While this is considered to be the most direct and accurate measure of mechanical wood properties aside from testing sawn boards, it is destructive of the genetic material and extremely time consuming during both the field sampling and laboratory assessment.

A more efficient and nondestructive measurement of MOE and MOR may be provided by the application of acoustic techniques similar to those used for log segregation. However, as the highly repeatable resonance based method used for assessing log stiffness requires two cut

ends, an alternative method must be used on standing trees (Grabianowski 2006). For this purpose, time-of-flight acoustic tools are employed to measure the propagation time of an induced stress wave between two probes placed a fixed vertical distance apart on the stem. The velocity of the stress wave is related to the longitudinal dynamic modulus of elasticity (MOE_d) of the outerwood based on the one-dimensional wave equation $MOE_d = \rho V^2$ (Toulmin and Raymond 2007). As the green density of the wood (ρ) is assumed to be constant, stress wave velocity (V) provides a surrogate measure of the dynamic longitudinal modulus of elasticity (Toulmin and Raymond 2007). Stress wave velocity is also correlated with wood strength (Kumar et al. 2002), since MOR is affected by many of the same wood properties affecting MOE and stress wave velocity.

Recent studies indicate that time-of-flight (TOF) acoustic tools may hold great potential for rapid screening for MOE and MOR in clonal field tests. Moderate to high phenotypic and additive genetic correlations have been found for several species between time of flight measurements in standing trees and both MOE and MOR determined from laboratory bending tests (Wang et al. 2001, Kumar et al. 2002, Lindstrom 2004, Chauhan and Walker 2006). Kumar et al. 2002 found a high heritability (0.46) for indirect stress wave measurements in standing trees. Jacques (2004) found strong genetic relationships between acoustic velocity and stiffness in a clonal larch study. In another clonal study, Lindstrom (2004) found that 80 percent of the phenotypic variance for acoustic velocity measured by a Fakopp TOF in young trees was explained by genetics, resulting in a high clonal repeatability (0.94).

Drill resistance measurements, such as those provided by the IML Resistograph (Chapter 1), may also have utility for screening clones for mechanical wood properties. This portable drilling device measures resistance in terms of power consumption (amplitude) as a 3 mm drilling needle is advanced into wood material at a constant rate. Mean amplitude measurements from this tool were shown in Chapter 1 as well as by Isik and Li 2003 to have a strong genetic relationship with wood density. Since MOR and MOE have been shown to be correlated with wood density (Yang and Evans 2003, Kumar 2004) the Resistograph may have value for mass screening clones for MOE and MOR.

This study focuses on the use of a TOF acoustic tool, the Fakopp TreeSonic, and a drill resistance tool, the Resistograph Model F400, for indirect assessment of structural wood traits in a clonal trial of loblolly pine. The primary objectives were to 1) examine the relationships between indirect field measurements and direct laboratory based measurements of MOE, MOR, and density; 2) estimate the relative contribution of genetic and environmental factors to the variation observed in these traits, and 3) determine the relative efficiency of clonal selection for wood traits based on the indirect measurements. If stress wave velocity and drilling amplitude can be used to efficiently select clones with superior mechanical properties, then they can serve as more cost effective alternatives to laboratory based methods for large scale clonal screening. Additionally, if these two tools provide useful and independent information on mechanical wood properties, then they could potentially be combined in a single index on which to base clonal selection.

2.2 Methods and Materials

2.2.1 Material and Data Collection

Three partially related full-sib families of loblolly pine were selected from the North Carolina State University – Cooperative Tree Improvement Program on the basis of superior volume production and rust resistance. Progeny from these three families were clonally replicated by means of hedging and subsequent vegetative propagation to produce rooted cuttings. The rooted cuttings were used for establishment of a clonal test in Berkley County, South Carolina in December 1997. The field design was a randomized complete block design with families planted as whole plots within each replication. Clones within these families were planted in single tree plots, such that each clone was represented with a single ramet in each block.

Ninety clones, consisting of 30 from each full-sib family, were randomly selected from the test at age-8 for stress wave testing with the Fakopp TreeSonic (Figure 2.1). Six ramets of each clone were sampled. For each sampled tree, diameter at breast height was measured. The start sensor of the TreeSonic was then driven into the stem at 1.9 m, while the stop sensor was placed directly below at 0.9 m (Figure 2.2). This resulted in a one meter test span approximately centered on breast height (1.4 m). The sensors were always placed at opposing 45° angles to the stem (Figure 2.2) and always on the same aspect to minimize environmental variation. Stress waves were induced by striking the start sensor with a hammer. The transit time (μs) of the stress wave between the start and stop sensors was then indicated by the microsecond timer. Three consecutive readings were taken at the same location for each tree and averaged to yield the final transit time measurement.

Each tree measured with the TreeSonic was also drilled with the Resistograph model F-400 to provide measurements of drill resistance (see Chapter 1). Drilling was conducted from bark to bark at breast height in the same direction for all trees (Figure 2.2). A constant forward speed of 25 cm/min was used with the Resistograph. Drilling files for each tree were stored on the onboard electronic unit of the Resistograph. These files contained resistance profile data such as tree identification and resistance (percent amplitude) measurements at each 0.1 mm increment of drilling depth.

From the original 90 clones, a sub-sample of 36 clones was used for analysis of mechanical wood traits. The sub-sample consisted of 12 clones from each full-sib family that were selected from the quartiles of the distribution of clone mean density as determined from increment cores (see Chapter 1). Ramets of the selected clones were felled in 3 blocks, resulting in 3 ramets sampled for each clone. Two bolts, approximately 1 m in length, were cut from each tree. The lower bolt was positioned with its large end at 1.4 m (4.5 ft) and the upper bolt positioned its large end at 3.8 m (12.5 ft), while allowing some deviation for avoidance of branch whorls and stem defects.

Bolts were transported to the USDA-Forest Service, Forestry Sciences Laboratory in Athens, GA for processing and analysis of mechanical wood traits. From each bolt, a 38 mm thick slab was cut from bark to bark through the pith. After drying to 12 percent moisture content, the slabs were split at the pith and static bending samples measuring 25.4 x 25.4 x 406 mm were cut consecutively from the bark to the pith from the opposite sides, making necessary adjustments for branches and other obvious defects. The clear wood static bending samples

were tested pith up at 12 percent EMC over a 355.6 mm span with center loading on a Tinius Olsen static bending machine following the procedures for alternate sample size under ASTM D-143 (ASTM 1980). A continuous load was applied at a head speed of 1.78 mm/minute rather than 1.29 mm/minute to reduce testing time. After testing, each sample was oven dried at 103 °C and basic density was calculated based on oven dry weight and specimen dimensions at 12 percent EMC. Modulus of elasticity (MOE) and modulus of rupture (MOR) were calculated using procedures outlined in ASTM D-143 (ASTM 1980) and using the following equations:

$$\text{[Eq. 2.1]} \quad \text{MOE (lbf / in}^2\text{)} = \frac{\text{PPL} \times \text{L}^3}{48 \times \left[(\text{W} \times \text{d}^3) / 12 \right] \times \text{D}}$$

$$\text{[Eq. 2.2]} \quad \text{MOR (lbf / in}^2\text{)} = \frac{1.5 \times \text{P} \times \text{L}}{\text{W} \times \text{d}^2}$$

where PPL is the proportional load (i.e. the loading at the elastic limit of deflection) measured in pounds of force (lbf), P is the maximum loading to failure (lbs), L is the length of sample (in), W is the width of the sample (in), d is the depth of the sample (in), D is the deflection of the sample at proportional loading (in). MOE and MOR values were converted to Pascal units for analysis.

2.2.2 Derivation of Analysis Variables

As the within tree variation due to ring position and height level was identified as a source of extraneous variation for MOE, MOR, and density measurements obtained from the bending samples (see section 2.3.1), a preliminary model was fit to the raw bending data to provide an adjustment for their effects as shown in Equation 2.3:

$$[Eq. 2.3] \quad y_{ijk} = ring_i + ht_j + ring \times height_{ij} + \epsilon_{ijk}$$

where y_{ijk} is the observation at the i th ring and the j th height, $ring_i$ is the main effect of the i th ring (1 through 8), ht_j is the main effect of the j th height level (either 0 or 1), and the interaction allows for different radial trends at different heights. The residuals from fitting the model (ϵ_{ijk}) provided position adjusted bending sample measurements of MOE, MOR, and density since they were standardized to the mean at their corresponding sample positions. Individual tree values of MOE, MOR, and density that served as the analysis variables were then calculated by averaging all of the position adjusted measurements for each tree.

Based on acoustic theory, the most direct surrogate measure of wood stiffness is provided by the square velocity of the stress wave. Therefore, squared stress wave speed (SWS) was used as the analysis variable in this study. Stress wave transit times (SWT) measured in microseconds per meter were converted to squared stress wave speed (SWS) measured in kilometers per second based on the following formula:

[Eq. 2.4]
$$\text{SWS} = \left[(1/\text{SWT}) * 1000 \right]^2$$

For the Resistograph data, increasing trends in the drilling profiles with drilling depth were removed using centered moving means and centered moving minimums as described in Chapter 1. Mean percent amplitude (AMP) of the adjusted profiles was then used as an analysis variable for drill resistance.

2.2.3 *Assessing Clone Mean Relationships*

Clone mean phenotypic relationships between all pairs of traits were assessed by fitting linear regression models. As the objective of this study was to assess within-family selection of clones, it was not desired to include the intertrait covariance caused by the full-sib families in these calculations. The differences among the three families could result in large groups of data points that would create misleading correlations. Family effects were therefore removed by including the effects as a family class variable in the linear models using PROC GLM as suggested by Isik et al. (2007). T-tests were performed at the 95% confidence level to assess the significance of these intertrait phenotypic correlations.

2.2.4 *Partitioning of Phenotypic Variance*

The phenotypic variance for each analysis variable was partitioned into its genetic and environmental components according to the following general linear mixed model:

[Eq. 2.5]
$$y_{ijk} = \mu + b_i + f_j + bf_{ij} + c(f)_{k(j)} + \varepsilon_{ijk}$$

where y_{ijk} is the individual phenotypic observation, μ is the grand mean, b_i is the fixed effect of the i th block, f_j is the fixed effect of the j th family, bf_{ij} is the fixed interaction effect between the i th block and j th family, $c(f)_{jk}$ is the random effect of the k th clone within the j th family and is $\sim \text{NID}(0, \sigma_{C(F)}^2)$, and ε_{ijk} is the random environmental error and is $\sim \text{NID}(0, \sigma_E^2)$.

Clone within family ($\sigma_{C(F)}^2$) and residual (σ_E^2) variance components were estimated using Restricted Expected Maximum Likelihood methods by fitting the mixed model in ASReml (Gilmour et al. 2002). By fixing the family effect (f_j), it was assumed that there was no variance associated with full-sib families. Thus, the estimated individual tree phenotypic variance ($\hat{\sigma}_{C(F)}^2 + \hat{\sigma}_E^2$) was interpreted as the total phenotypic variance. Correspondingly, the phenotypic variance of clone means was calculated as $\hat{\sigma}_{C(F)}^2 + \hat{\sigma}_E^2 / b$, where b is the number of ramets per clone.

Variance components from the mixed model were used to calculate the phenotypic coefficient of variance for clone means ($CV_{\bar{C}(F)}$), percent of the variance explained by clonal differences ($\% \sigma_{C(F)}^2$), and repeatability of clone means ($H_{\bar{C}(F)}^2$) for each trait. These parameters were calculated as shown in Equations 2.6 through 2.8 below:

$$\text{[Eq. 2.6]} \quad CV_{\bar{C}(F)} = \frac{\sqrt{\sigma_{C(F)}^2 + \sigma_E^2 / b}}{\bar{X}} \times 100$$

$$\text{[Eq. 2.7]} \quad \% \sigma_{C(F)}^2 = \frac{\sigma_{C(F)}^2}{\sigma_{C(F)}^2 + \sigma_E^2}$$

$$\text{[Eq. 2.8]} \quad H_{\bar{C}(F)}^2 = \frac{\sigma_{C(F)}^2}{\sigma_{C(F)}^2 + \sigma_E^2 / b}$$

Bivariate models including the same effects shown in Equation 2.5 were fit in ASReml (Gilmour et al. 2002) to estimate genetic and environmental covariances between traits. These covariances were then used to calculate genetic correlations between pairs of traits according to Equation 2.9:

$$\text{[Eq. 2.9]} \quad r_{G(x,y)} = \frac{\sigma_{C(F)xy}}{\sqrt{\sigma_{C(F)x}^2 \sigma_{C(F)y}^2}}$$

where $\sigma_{C(F)xy}$ is the clone within family covariance for traits x and y , $\sigma_{C(F)x}^2$ is the clone within family variance for trait x , and $\sigma_{C(F)y}^2$ is the clone within family variance for trait y . Standard errors for genetic correlations as well as for other ratios were estimated using the delta method (Lynch and Walsh 1998).

2.2.5 *Estimating Indirect Selection Efficiency*

Three indirect clonal selection scenarios for MOE and MOR target traits were assessed.

These selection scenarios were: 1) indirect selection on SWS only, 2) indirect selection on AMP only, and 3) combined indirect selection on both SWS and AMP. The relative efficiency of clonal selection (Q) from each scenario was assessed by the ratio of genetic gain resulting from the indirect selection scenario to the genetic gain from direct selection on the target trait. A selection scenario with $Q = 1$ would therefore indicate that the scenario is equally effective at selecting clones for MOE and MOR as direct selection on these target traits. The higher this ratio, the more efficient the indirect measurements assessed in this study are at predicting clone genetic values for MOE and MOR.

Genetic gain from direct clonal selection on the target trait was calculated according to Equation 2.10 while genetic gain from indirect selection on a single trait (scenarios 1 and 2) was calculated according to Equation 2.11:

[Eq. 2.10]
$$\Delta G_{y(direct)} = i_y H_{\bar{C}(F)y}^2 \sigma_y$$

[Eq. 2.11]
$$\Delta G_{y(indirect)} = i_x H_{\bar{C}(F)x} H_{\bar{C}(F)y} r_{G(x,y)} \sigma_y$$

where $H_{\bar{C}(F)y}^2$ is the clone mean repeatability for the target trait, $H_{\bar{C}(F)x}$ is the square root of the clone mean repeatability for the indirect selection trait, σ_y is the square root of the clone mean phenotypic variance for the target trait, $r_{G(x,y)}$ is the genetic correlation between the target trait (y) and measured trait (x), and i_x and i_y are the corresponding standardized

selection differentials. Thus, for indirect selection based on a single trait (scenarios 1 and 2), the ratio Q simplifies to the following expression assuming that $i_x = i_y$:

$$[\text{Eq. 2.12}] \quad Q = \frac{i_x H_{\bar{C}(F)x} H_{\bar{C}(F)y} r_{G(x,y)} \sigma_y}{i_y H_{\bar{C}(F)y}^2 \sigma_{\bar{y}}} = \frac{H_{\bar{C}(F)x} r_{G(x,y)}}{H_{\bar{C}(F)y}}$$

For combined indirect selection based on both SWS and AMP (scenario 3), it was necessary to use a more generalized form of the selection equations based on selection index methodology. The form of the selection index used to predict the clone genetic values for the target trait in scenario 3 is shown in Equation 2.13:

$$[\text{Eq. 2.13}] \quad I = b_{AMP} P_{AMP} + b_{SWS} P_{SWS}$$

were b_{AMP} and b_{SWS} are the partial regression coefficients relating the clone mean phenotypes for AMP and SWS to the clone genetic value for the target trait and P_{AMP} and P_{SWS} are the corresponding clone mean phenotypes for AMP and SWS. The partial regression coefficients were solved by the function $V^{-1}C$, where V is the phenotypic variance-covariance matrix of clone means and C is the covariance matrix between genetic and phenotypic values. Genetic gain in the target trait resulting from clonal selection based on this index can be derived to be equivalent to Equation 2.14:

$$\begin{aligned}
\text{[Eq. 2.14]} \quad \Delta G_{y(\text{indirect})} &= i \sqrt{b'Vb} \\
&= i \sqrt{\begin{bmatrix} b_{AMP} & b_{SWS} \end{bmatrix} \begin{bmatrix} \sigma_{\bar{y} \text{ AMP}}^2 & \sigma_{\bar{y} \text{ AMP},SWS} \\ \sigma_{\bar{y} \text{ AMP},SWS} & \sigma_{\bar{y} \text{ SWS}}^2 \end{bmatrix} \begin{bmatrix} b_{AMP} \\ b_{SWS} \end{bmatrix}}
\end{aligned}$$

where V is the phenotypic variance-covariance matrix of the clone means and b is the vector of partial regression parameter estimates. Therefore, Q for scenario 3 simplifies to Equation 2.15 when assuming equal selection intensities for all traits:

$$\text{[Eq. 2.15]} \quad Q = \frac{\sqrt{b'Vb}}{H_{\bar{C}(F)y}^2 \sigma_y}$$

2.3 Results

2.3.1 Bending Measurements

Preliminary analysis of variance on the results of the bending tests indicated a large amount of within tree variation for MOE, MOR, and basic density measured from the bending samples (Table 2.1a and Table 2.2a). Least squared means depicted the radial and vertical trends responsible for the within tree variation (Table 2.3). As samples often came from different locations in each tree, this large amount of within tree variation would have contributed to large standard errors of means for clones and therefore to low clonal repeatabilities. Adding ring position and height level as covariates greatly reduced the within tree error component for MOE (Table 1.2b) and MOR (Table 2.2b). Thus the standardization for bending sample measurements provided by Equation 2.3 was deemed necessary.

2.3.2 Descriptive Statistics

Overall means for MOE, MOR, and DEN of these 8-year-old trees were 5.2 GPa, 56 MPa, and 389 kg/m³, respectively (Table 2.4). The range of the clone means for MOE and MOR traits, in addition to their relatively large clone mean coefficients of variance, suggests that there was sufficient variation among clones to justify clonal selection to improve mechanical wood properties (Table 2.4). Analysis of variance results on the bending data after adjustments for ring and height effects indicated highly significant differences among both families and clones within families for MOE and MOR (Table 2.1b and Table 2.2b). The adjustment for ring and height effects greatly improved the repeatability of the MOE, MOR, and density measurements obtained from the bending samples. Clone means for stress wave speed (SWS) and amplitude (AMP) exhibited greater variation than mechanical wood traits as indicated by the clone mean coefficients of variance in Table 2.4.

2.3.3 Relationship between direct and indirect measures

Fitting regression models to the individual tree data indicated linear phenotypic relationships between direct and indirect and measurements of mechanical wood traits. At the individual tree level, SWS had a moderate and significant phenotypic correlation with MOE (0.42) and weak but significant phenotypic correlated with MOR (0.26). However, no correlation was detected between SWS and basic density at the individual tree level. AMP had a moderate significant phenotypic correlation with density (0.44), but was not significantly correlated with either MOE or MOR for individual trees at alpha-level of 0.05.

The estimates of the phenotypic correlations at the clone mean level among all assessed traits

are given in Table 2.5. Figure 2.3 and Figure 2.4 show the moderate and highly significant clone mean correlations were found between SWS and both MOE (0.67) and MOR (0.50). No phenotypic correlation was found between clone means for SWS and either basic density (Figure 2.5) or amplitude (Table 2.5). A weak negative clone mean correlation between SWS and diameter at breast height was not significant at the 0.05 alpha-level.

The clone mean correlations estimated between amplitude and mechanical wood traits were conversely related to those found for SWS. Clone mean AMP was most strongly related to basic density ($r = 0.70$) and had a weaker but significant correlation with MOR (Table 2.5). Yet, no significant clone mean correlation was detected between AMP and MOE. This was similar to the results found for density, which was moderately correlated with MOR (0.54) at the clone mean level, but with a weak and non-significant correlation with MOE at the 0.05 alpha-level. Although AMP and SWS independently explained only 11 and 25 percent of the variation in clone mean MOR (Figure 2.4), respectively, these traits were uncorrelated; a multiple linear regression equation using both AMP and SWS covariates explained 35 percent of the variation in MOR with variance inflation factors essentially equal to one. AMP was the only trait that was found to have a significant phenotypic correlation with diameter at breast height.

Partitioning of the phenotypic covariance between SWS and MOE indicated a fairly strong genetic correlation (0.73), whereas a moderate genetic correlation (0.50) was estimated between SWS and MOR (Table 2.5). No genetic relationship was detected between SWS and either AMP or basic density (Table 2.5). Corresponding with the results of Chapter 1,

AMP was found to have a strong genetic relationship with density (0.99). In addition, AMP also had a moderate genetic correlation with both MOE (0.45) and MOR (0.68). Interestingly, the genetic relationship estimated between AMP and MOE was stronger than that between DEN and MOE, while the opposite situation was found with MOR. MOE was estimated to have a negative genetic correlation with DBH (-0.48), and was the only trait exhibiting a genetic relationship with growth. Standard errors of the estimated genetic correlations were sizeable for some pairs of traits (Table 2.5). However, the majority of these correlations were substantially larger than their standard errors, allowing inferences about the relationships to be made.

2.3.4 Genetic Parameters and Relative Efficiencies

Genetic parameters estimated from fitting the linear mixed model (Equation 2.5) are given in Table 2.6. For SWS, 48% of the total phenotypic variance for SWS was due to the differences among clones within families. Therefore, the clone means for SWS were highly repeatable (0.85). In fact, the percentage of clonal variation was slightly greater for SWS than for either MOE or MOR determined directly from static bending (Table 2.6). The greater proportion of environmental variance contributing to MOE and MOR measurements combined with the fewer number of clones measured for these traits resulted in lower clone mean repeatabilities, with values of 0.69 and 0.62 for MOE and MOR, respectively. Amplitude was the least repeatable measurement, with 20% of the total phenotypic variance being due to clonal differences.

Percent genetic gains from direct selection were 19.9, 11.3, 11.1 percent for MOE, MOR,

and density, respectively (Table 2.7). It was possible to achieve a large amount of genetic gain for MOE due to the large clone mean coefficient of variance for this trait (Table 2.4). Clonal selection based on SWS alone resulted in relative efficiencies of 0.81, 0.59, and 0.03 for target traits of MOE, MOR, and density, respectively (Table 2.7). For clonal selection based on AMP, relative efficiencies of 0.42, 0.67, and 0.89 were estimated for MOE, MOR, and density target traits, respectively. Combined selection based on an index containing both SWS and AMP resulted in relative efficiencies of 0.91, 0.90, and 0.89 for MOE, MOR, and density, respectively (Table 2.7). SWS did not have any significant value in the selection index for wood density. Inclusion of AMP with SWS in a clonal selection index for MOE yielded a slight improvement in the genetic gain for MOE. While selection based on solely SWS or AMP did not provide a high efficiency relative to direct clonal selection on MOR, combining these indirect measures into a clonal selection index resulted in a considerable increase in selection efficiency. The fitted selection indices are shown in Table 2.7.

It should be noted that the repeatabilities used to calculate relative efficiencies were based on the number of ramets sampled in the study. Thus, repeatabilities for MOE, MOR, and DEN were based on the measurement of 3 ramets per clone while estimates for SWS and AMP were based on 6 ramets per clone. However, it was assumed for the efficiencies given in Table 2.7 that the number of clones, and therefore the selection intensities, were the same for both direct and indirect measurements. However, SWS and AMP measurements were much cheaper than corresponding direct measurements of wood traits such that more clones could be measured for same the cost, resulting in greater selection intensities and genetic gain. Therefore, the efficiencies presented here may be an underestimate of the true potential gain.

2.4 Discussion

Considerable variation among clone means for MOE and SWS in this 8-year-old test is consistent with previous research. Jacques et al. (2004) found clone mean coefficients of variance of 13.5 and 13.1 percent for static bending MOE and acoustic velocity measurements in standing trees, respectively. At the individual tree level, Chauhan and Walker (2006) found coefficients of variance of 25.3, 12.6, and 5.6 percent for MOE, Fakopp acoustic velocity, and basic density, respectively, for 8-year-old trees in a radiata pine stand. Correspondingly, clone means for basic density were less variable than for the other traits assessed in the current study. However, clone mean amplitude values were well correlated with density and yet provided a greater separation of clone means. Thus, it can be seen that the indirect measures provided by SWS and AMP yield sufficient separation of clone mean phenotypes to facilitate clonal screening for mechanical wood traits.

The main hindrance to direct clonal screening for mechanical wood traits, however, is not the lack of variation present for these traits, but instead it is the high cost associated with directly measuring these traits on a large scale. Efficient indirect measurements of mechanical wood traits must therefore reduce measurement cost while concomitantly providing a reliable phenotypic criterion for selection. It was shown in Chapter 1 that the Resistograph greatly reduced the measurement cost relative to measuring wood density from increment cores. By extension of the previous results, the Resistograph also reduces the cost of sampling for mechanical wood traits, since destructive sampling and laboratory testing of clearwood samples is more time consuming than the sampling and analysis of breast height increment cores. The Fakopp TreeSonic is similarly capable of reducing measurement costs as

indicated by the measurement rate of 800 trees per day achieved by a two-man field crew in this study.

To provide a quality phenotypic criterion for selection, these tools need to produce measurements that are sufficiently repeatable at the clone mean level and that have strong genetic relationships with the wood traits targeted for improvement. Results from Chapter 1 showed amplitude measurements were affected by a large degree of environmental variance, such that clone means based on 6 ramets were not highly repeatable ($H^2_{\bar{\epsilon}(f)} = 0.60$). However, after partitioning out the environmental covariance, Resistograph amplitude measurements were found to be highly genetically correlated with wood density both in this study ($r_G = 0.99$) and in a study by Isik and Li (2003). This strong genetic correlation implies that the Resistograph should have some utility at screening any trait that has a sufficient genetic correlation with wood density. Thus, the gains in MOR and MOE resulting from selection on the Resistograph are to be expected given the positive genetic correlations found between these traits and density. Kumar (2004) found similar but slightly stronger genetic relationships between wood density and mechanical wood traits for radiata pine.

Unlike amplitude measurements, Fakopp SWS measurements produced highly repeatable clone means (0.85) since nearly half of the within family phenotypic variance for SWS was attributable to the variance among clones. Lindstrom et al. (2004) found a greater clonal repeatability (0.95) for Fakopp SWS measurements in young radiata pine. Strong genetic control for SWS is also in agreement with Kumar et al. (2002), who found a high individual tree heritability (0.46) for measurements from a time-of-flight tool.

The greater repeatability found at the individual tree and clone mean level for SWS compared to MOE and MOR in this study may be due to the many potential sources of variability in the bending measurements. In addition to wood strength and stiffness, results of static bending tests can be affected by knots, compression wood, slope of the grain, annual ring orientation, compression failures, pitch pockets, and other defects (Green et al. 1999). While samples containing defects affecting the bending tests were removed from the analysis, this certainly did not preclude effects from all of these factors. Additionally, poor standardization of the location of these samples due to defects within the stem can result in considerable environmental variation among trees. The high repeatability of the time-of-flight tools suggests that they should be efficient at clonal screening if sufficient genetic correlations exist between SWS and mechanical wood traits.

The fairly strong genetic correlation estimated between SWS and MOE (0.73) was of a similar absolute magnitude to the additive genetic correlation reported by Kumar et al. (2002) (-0.69). This negative correlation was found by Kumar et al. (2002) since stress wave transit time was used as the analysis variable in place of the derived velocity measurements used in the current study. Compared to the findings of Kumar et al. (2002), the genetic correlation between SWS and MOR was considerably weaker. This weaker genetic relationship between SWS and MOR seems to be intuitive given that MOR is largely determined by wood density (Yang and Evans 2002), a trait with which SWS was largely uncorrelated.

The lack of a strong association between SWS and wood density has been presented in previous studies (Chauhan and Walker 2006, Lindstrom et al. 2004, Kumar et al. 2002). A review of acoustic techniques provided by Huang et al. (2003) suggests that SWS may be

more directly affected by the microfibril angles in the S2 layer of the cell wall and by tracheid length, which are traits that have weak to no genetic relationships with density in loblolly pine (Megraw 1985, Myszewski 2004). The absence of a relationship between SWS and density explains the independence of SWS and AMP at both the phenotypic and genetic level and their independent contributions to the prediction of mechanical wood traits. A linear combination of SWS and AMP should therefore be useful at improving predictions of genetic values for MOE and MOR.

Assessing the relative efficiencies resulting from selection on SWS and AMP confirms that a combination of these indirect measures is most efficient at selection for MOE and MOR. This was especially evident for MOR, given that clonal index selection on both SWS and AMP resulted in 52 and 34 percent greater genetic gain than independent selection on SWS or AMP, respectively. Thus, if the primary objective is to improve wood strength, then it appears that both SWS and AMP would need to be assessed. However, if MOE is the primary trait being valued, then time-of-flight tools may be solely sufficient since the efficiency of clonal selection was high when based on SWS alone. While index selection provided 2 percent greater gain, this increment of gain must be weighed against the additional cost of obtaining amplitude measurements. This additional cost should be fairly trivial (see Chapter 1). If there is any value for improving wood density aside from its effect on strength and stiffness properties, then an alternative to time-of-flight methods must be employed since SWS provided little value for selecting clone for density.

As genetic correlations among MOE, MOR, and density all were positive, simultaneous improvement for all of these properties could easily be achieved by selection on SWS and AMP. However, in addition to these wood traits, selection of clones with high value for production of solid wood products would also need to consider important growth and stem form traits. Unfortunately, a negative genetic correlation was estimated between wood stiffness and growth in this study as well as in previous research (Kumar et al. 2002, Kumar 2004). For this reason, multi-trait index selection procedures should be employed to obtain optimal simultaneous improvement of growth, form, and wood quality traits. These indices are often more efficient in the presence of negative correlations and can also be restricted such that maximum genetic gain can be obtained in one trait without loss in the other traits (Cunningham et al. 1970).

The traits that define the index for selection are not constrained to those defining the aggregate genotype (Falconer and Mackay 1996). Thus, indirect measures of mechanical wood traits such as AMP and SWS will be critical for providing the phenotypic selection criteria in these clonal selection indices. This is necessary since growth and stem form traits can be rapidly measured on a number of clones that would be impractical to assess using direct assessment of mechanical wood traits. However, to construct the clonal selection indices, the genetic covariances among traits in the index and aggregate genotypic value must be known (Falconer and Mackay 1996). The genetic parameter estimates provided in this study can be directly applied to the development of the variance-covariance structure for such clonal selection indices.

2.5 Conclusions

Fakopp stress wave speed and Resistograph amplitude measurements were found to provide independent information regarding the variation in MOE and MOR. Simple clonal selection indices combining these two indirect measurements predicted clonal genetic values for MOE, MOR, and density with high accuracy compared to direct measurements on clearwood bending samples. Selection to improve MOE was fairly efficient when based solely on SWS, but a combination of SWS and AMP is needed for prediction of MOR. SWS was not found to have any value in terms of selecting clones for wood density. While this study indicates that improving solid wood quality in clonal deployment populations using indirect measurements will be successful, the ultimate goal is to improve the overall value of clones for sawtimber production. Thus, additional research will need to determine the utility of indirect measurements when important growth and stem form traits are simultaneously being considered for selection.

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Figure 2.1. Fakopp TreeSonic time-of-flight tool being used to measure stress wave velocity in a clonal trial to provide an indirect measurement of mechanical wood traits.

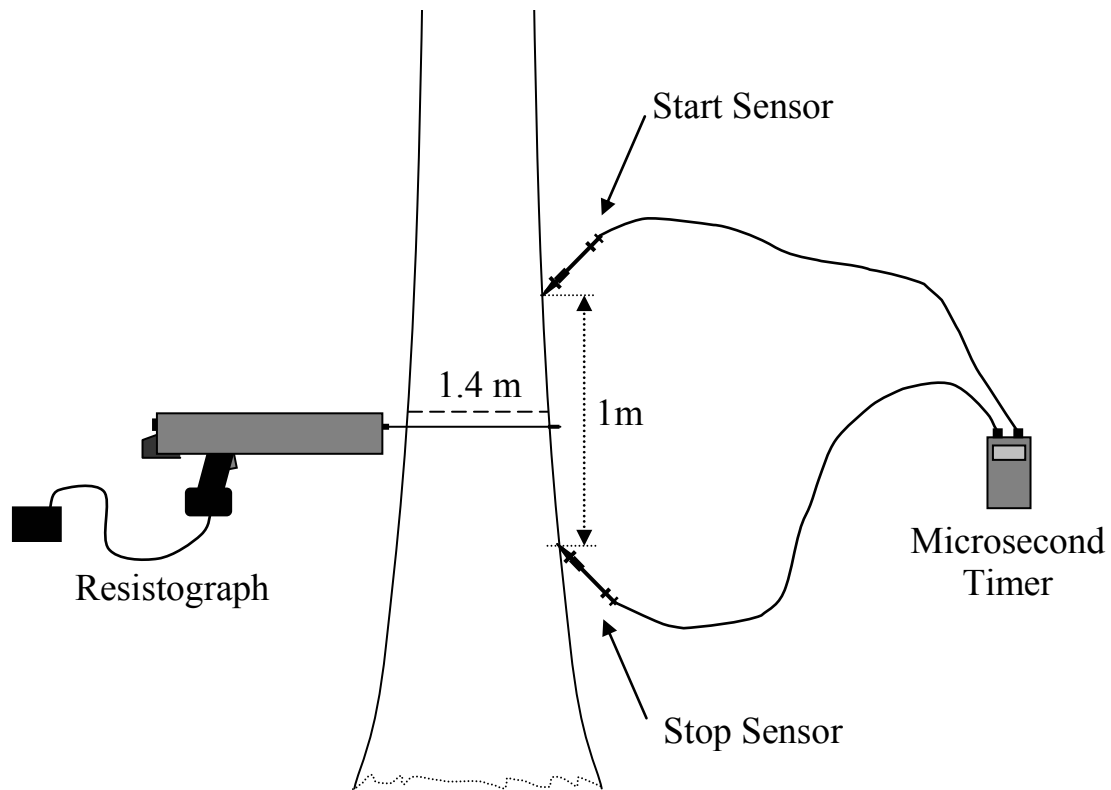


Figure 2.2. Measurement protocol for breast height (horizontal dashed line) sampling with the TreeSonic and Resistograph. Note that the TreeSonic stress wave measurements were actually taken perpendicular to the direction of the drilling holes.

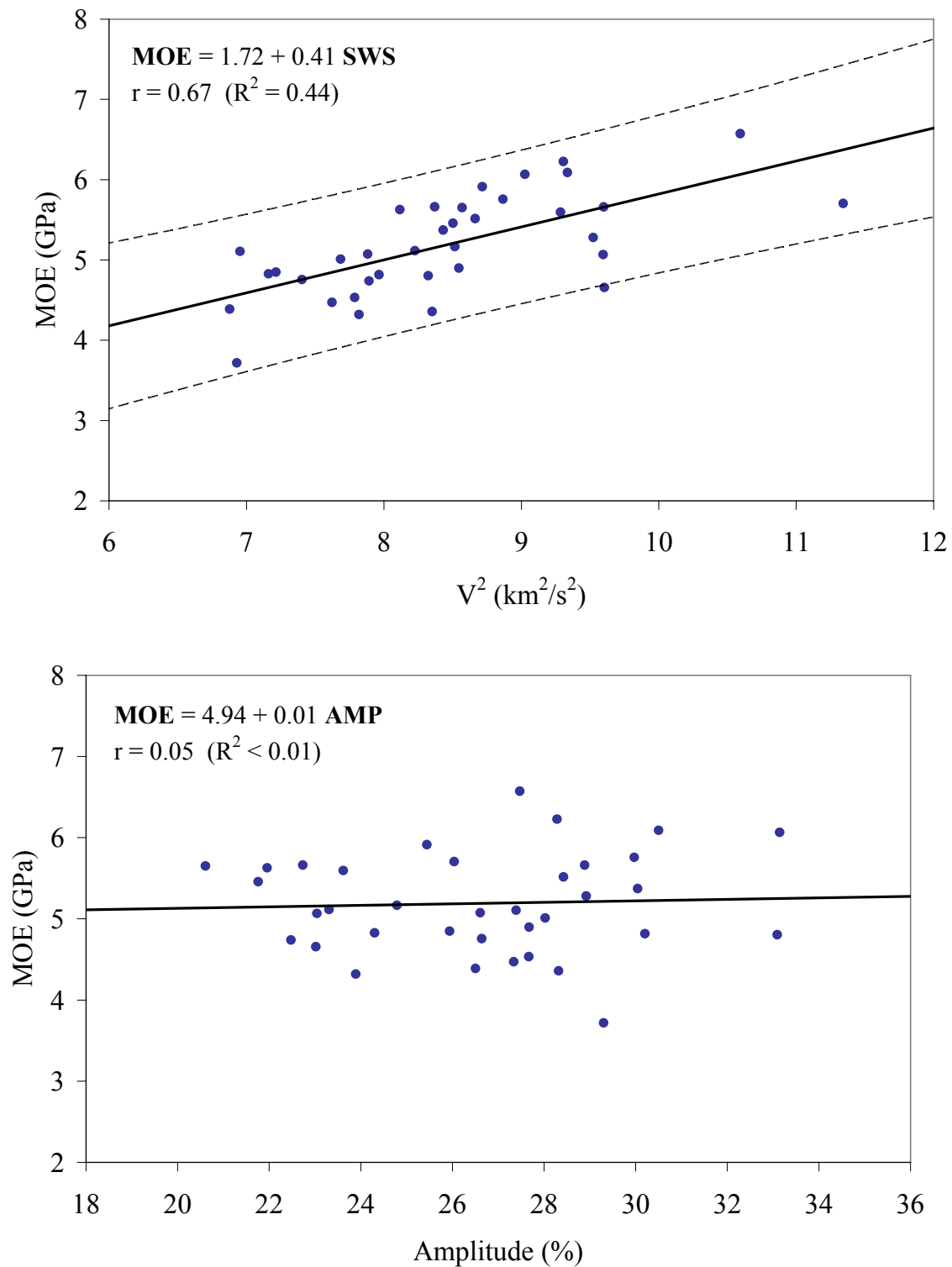


Figure 2.3. Fitted clone mean regression models (solid black lines) between MOE and indirect measurements showing 95% prediction interval (dashed lines) if applicable.

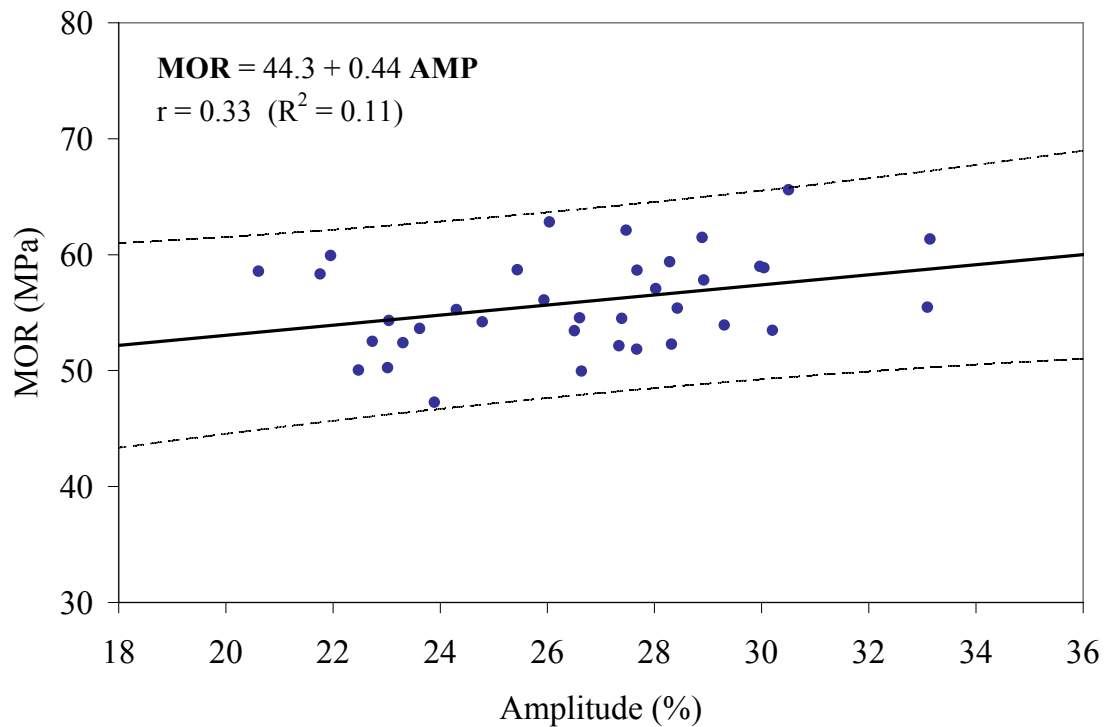
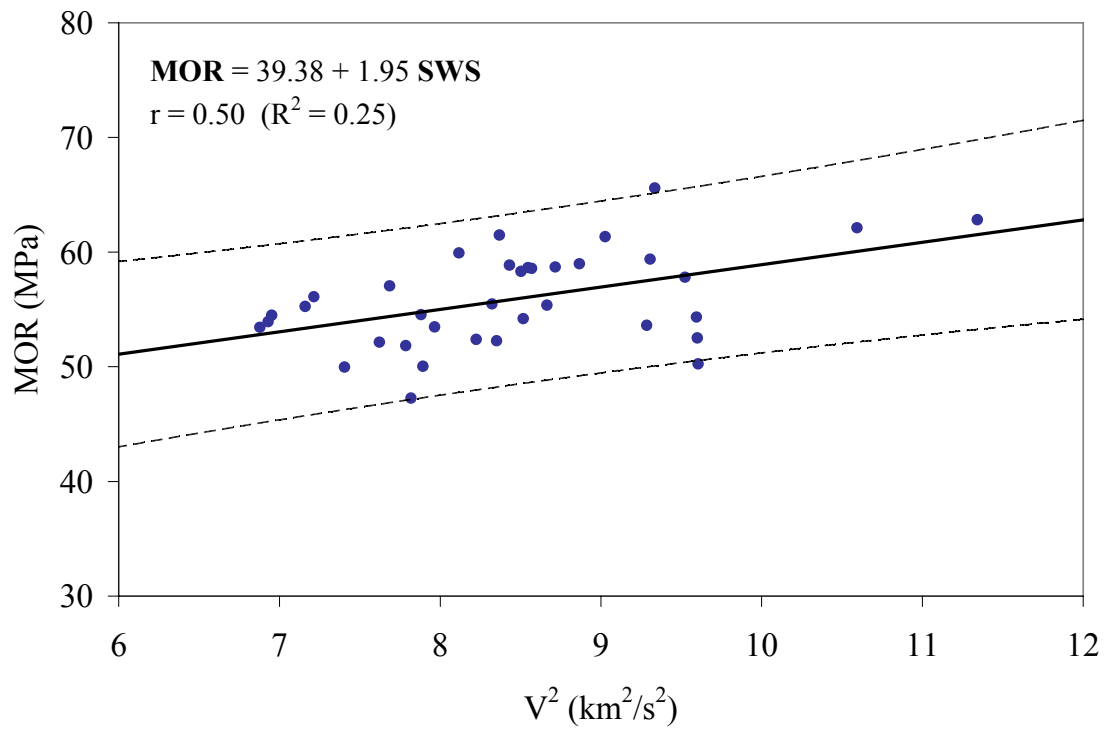


Figure 2.4. Fitted clone mean regression models (solid black lines) between MOR and indirect measurements showing 95% prediction interval (dashed lines) if applicable.

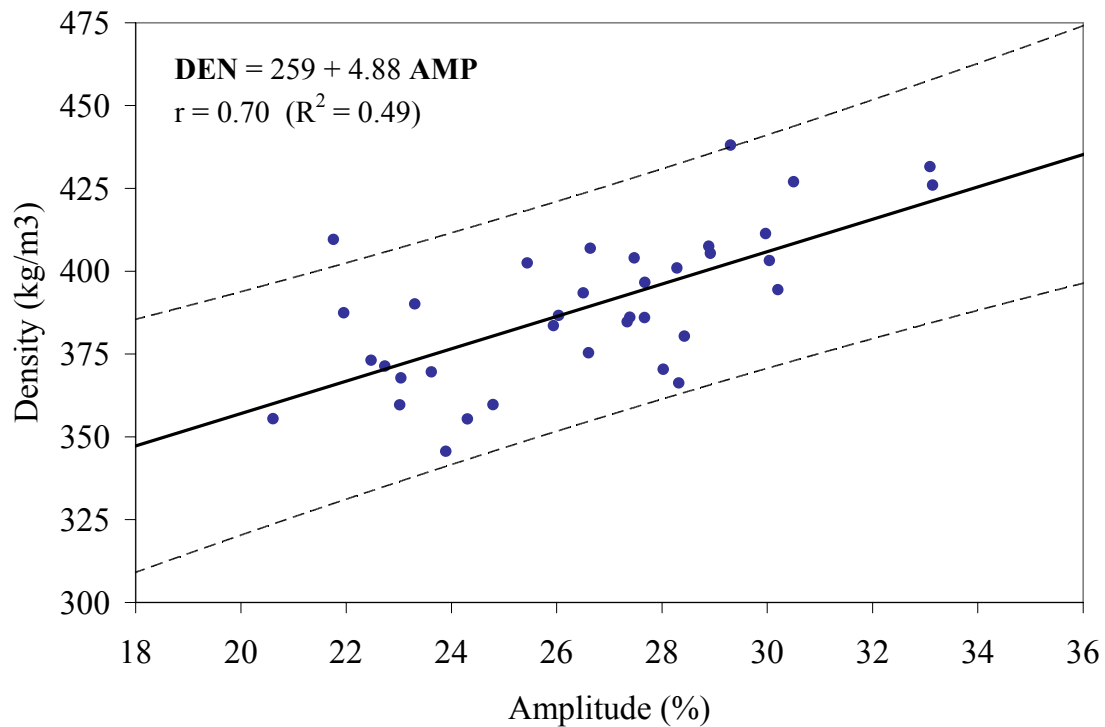
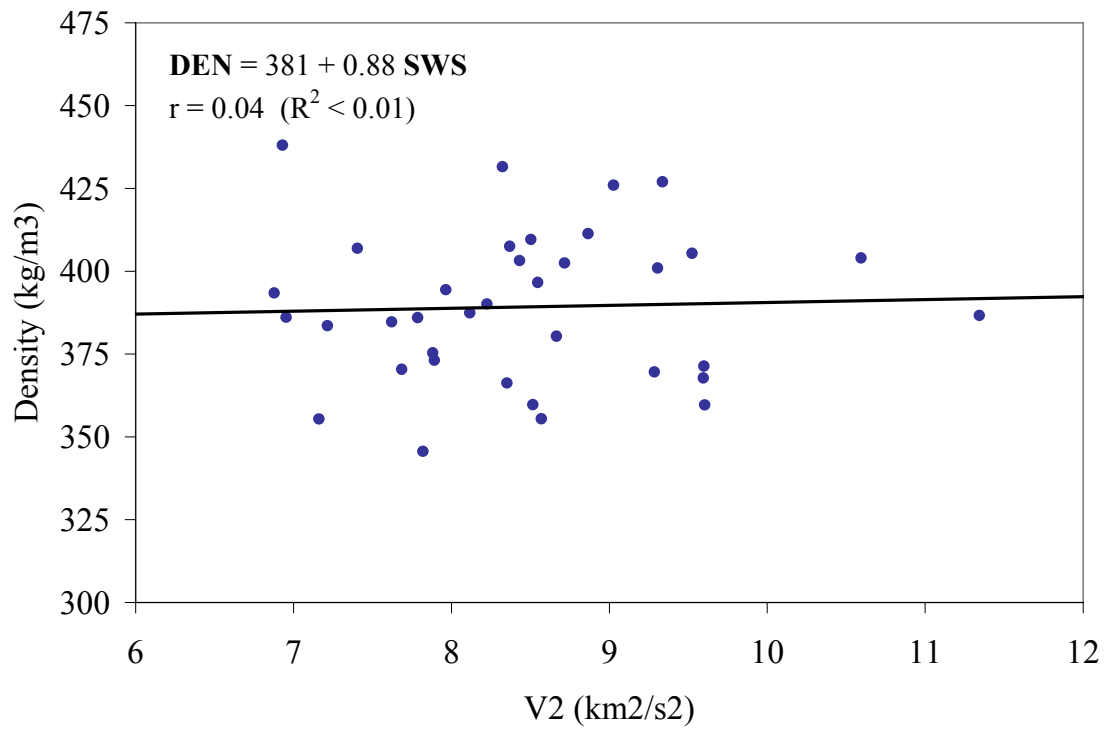


Figure 2.5. Fitted clone mean regression models (solid black lines) between density and indirect measurements showing 95% prediction interval (dashed lines) if applicable.

Table 2.1. Analysis of variance and expected mean squares on bending test data for MOE

a) Reduced Model Type III ANOVA for MOE ($R^2 = 0.38$)

Source	DF	MS	F	P-value
Block	2	4.9	3.1	0.05
Family	2	7.7	4.9	<0.01
Block*Family	4	0.6	0.4	0.84
Clone(Family)	33	2.5	1.6	0.03
Between Tree	54	1.0	0.6	0.97
Within Tree	191	1.6		

b) Full Model Type III ANOVA for MOE ($R^2 = 0.82$)

Source	DF	MS	F	P-value
Block	2	2.4	4.9	<0.01
Family	2	14.0	28.8	<0.01
Block*Family	4	0.6	1.3	0.27
Clone(Family)	33	2.5	5.1	<0.01
Ring	6	17.5	35.2	<0.01
Height	1	123.4	253.1	<0.01
Between Tree	54	0.9	1.8	<0.01
Within Tree	184	0.5		

Source	Expected Mean Square ¹	F-Test
Block	$V_{Ew} + nV_{Eb} + fc nQ_B$	$MS_{Block} / MS_{Between Tree}$
Family	$V_{Ew} + nV_{Eb} + bnV_{C(F)} + bncQ_F$	$MS_{Family} / MS_{Clone(Family)}$
Block*Family	$V_{Ew} + nV_{Eb} + cnQ_{BF}$	$MS_{Block*Family} / MS_{Between Tree}$
Clone(Family)	$V_{Ew} + nV_{Eb} + bnV_{C(F)}$	$MS_{Clone(Family)} / MS_{Between Tree}$
Between Tree	$V_{Ew} + nV_{Eb}$	$MS_{Between Tree} / MS_{Within Tree}$
Within Tree	V_{Ew}	

¹ n = number of samples per tree, b = number of ramets per clone, c = number of clones, f = number of families.

Note: Ring x height interaction was found to be insignificant and was dropped from the full model.

Table 2.2. Analysis of variance and expected mean squares on bending test data for MOR

a) Reduced Model Type III ANOVA for MOR (R² = 0.42)

Source	DF	MS	F	P-value
Block	2	91	1.3	0.29
Family	2	748	10.3	<0.01
Block*Family	4	27	0.4	0.83
Clone(Family)	33	126	1.7	0.01
Between Tree	54	74	1.0	0.46
Within Tree	191	73		

b) Full Model Type III ANOVA for MOR (R² = 0.69)

Source	DF	MS	F	P-value
Block	2	82	2.1	0.13
Family	2	1048	26.4	<0.01
Block*Family	4	18	0.5	0.74
Clone(Family)	33	122	3.1	<0.01
Ring	6	1029	25.9	<0.01
Height	1	755	19.0	<0.01
Between Tree	54	57	1.4	<0.04
Within Tree	184	40		

Source	Expected Mean Square ¹	F-Test
Block	$V_{Ew} + nV_{Eb} + fc nQ_B$	$MS_{Block} / MS_{Between Tree}$
Family	$V_{Ew} + nV_{Eb} + bnV_{C(F)} + bncQ_F$	$MS_{Family} / MS_{Clone(Family)}$
Block*Family	$V_{Ew} + nV_{Eb} + cnQ_{BF}$	$MS_{Block*Family} / MS_{Between Tree}$
Clone(Family)	$V_{Ew} + nV_{Eb} + bnV_{C(F)}$	$MS_{Clone(Family)} / MS_{Between Tree}$
Between Tree	$V_{Ew} + nV_{Eb}$	$MS_{Between Tree} / MS_{Within Tree}$
Within Tree	V_{Ew}	

¹ n = number of samples per tree, b = number of ramets per clone, c = number of clones, f = number of families.

Note: Ring x height interaction was found to be insignificant and was dropped from the full model.

Table 2.3. Least squared means for ring and height effects showing the effects of ring position and height within trees for MOE, MOR, and density. Standard errors are shown in parentheses.

Bolt 1: base between 1.5 -2.0 m

Ring Position	Number of Samples	Modulus of Elasticity (GPa)	Modulus of Rupture (MPa)	Basic Density (kg/m ³)
2	10	3.41 (0.27)	45.1 (2.30)	355 (8.48)
3	24	3.75 (0.19)	46.6 (1.55)	365 (6.11)
4	50	4.01 (0.15)	49.2 (1.14)	377 (4.91)
5	43	4.70 (0.15)	55.2 (1.22)	397 (5.11)
6	48	5.14 (0.15)	59.4 (1.16)	423 (4.94)
7	24	5.41 (0.19)	61.0 (1.57)	449 (6.19)
8	17	5.81 (0.22)	66.1 (1.82)	462 (7.00)

Bolt 2: base between 3.8 -4.4 m

Ring Position	Number of Samples	Modulus of Elasticity (GPa)	Modulus of Rupture (MPa)	Basic Density (kg/m ³)
3	12	4.90 (0.26)	52.1 (2.21)	357 (8.22)
4	32	5.45 (0.16)	52.6 (1.28)	362 (5.32)
5	34	6.18 (0.15)	59.3 (1.21)	378 (5.13)
6	24	6.69 (0.18)	63.9 (1.49)	382 (5.91)
7	15	7.11 (0.25)	62.2 (2.16)	389 (7.87)

Table 2.4. Descriptive statistics for wood traits based on clone means including the grand mean, range of clone means, and phenotypic coefficients of variance for clone means.

Trait	Mean	Min	Max	$CV_{\bar{C}(F)}$
Modulus of elasticity (GPa)	5.2	4.0	6.4	13.15
Modulus of rupture (MPa)	56	42	68	8.69
Basic density (kg/m ³)	389	340	441	4.01
Stress wave speed (km ² /s ²)	8.5	5.5	11.6	13.55
Adjusted amplitude (%)	28	22	36	11.86

Table 2.5. Clone mean phenotypic correlations (above diagonal) and genetic correlations (below diagonal) between wood traits, relative wood traits, and breast height diameter.

	MOE	MOR	DEN	AMP	SWS	DBH
MOE	-	0.74 ($<.0001$)	0.20 (0.25)	0.05 (0.87)	0.67 ($<.0001$)	-0.28 (0.10)
MOR	0.70 (0.16)	-	0.54 ($<.001$)	0.33 (0.04)	0.50 ($<.01$)	-0.19 (0.26)
DEN	0.25 (0.25)	0.77 (0.17)	-	0.70 ($<.0001$)	0.08 (0.63)	0.04 (0.82)
AMP	0.45 (0.30)	0.68 (0.31)	0.99 (0.21)	-	-0.03 (0.84)	0.47 ($<.01$)
SWS	0.73 (0.19)	0.50 (0.29)	-0.03 (0.28)	0.01 (0.15)	-	-0.29 (0.09)
DBH	-0.48 (0.30)	0.04 (0.37)	0.18 (0.30)	0.09 (0.17)	-0.11 (0.14)	-

Note: Clone mean correlations are followed by their p-value and genetic correlations are followed by their approximate standard errors. MOE = modulus of elasticity, MOR = modulus of rupture, DEN = basic density, AMP = mean amplitude, SWS = stress wave speed, DBH = diameter at breast height.

Table 2.6. Genetic parameter estimates from fitting the linear mixed model quantifying the environmental and genetic variance for wood traits and indirect measurements.

Parameter Estimate	Modulus of Elasticity (GPa)	Modulus of Rupture (MPa)	Basic Density (kg/m ³)	Stress Wave Speed (km ² /s ²)	Adjusted Amplitude (%)
$\sigma_{C(F)}^2$	0.22 (0.08)	9.12 (3.95)	357 (125)	1.13 (0.20)	6.4 (1.65)
σ_E^2	0.29 (0.05)	16.8 (3.15)	375 (71)	1.18 (0.08)	26.1 (1.77)
$\sigma_{\bar{Y}}^2$	0.31 (0.08)	14.7 (3.93)	484 (123)	1.33 (0.20)	10.7 (1.62)
% $\sigma_{C(F)}^2$	43%	35%	49%	48%	20%
$H_{\bar{C}(F)}^2$	0.69 (0.10)	0.62 (0.12)	0.74 (0.08)	0.85 (0.03)	0.60 (0.07)

Note: estimates are followed by their standard errors. Parameter estimates are the clone within family variance ($\sigma_{C(F)}^2$), environmental variance (σ_E^2), clone mean phenotypic variance ($\sigma_{\bar{Y}}^2$), percent of phenotypic variance explained by clonal variance (% $\sigma_{C(F)}^2$), and clone mean repeatabilities ($H_{\bar{C}(F)}^2$).

Table 2.7. Percent genetic gains ($i = 2.063$) and relative efficiencies (in parentheses) resulting from direct and indirect selection for target wood traits showing fitted selection indices.

Selection Trait	Target Trait		
	MOE	MOR	DEN
Direct	19.9% (1.00)	11.3% (1.00)	11.1% (1.00)
SWS	16.1% (0.81)	6.7% (0.59)	0.3% (0.03)
AMP	8.4% (0.42)	7.6% (0.67)	9.9% (0.89)
SWS & AMP	18.1% ¹ (0.91)	10.2% ² (0.90)	9.9% ³ (0.89)

¹Selection Index: $\hat{G}_{MOE} = 0.05AMP + 0.27SWS$, $r_{GI} = 0.76$, $r_{GP} = 0.83$.

²Selection Index: $\hat{G}_{MOR} = 0.49AMP + 1.25SWS$, $r_{GI} = 0.71$, $r_{GP} = 0.79$.

³Selection Index: $\hat{G}_{DEN} = 4.38AMP + 0.32SWS$, $r_{GI} = 0.76$, $r_{GP} = 0.86$.

Note: r_{GI} = correlation between the indirect selection index and the genotypic value.

r_{GP} = correlation between the phenotypic value for the target trait and the genotypic value.

Chapter 3

Multi-trait Clonal Selection for Simultaneous Improvement of Growth and Solid Wood Quality Traits

3.1 Introduction

Deployment of fast-growing genotypes in conjunction with intensive plantation silviculture has enabled the production of sawlog sized trees from relatively short rotation (< 25 years) southern pine plantations. However, increasing harvests of sawtimber from these young plantations has necessitated the utilization of greater quantities of juvenile wood, which is characterized by considerably reduced strength, stiffness, and density compared to mature wood (Kretschmann and Bendtsen 1992, Larson et al. 2001). An increasing reliance on this fast grown wood resource can therefore be expected to have negative economic implications for manufacturers of solid wood products (Kretschmann and Bendtsen 1992). Thus, genetic gain for a single trait such as volume production may not equate to large economic gains in terms of sawtimber production if important solid wood properties are ignored.

Selection and deployment of clones based on both rapid growth and more desirable solid wood properties would aid in maximizing the value of southern pine plantations for sawtimber production. Efficient clonal selection for these traits would require mass screening of clones from elite crosses using large clonally replicated field tests (Isik et al. 2005). Such tests have been established for loblolly pine and used to screen a large number of clones for growth traits, revealing considerable clonal variance and moderately high clone mean repeatabilities for volume (Isik et al. 2005). Similarly, Chapters 1 and 2 indicated that large genetic gains could be obtained from clonal screening for important solid wood properties including MOE, MOR, and density. However, the capacity for multi-trait selection to simultaneously improve volume and solid wood properties and thereby enhance overall merit for sawtimber production has yet to be assessed.

Multi-trait selection of clones for deployment could be performed using either independent culling or selection index methods. Independent culling considers each trait of interest separately by establishing minimum threshold values for each trait. These threshold values determine whether an individual or genetic group should be retained or culled (Van Vleck et al. 1987). Thus, any clone with a trait below its respective threshold value would be discarded regardless of its value for other traits. Alternatively, index selection considers all traits simultaneously based on their repeatabilities, economic values, and genetic correlations to derive a single index value, called the selection index, that can then be used to rank clones for selection (Van Vleck et al. 1987). This selection index is a linear combination of the phenotypic values for each trait weighed by coefficients that maximize the correlation between the index and the aggregate genotypic value of the target traits. Derivation of the index weights is based on multiple regression theory which assumes that the genetic and phenotypic variances and covariances for the traits are known (Falconer and Mackay 1996). In theory, the selection index methodology provides the most efficient form of multiple trait selection (Hazel and Lush 1942, Young 1961), especially under high selection intensities which are expected when selecting clones for deployment.

While the traits to be included in the aggregate genotypic value are those which have a direct impact on profitability or cost, the traits that form the selection index are those which have been practically chosen to provide the phenotypic information on which to base selection (Wei and Borralho 1999). The traits in the selection index are therefore not restricted to the same traits that define the aggregate genotypic value, but instead can be based on indirect traits that can be more easily assessed. This approach was exemplified by Wei and Borralho

(1999), in which easily measured traits (height, breast height diameter, Pilodyn penetration, and relative bark thickness) were used as the selection criteria to improve the aggregate genotypic value of traits directly affecting the value of pulp production (volume, density, and pulp yield).

Likewise, a practical assessment of multi-trait selection of clones to improve sawtimber production must be based on the use of indirect selection criteria due to the cost associated with direct assessment of solid wood properties. Recent studies indicate that time-of-flight acoustic tools provide an indirect selection criterion for MOE and MOR that can be rapidly measured on a large number of standing trees (Wang et al. 2000, Kumar et al. 2002, Lindstrom 2004, Chauhan and Walker 2006, Chapter 2). Drill resistance measurements in standing trees provided by the IML Resistograph have been demonstrated to have utility for providing rapid indirect measurements of wood density (Isik and Li 2003, Chapter 1). It was shown in Chapter 2 that combined clonal selection based on both time-of-flight measurements and drill resistance measurements resulted in high relative selection efficiencies (~ 0.90) for MOE, MOR, and wood density. However, the utility of these indirect measurements in the context of index selection for both growth and wood quality traits has not been evaluated.

This study utilized growth measurements as well both direct and indirect measurements of wood density, modulus of elasticity, and modulus of rupture from a clonal test of loblolly pine to assess genetic gains from multi-trait selection for both growth and solid wood quality. The objectives were to 1) estimate the genetic relationships among growth and solid wood

quality traits and determine their effects on combined index selection, 2) assess the utility of the Fakopp TreeSonic time-of-flight tool and IML Resistograph drill resistance tool for providing indirect selection criteria for these clonal selection indices, and 3) examine selection response surfaces resulting from index selection to determine the effects of differing levels of emphasis placed on growth and wood traits. If genetic correlations between growth and wood quality traits are not highly antagonistic and if indirect measurements are capable of providing quality information on solid wood properties, then the selection and deployment of clones has the potential to greatly improve the value of southern pine plantations for the production of solid wood products.

3.2 Methods and Materials

3.2.1 Materials and Data Collection

Three partially related full-sib families of loblolly pine were selected from the North Carolina State University – Cooperative Tree Improvement Program on the basis of superior volume production and rust resistance. Progeny from these three families were clonally replicated by means of hedging and subsequent vegetative propagation to produce rooted cuttings. The rooted cuttings were used for establishment of a clonal test in Berkley County, South Carolina in December 1997. The field design was a randomized complete block design with families planted as whole plots within each replication. Clones within these families were planted in single tree plots, such that each clone was represented with a single ramet in each block.

Ninety clones, consisting of 30 from each full-sib family, were randomly selected from the

test at age 8. Indirect measurements of solid wood properties were obtained from these clones using the Fakopp TreeSonic and IML Resistograph. Six ramets were measured for each clone. TreeSonic stress wave measurements were obtained over a one meter longitudinal test span centered on breast height. To accomplish this, the start sensor of the TreeSonic was driven into the stem at 1.9 m, while the stop sensor was placed directly below at 0.9 m. The sensors were always placed at opposing 45° angles to the stem and always on the same aspect to minimize unwanted variation. Stress waves were induced by striking the start sensor with a hammer and the transit time (μs) of the stress wave between the start and stop sensors was recorded by a microsecond timer. Three consecutive readings were taken at the same location for each tree and averaged to yield the final transit time measurement. Transit times were converted to the squared stress wave speed (SWS) analysis variable as shown in Chapter 2.

Drilling with the IML Model F400 Resistograph was conducted from bark to bark at breast height in the same direction for all trees (see Figure 2.2 in Chapter 2). A constant forward speed of 25 cm/min was used. Drilling files for each tree were stored in the onboard electronic unit of the Resistograph. These drilling files contained resistance profile data including tree identification and resistance (percent amplitude) measurements at each 0.1 mm increment of drilling depth. Increasing trends in the drilling profiles obtained from the Resistograph with drilling depth were removed using centered moving averages and centered moving minimums as described in Chapter 1. Mean percent amplitude values (AMP) of the adjusted profiles were then used as the analysis variable for drill resistance.

Diameter at breast height and total height were also measured at age 8 on the same trees sampled for indirect wood quality traits. These diameter and height measurements were used to estimate total stem volume based on the volume equation of Geobel and Warner (1966) as shown in Equation 3.1.

$$\text{[Eq. 3.1]} \quad VOL = 0.0337 + \left(0.0196 \times D^2 \times \frac{H}{10} \right)$$

Where VOL is the estimate of total inside bark stem volume (ft^3), D is the diameter at breast height (in), and H is the total height (ft). All growth measurements were converted to metric units for analysis. These volume estimates were assumed to provide a direct selection criterion for volume (i.e. the objective was to improve this function of height and diameter).

From the original 90 clones, a sub-sample of 36 clones consisting of 12 from each full-sib family was obtained for direct measurement of solid wood traits. This sub-sample was randomly selected from the quartiles of the distribution of clone mean density within each family based on previously obtained increment cores to ensure a representative sample. Ramets of the selected clones were felled in 3 blocks, resulting in 3 ramets sampled for each clone. Diameters measured at 13 sample positions from the stump to a 2 inch top on each felled stem were used to assess the accuracy of the volume estimates provided by Geobel and Warner (1966). Two bolts, approximately 1 m in length, were then cut from each tree. The lower bolt was positioned with its large end at 1.4 m (4.5 ft) and the upper bolt positioned with its large end at 3.8 m (12.5 ft), while allowing some deviation for avoidance of branch

whorls and other stem defects.

Bolts were transported to the USDA-Forest Service, Forestry Sciences Laboratory in Athens, GA for processing and analysis of wood traits. Static bending samples measuring 25.4 x 25.4 x 406 mm were processed from these bolts as described in Chapter 2. After removing defected samples, an average of 3.4 samples per tree and 9.3 samples per clone were available for bending tests. The clear wood static bending samples were tested at 12 percent EMC and pith up over a 355.6 mm span with center loading on a Tinius Olsen static bending machine following the procedures for alternate sample size under ASTM D-143 (ASTM 1980). A continuous load was applied at a head speed of 1.78 mm/minute rather than 1.29 mm/minute to reduce testing time. Modulus of elasticity (MOE) and modulus of rupture (MOR) were calculated using procedures outlined in ASTM D-143 (ASTM 1980). After testing, each sample was oven dried at 103°C and basic density was calculated based on oven dry weight and specimen dimensions at 12 percent EMC.

MOE, MOR, and density measurements obtained from the bending samples were adjusted for within-tree position effects by fitting a preliminary linear model with ring and height effects as described in Chapter 2. The residuals from fitting the model provided position adjusted bending sample measurements of MOE, MOR, and density that were standardized to the mean at their corresponding sample positions. Individual tree values of MOE, MOR, and density that will serve as analysis variables were then calculated by averaging all of the position adjusted measurements for each tree.

3.2.2 Derivation of Variances and Covariances

Genetic and environmental variances and covariances for the assessed traits were determined by using ASREML to fit univariate and bivariate forms of the mixed model shown in the following equation:

$$\text{[Eq. 3.2]} \quad y_{ijk} = \mu + b_i + f_j + bf_{ij} + c(f)_{k(j)} + \varepsilon_{ijk}$$

where y_{ijk} is the individual phenotypic observation, μ is the grand mean, b_i is the fixed effect of the i th block, f_j is the fixed effect of the j th family, bf_{ij} is the fixed interaction effect between the i th block and j th family, $c(f)_{k(j)}$ is the random effect of the k th clone within the j th family, and ε_{ijk} is the random environmental error. Fitting the univariate models provided estimates of the clone within family genetic variance ($\hat{\sigma}_{C(F)}^2$) and residual environmental variance ($\hat{\sigma}_E^2$) for each trait. Fitting the bivariate models for each pair of traits provided estimates of the clone within family genetic covariances ($\hat{\sigma}_{C(F)xy}$) and environmental covariances ($\hat{\sigma}_{Exy}$) among traits.

By fixing the family effect (f_j), it was assumed that there was no variance associated with full-sib families. Therefore, the selection being imposed was within family selection of clones, as would be expected when the families have already been tested and identified as superior prior to test establishment. Thus, the total phenotypic variance of clone means within families was calculated as $\hat{\sigma}_{C(F)}^2 + \hat{\sigma}_E^2 / b$, where b is the number of ramets per

clone. Similarly, the phenotypic covariance among clone means was derived as $\hat{\sigma}_{C(F)xy} + \hat{\sigma}_{Exy} / b$. When deriving the theoretical variances and covariances it was assumed that clone means were based on 6 ramets for all measurements ($b = 6$).

Estimated variance components were summarized by calculating the clone mean phenotypic coefficient of variance ($CV_{\bar{C}(F)}$) and clonal repeatabilities ($H_{\bar{C}(F)}^2$) as performed in the previous chapters. Covariance components were summarized by calculating clone mean phenotypic (r_P) and genetic (r_G) correlations as performed in previous chapters.

3.2.3 Objectives for Clonal Selection

Three specific objectives (H_i) for clonal selection were considered that defined the linear combination of traits used for the aggregate genotypic values. These specific objectives were 1) simultaneous improvement of volume and MOE as shown Equation 3.3, 2) simultaneous improvement of volume and MOR as shown in Equation 3.4, and 3) simultaneous improvement of volume and wood density as shown in Equation 3.5:

$$[\text{Eq. 3.3}] \quad H_{MOE} = a_{VOL} G_{VOL} + a_{MOE} G_{MOE} + 0G_{MOR} + 0G_{DEN}$$

$$[\text{Eq. 3.4}] \quad H_{MOR} = a_{VOL} G_{VOL} + 0G_{MOE} + a_{MOR} G_{MOR} + 0G_{DEN}$$

$$[\text{Eq. 3.5}] \quad H_{DEN} = a_{VOL} G_{VOL} + 0G_{MOE} + 0G_{MOR} + a_{DEN} G_{DEN}$$

where VOL is the volume determined by the Geobel and Warner (1966) equation; MOE, MOR, and DEN are the solid wood traits determined from bending samples; G is the genetic value for the subscripted trait; and a is the relative economic weight for each subscripted trait. Subscripts on H indicated only the target wood trait as volume was ubiquitously targeted. Non-target traits were retained in the aggregate genotypic values with zero weights.

The relative economic weights for the target traits in these objectives were defined as shown in Equation 3.6:

$$\text{[Eq. 3.6]} \quad a_{VOL} = \frac{w_{VOL}}{\sigma_{C(F)VOL}}, \quad a_{MOE} = \frac{w_{MOE}}{\sigma_{C(F)MOE}}, \quad a_{MOR} = \frac{w_{MOR}}{\sigma_{C(F)MOR}}, \quad a_{DEN} = \frac{w_{DEN}}{\sigma_{C(F)DEN}}$$

where w is the proportional economic weight (ranging from 0 to 1) placed on one genetic standard deviation of the subscripted trait and $\sigma_{C(F)}$ is the genetic standard deviation of the subscripted trait. Proportional economic weights in each objective were constrained such that their sum was equal to one. This derivation of the economic weights is a simple extension of the equal emphasis method discussed by Cotterill and Jackson (1985). It was employed in this analysis to adjust the genetic values for scale effects such that proportional economic weights could be used to reflect the relative levels of emphasis placed on each trait in the objective (H_i).

3.2.4 Construction of Selection Indices

Three clonal selection indices (I_i) using indirect criteria for wood traits were considered for providing gain in the aggregate genotypic values: 1) combined selection on volume and stress wave speed as shown in Equation 3.7, 2) combined selection on volume and amplitude as shown in Equation 3.8, and 3) combined selection on volume, stress wave speed, and amplitude as shown in Equation 3.9.

$$[\text{Eq. 3.7}] \quad I_{\text{SWS}} = b_{\text{VOL}}P_{\text{VOL}} + b_{\text{SWS}}P_{\text{SWS}}$$

$$[\text{Eq. 3.8}] \quad I_{\text{AMP}} = b_{\text{VOL}}P_{\text{VOL}} + b_{\text{AMP}}P_{\text{AMP}}$$

$$[\text{Eq. 3.9}] \quad I_{\text{SWS.AMP}} = b_{\text{VOL}}P_{\text{VOL}} + b_{\text{SWS}}P_{\text{SWS}} + b_{\text{AMP}}P_{\text{AMP}}$$

where P is the clone mean phenotype for the subscripted trait and the b 's are the index weights for the subscripted traits which maximize the correlation between the index value (I_i) and aggregate genotypic value (H_i). Subscripts on I indicated only the selection criteria for wood traits as volume was used for selection in all indices. The index weights were derived based on Equation 3.10.

$$[\text{Eq. 3.10}] \quad b = V^{-1}Ca$$

where V^{-1} is the inverse of the phenotypic variance-covariance matrix for traits in the

selection index, C is the genetic covariance matrix between traits in I_i and H_i , and a is the vector of relative economic weights for each trait. For each objective, an additional index (I_{MOE} , I_{MOR} , or I_{DEN}), which incorporated the direct measure of the wood trait in the objective, was used as a basis for assessing the magnitude of gains from I_{SWS} , I_{AMP} , and $I_{SWS.AMP}$.

3.2.5 Response Surface Analysis

It was not desired to implicitly define economic weights for volume and wood quality traits for the construction of the indices, as to do so would require assumptions about the specific attributes and objectives of grower, processor, and end user. These attributes and objectives are variable and are subject to change over time (Lindstrom 2004). Therefore, it was desired to examine genetic gains from index selection over a range of emphasis placed on volume and wood quality. Thus, for each specific objective (H_i), the proportional economic weights (w) placed on volume and wood quality were varied conversely from 0 to 1. The effects of different levels of emphasis for the traits in H_i on combined index selection were then assessed by examining selection response surfaces for each target trait. These surfaces were created by plotting the vector of genetic gains for each trait in H_i over the changing economic weights. This vector of genetic gains was calculated as shown in Equation 3.11:

$$[\text{Eq. 3.11}] \quad \Delta G = C'b \frac{i}{\sqrt{b'Vb}}$$

where ΔG is the vector of genetic gains for each trait, C' is the transpose of the genetic covariance matrix of traits in the index with traits in the aggregated genotypic value, V is the

phenotypic variance-covariance matrix for traits in the index, b is the vector of index weights, and i is the selection intensity. The selection intensity was set to 2.665, which was equivalent to selecting one percent of the screened clones for deployment. Since non-target wood traits remained in the aggregate genotypic values with zero weights, the vector of genetic gains also included correlated responses for the non-target wood traits caused by selection on the index.

The accuracy of each selection index was assessed by calculating the correlation between the index and the objective aggregate genotypic value as shown in Equation 3.12:

$$\text{[Eq. 3.12]} \quad R_{HI} = \frac{\sqrt{b'Vb}}{\sqrt{a'Aa}}$$

where A is the genetic variance-covariance matrix for traits in the aggregate genotypic value, a is the vector of economic weights, and the other terms were previously defined. For each objective, the best performing selection index based on indirect wood quality measurements was identified in terms of this correlation.

3.3 Results

3.3.1 Parameter estimates

Fitting the univariate mixed models showed that there was substantial variation among clone means for volume estimates ($CV_{\bar{C}(F)} = 18.6\%$) and that a large proportion of this variation was under genetic control as indicated by the moderately high clone mean repeatability

(Table 3.1). Stem volume measured from felled trees confirmed that volume estimates from the Geobel and Warner (1966) equation were accurate. The individual tree phenotypic correlation between volume on felled stems and volume estimates was 0.98, while the genetic correlation was 1.00. Repeatability estimates were also comparable such that Geobel and Warner (1966) estimates essentially provided a direct measure of volume. However, clonal variance for volume measured from the felled trees was lower as a result of excluding trees that were of insufficient diameter to yield bolts for processing of bending samples.

Clone means for solid wood properties had higher repeatabilities compared to growth traits (0.77–0.85), but exhibited less variability than volume (Table 1.3). The variation among clone means was drastically lower for wood density ($CV_{\bar{C}(F)} = 4.0\%$) and MOR ($CV_{\bar{C}(F)} = 8.7\%$), but remained fairly high for MOE ($CV_{\bar{C}(F)} = 13.2\%$). Although both indirect measurements of solid wood properties provided good separation of clone means for selection, stress wave speed measurements provided much more repeatable clone means than amplitude measurements (Table 3.1).

MOE and MOR had negative clone mean phenotypic correlations with all growth traits (Table 3.2). However these correlations were generally weak and non-significant at the 0.05 alpha-level. Larger negative correlations were found at the genetic level between MOE and growth traits (-0.21 to -0.48), suggesting possible difficulties for simultaneous improvement of wood stiffness and tree growth. However, both MOR and density had weak positive genetic correlations with growth traits (Table 3.2). Positive phenotypic and genetic relationships were estimated among the solid wood properties targeted for improvement,

including strong correlations between MOE and MOR and between MOR and density (Table 3.2). Strong correlations were also found among growth traits.

Resistograph drill resistance measurements (amplitude) had a strong positive genetic correlation with density (0.99), as well as moderate positive genetic relationships with MOR and MOE. These correlations were weaker at the phenotypic level due to the poor repeatability of clone means for amplitude (Table 3.2). Fakopp stress wave speed had a moderately strong genetic correlation with MOE (0.73) and a moderate genetic relationship with MOR (0.50), and these genetic correlations were comparable in magnitude to the correlations observed at the clone mean phenotypic level due to the high repeatability for stress wave speed measurements. Stress wave speed did not provide any significant amount of information regarding the variation for wood density at the phenotypic or genetic level. These indirect wood quality measurements generally had weak genetic correlations with growth traits.

3.3.2 Single trait selection

Genetic gain estimates shown in Table 3.3 suggest that large genetic gains could be achieved for either volume (34.5%) or MOE (21.7%) when based on clonal selection for single traits. However, due to the negative genetic correlation estimated between these traits, clonal selection based solely on volume resulted in an undesirable negative correlated response for MOE (-6.03%). Correspondingly, clonal selection based solely on MOE resulted in a genetic loss of 11.2% for volume. This negative correlated response for volume was greatly reduced when selection was based on stress wave speed instead of MOE (Table 3.4). Correlated

responses for MOR and density caused by clonal selection on volume were positive but small. Selection for any one of the solid wood traits resulted in favorable correlated responses in the other wood traits, with degrees corresponding to the strength of the genetic correlations among the wood traits. Selection based on amplitude resulted in moderate genetic gains for all solid wood traits, while selection for stress wave speed focused genetic gain on MOE (Table 3.4).

3.3.3 Simultaneous selection for volume and MOE

Clonal selection based on an index containing volume and MOE (I_{MOE}) resulted in high correlations with the objective (H_{MOE}), which ranged from 0.90 to 0.84 with increasing weight on volume (Figure 3.1). The most efficient selection index based on indirect wood quality measurements included both SWS and AMP as selection criteria ($I_{SWS,AMP}$). This index resulted in correlations with H_{MOE} that ranged from 0.75 to 0.84. Therefore, the relative efficiency of $I_{SWS,AMP}$ ranged from 0.85 to 1.00, with higher efficiencies obtained as the proportional economic weight on volume increased. Relative efficiencies for all indices tended to approach 1 as the proportional weight for volume increased since volume was assumed to be measured directly. SWS provided higher quality information for improving MOE compared to AMP as can be seen by the poor efficiency of I_{AMP} when lower weights are placed on volume (Figure 3.1).

Theoretical response surfaces resulting from direct (I_{MOE}) and indirect ($I_{SWS,AMP}$) index selection are shown in Figure 3.2 and Figure 3.3, respectively. Selection on I_{MOE} and $I_{SWS,AMP}$ resulted in very similar response surfaces for volume and MOE, as well as non-

target wood traits. For $I_{SWS,AMP}$, antagonistic selection resulted in negative genetic gains for either volume or MOE when the value of a standard deviation for that trait was reduced below 0.3. However, the proportional economic weight for volume could be reduced to 0.7 such that no loss occurred for MOE, while still obtaining 32 percent gain for volume (a loss of only 3 percent). Likewise, w_{MOE} could be reduced to 0.70 with only a 2 percent loss from maximum gain for MOE. However, gains for either MOE or volume reduced rapidly with further decreases in the economic weights.

Obtaining significant genetic gains for both volume and MOE required more moderate proportional economic weights ($w_{VOL} \sim 0.5$ to 0.6) to be used, and resulted in large reductions from maximum gain for either trait. For example, Table 3.5 shows that using proportional weights of 0.5 for both traits provided the objective: $H_{MOE} = 0.0319VOL + 1.0638MOE$. The selection index using indirect wood quality criteria that maximized R_{HI} for this objective was: $I_{MOE} = 0.0151VOL + 0.3111SWS + 0.0464AMP$. Selection based on this index resulted in genetic gains of 20.8 and 9.5 percent for volume and MOE, respectively. This equates to a loss of 13.7 percent from maximum gain for volume and a loss of 12.2 percent from maximum gain for MOE.

Selection based on $I_{SWS,AMP}$ resulted in positive correlated responses for other solid wood traits. Correlated responses for MOR and DEN remained fairly constant around 10 and 5 percent, respectively, but diminished gradually towards zero as the proportional weight for volume increased above 0.5.

3.3.4 *Simultaneous selection for volume and MOR*

Clonal selection based on an index containing volume and MOR (I_{MOR}) resulted in correlations with the objective (H_{MOR}) that ranged from 0.88 to 0.84 with increasing weight on volume (Figure 3.4). The most efficient selection index based on indirect selection criteria for improving MOR included both SWS and AMP ($I_{SWS,AMP}$). This index resulted in correlations with H_{MOR} that ranged from 0.72 to 0.84. Therefore, efficiency of $I_{SWS,AMP}$ relative to I_{MOR} ranged from 0.82 to 1.00. Neither SWS nor AMP was solely sufficient for improving H_{MOR} as indicated by the low estimates of R_{HI} for I_{SWS} and I_{AMP} when lower economic weights were placed on volume (Figure 3.4).

Response surfaces for direct (I_{MOE}) and indirect ($I_{SWS,AMP}$) index selection were similar (Figure 3.5 and Figure 3.6). Compared to MOE, gain for MOR resulting from selection on $I_{SWS,AMP}$ was fairly insensitive to changing economic weights, diminishing gradually from +10.3% towards zero as the value for a standard deviation of volume increased. Reducing w_{VOL} below 0.6 generated additional marginal gains for MOR but required large losses in volume gain. Correlated responses for wood density closely followed the response surface for MOR, while correlated responses for MOE were highly sensitive to changes in the economic weights, ranging from +16 to -7 percent. Interestingly, when selection was based on $I_{SWS,AMP}$, inclusion of MOR in the objective for clonal selection did not improve gains for MOR above those obtained by including MOE in the objective. However, using H_{MOR} did result in less drastic losses in volume gain as increased emphasis was placed on wood quality.

3.3.5 *Simultaneous selection for volume and density*

Clonal selection based on an index containing volume and density (I_{DEN}) resulted in correlations with the objective (H_{DEN}) that ranged from 0.93 to 0.84 with increasing weight on volume (Figure 3.7). The selection index using only AMP as an indirect selection criteria (I_{AMP}) resulted in correlations with H_{DEN} that ranged from 0.85 to 0.84. Thus, the relative efficiency of I_{AMP} ranged from 0.91 to 1.00. SWS was not useful as a selection criterion for H_{DEN} as can be seen by the very poor accuracy of I_{SWS} when lower value was placed on a genetic standard deviation for volume (Figure 3.7). The correlation between I_{SWS} and H_{DEN} when the proportion economic weight on volume was 0 was primarily due to the positive correlation between volume in the index and density.

Again the response surfaces between the objective (H_{DEN}) and the best performing indirect selection index (I_{AMP}) were similar (Figure 3.8 and Figure 3.9). Gain for wood density resulting from selection on I_{AMP} was fairly insensitive to changes in the economic weights, approaching 0 gradually as w_{DEN} approached 0. Setting proportional economic weights to 0.5 for both volume and density resulted in 73 percent of the maximum genetic gain for both traits (Figure 3.9). Using these weights also resulted in no significant negative response for MOE. Further increasing the value of a genetic standard deviation for density resulted in marginal gains for density at the cost of large losses in gain for volume. Correlated response for MOR closely followed the response surface for density.

3.4 Discussion

Parameters estimated in this study suggest that a large amount of gain can be obtained in loblolly pine plantations from deployment of clones selected either for growth or wood quality traits. The moderately high clonal repeatability estimated for volume (0.69) in this study corresponds well with the range of estimates (0.60-0.70) reported by Isik et al. (2003) and Isik et al. (2005). The considerable variation found among clone mean volume and the large estimates of genetic gain resulting from clonal selection are also closely supported by these studies. High clonal repeatabilities estimated for wood quality traits in the current study are also promising as long as rapid and reliable indirect selection criteria are available. Fortunately, stress wave speed and Resistograph amplitude values provided quality indirect measurements for the assessed wood quality traits as indicated by the striking similarity of response curves between direct and indirect index selection. The greater variation found among clone means for MOE compared to other solid wood traits is supported by previous studies (Kumar et al. 2002, Chauhan and Walker 2006), and as a result, MOE appears to be the easiest trait to improve by the use of clonal selection.

While the estimated gains from clonal selection for single traits are promising, the capacity of clonal index selection to yield simultaneous improvement for growth and solid wood traits is largely dependent on the genetic correlations among the traits in the aggregate genotypic value. Negative correlations of a sufficient magnitude among growth and solid wood quality traits would result in antagonistic selection that could limit simultaneous improvement. Negative additive genetic correlations have been reported between wood density and growth traits (Kumar et al. 2002, Isik and Li 2003, Myszewski et al. 2004). Negative additive

genetic correlations were also reported for breast height diameter with both modulus of elasticity and modulus of rupture (Kumar et al. 2002, Kumar 2004). However, these genetic correlations are often associated with large standard errors such that general inferences about the relationships between growth and solid wood properties at any particular age are difficult.

Out of the wood traits assessed in this study, negative genetic correlations with growth traits were estimated only for MOE. It is possible that the number of rings contained in the bending samples, a function of diameter growth, may at least partially account for this negative relationship, as an ANOVA performed in Chapter 2 identified the number of rings in the sample as a major cause of variation for MOE. This effect is already being considered in optical lumber grading systems and procurement activities, in which the number of rings per inch is regarded to be an important criterion for wood quality.

As a result of the negative genetic correlations between MOE and growth traits, the wood trait that was easiest to improve by single trait clonal selection is also the most difficult to improve simultaneously with volume. Only a narrow range of moderate proportional economic weights resulted in significant genetic gain for both MOE and volume, but it appears that this simultaneous gain will come at a large cost to optimal gain for both traits. This result is somewhat unfortunate as it has been repeatedly recommended that selection for improving solid wood quality should focus directly on this mechanical wood trait (Tsehaye et al. 2000a, Lindstrom 2002, and others).

One option if confronted with this antagonistic selection for both volume and MOE would be

to use restricted selection indices to maximize gain for one trait under the constraint that no loss occurs for the other. The methodology for deriving these index weights to meet this objective was presented by Cunningham et al. (1970). It was demonstrated in the current study that an index yielding no loss for MOE did not require a large sacrifice for volume gain (Table 3.5). Alternatively, if positive gains for both volume and wood quality traits are desired, then desired gain indices could be used to derive the economic weights in the traits in the aggregate genotypic value that would yield specified gains for those traits (Baker 1986). Practical desired gains from clonal index selection can be approximated by examining the selection response surfaces presented in this study, assuming that the underlying parameter estimates are accurate.

Although MOR and density were relatively genetically independent of growth traits, potential gains for these wood traits may be largely overshadowed by the potential gains for volume. Due to the considerably lower variation among clone means for MOR and density relative to volume, large increases in the economic weights for these wood traits will result in small improvements in wood quality compared to large reductions in volume. Therefore, if there is a large value differential that emphasizes volume production as there has been in tree improvement programs for loblolly pine (Li et al. 1999), then these large reductions in volume cannot be rationalized, as the economic weight for volume would dominate the results of index selection.

Positive correlations among MOE, MOR, and density facilitated the improvement of all of these traits to some degree by including only one of the wood traits in the objective for clonal

selection. Strong positive correlations have been found among these wood traits in previous studies (Kumar et al. 2002, and others). MOR was especially strongly correlated with both MOE and density, and thus exhibited large correlated responses from selection targeting either of these traits. For this reason, it does not appear that there is a large advantage for specifying MOR in the objective for clonal selection so long as one of these other solid wood traits is being targeted for improvement.

In general, a proportional economic weight for volume of 0.7 resulted in no large loss for MOE while providing moderate gains for other wood traits and nearly optimal gains for volume. The aggregate genotypic values, selection indices, and corresponding gains based on this proportional economic weight are given in Table 3.5. Alternatively, economic weights of 0.5 for volume provided a compromise that resulted in moderate but suboptimal gains for both wood quality and growth traits in the genetic values (Table 3.5). However, determination of the amount of emphasis to place on growth as opposed to wood quality traits will have to be based on the specific attributes and objectives of the growers.

3.5 Conclusion

Simultaneous improvement for both solid wood quality and growth can be obtained by index selection of clones. Fortunately, Fakopp stress wave speed and Resistograph amplitude values provide highly efficient indirect measurements for solid wood traits when used in these selection indices. However, negative genetic relationships between MOE and growth traits and the lower responsiveness of MOR and density to clonal selection suggest that substantial genetic gains for solid wood quality from index selection will come at an

undesirable loss of volume gain. Sufficient economic value will have to exist for wood quality traits to justify obtaining non-optimal gains for volume. If there is a large value differential favoring growth, then data provided by amplitude and stress wave speed could be used to restrict selection indices such that gain for volume is maximized at no loss in wood quality. Even with such a restriction, deployment of the selected clones would still provide greater uniformity of the wood produced.

3.6 Acknowledgements

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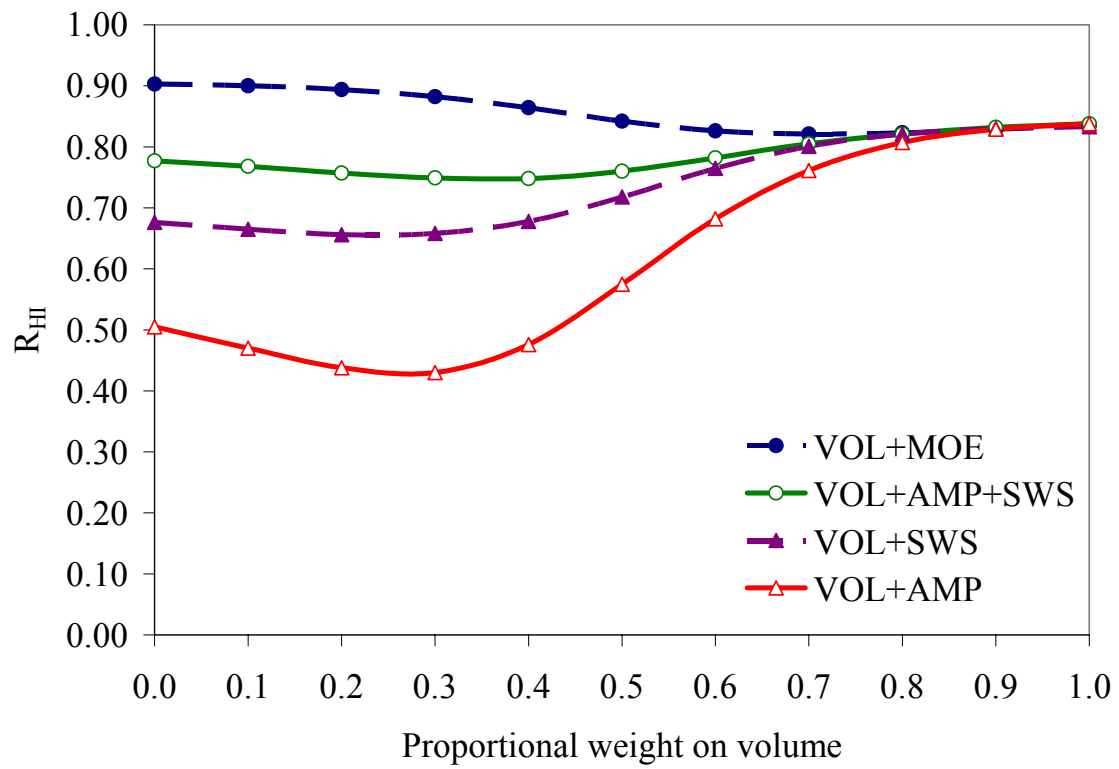


Figure 3.1. Correlations between the aggregate genotypic value H_{MOE} and fitted selection indices over a range of economic weights.

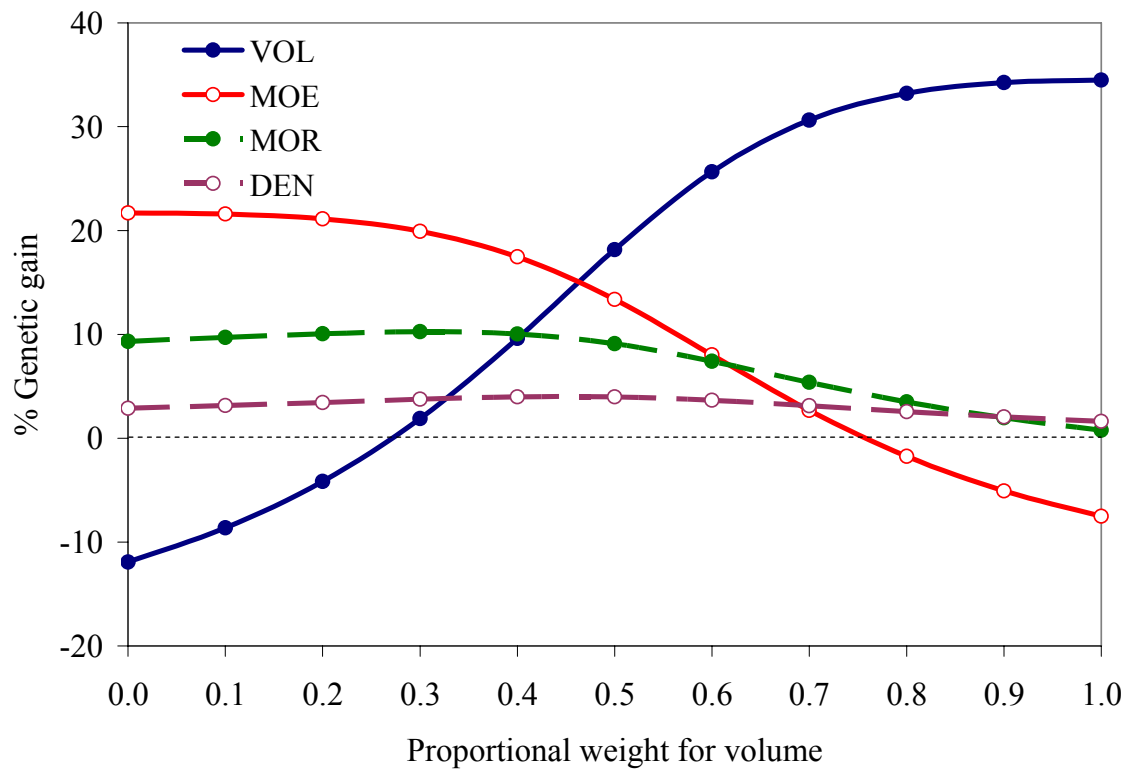


Figure 3.2. Selection response surfaces for individual traits resulting from selection on I_{MOE} to improve H_{MOE} . Dashed lines indicate correlated responses of non-target wood traits.

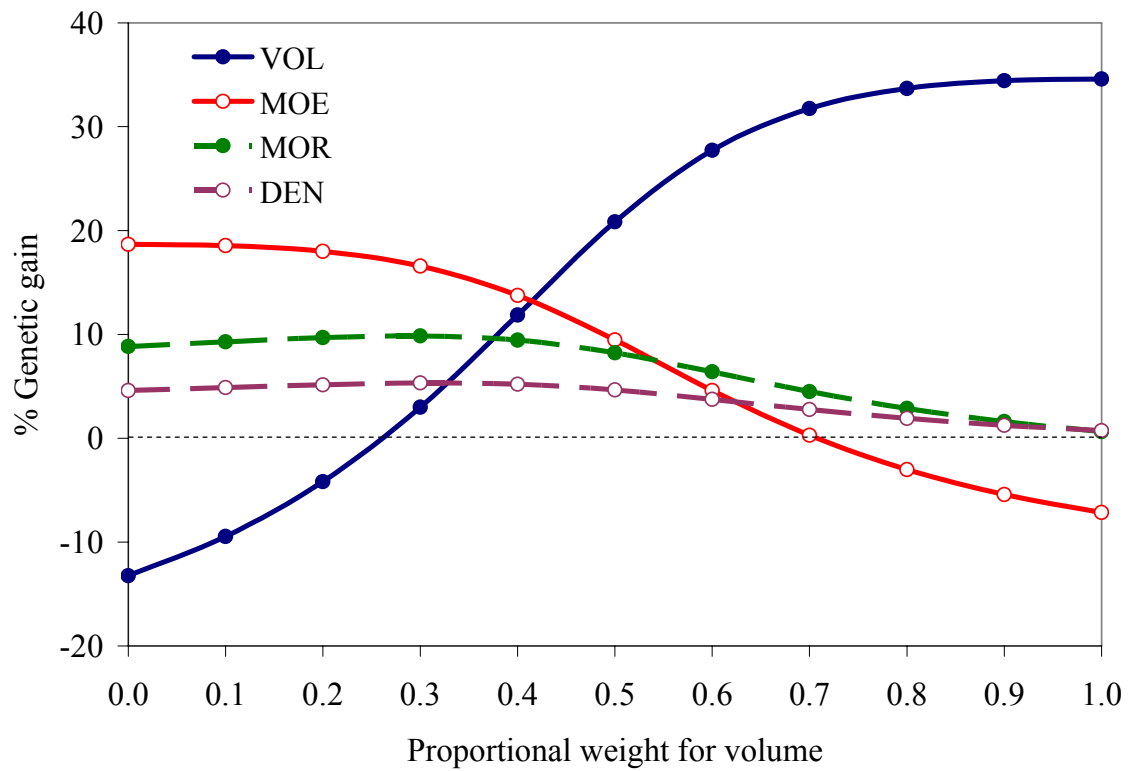


Figure 3.3. Selection response surfaces for individual traits resulting from selection on $I_{SWS.AMP}$ to improve H_{MOE} . Dashed lines indicate correlated responses of non-target wood traits.

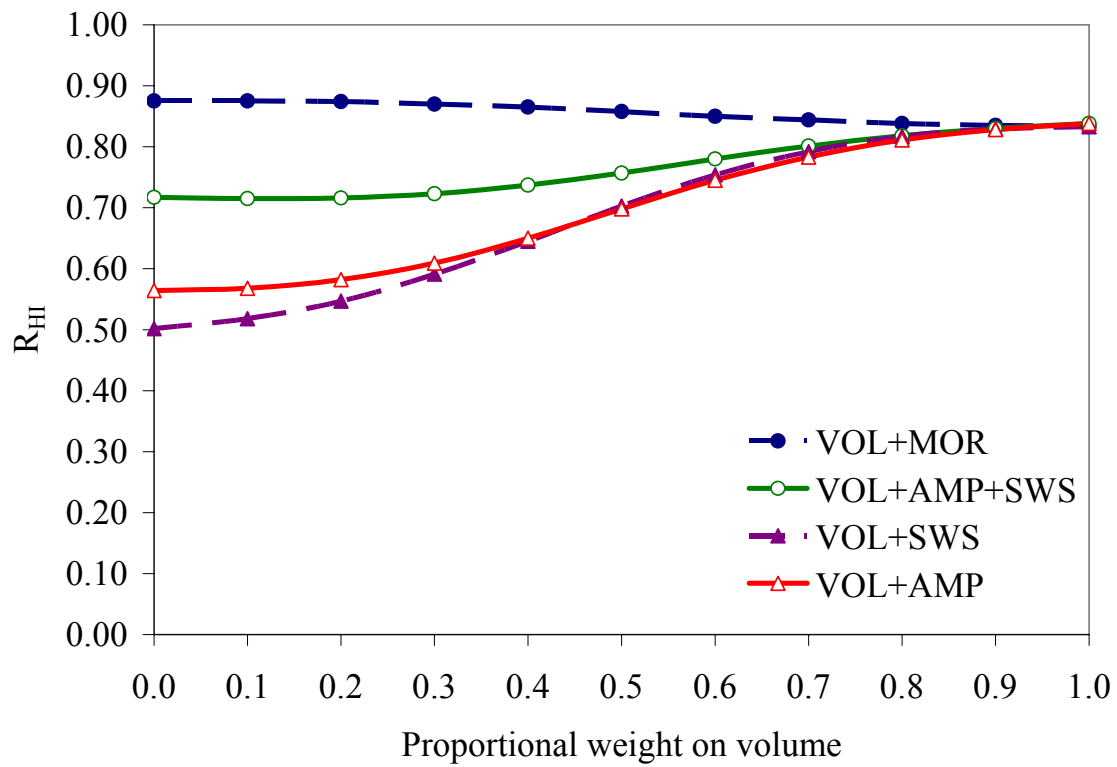


Figure 3.4. Correlations between the aggregate genotypic value H_{MOR} and fitted selection indices over a range of economic weights.

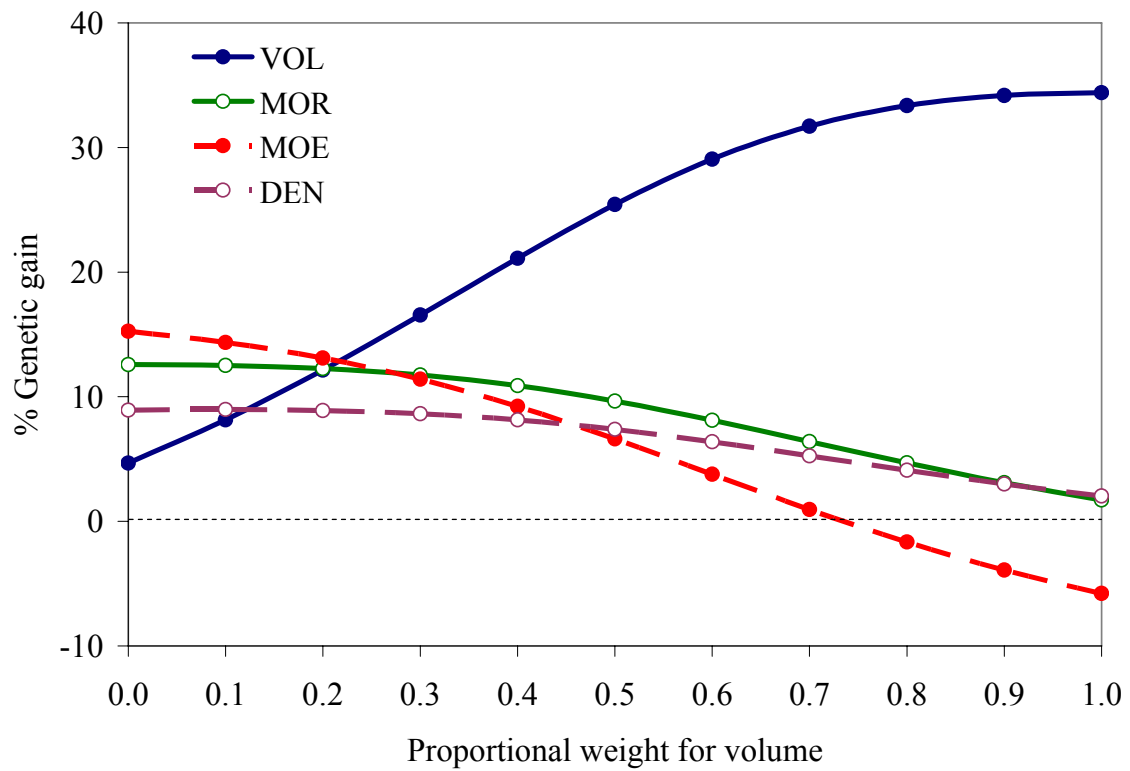


Figure 3.5. Selection response surfaces for individual traits resulting from selection on I_{MOR} to improve H_{MOR} . Dashed lines indicate correlated responses of non-target wood traits.

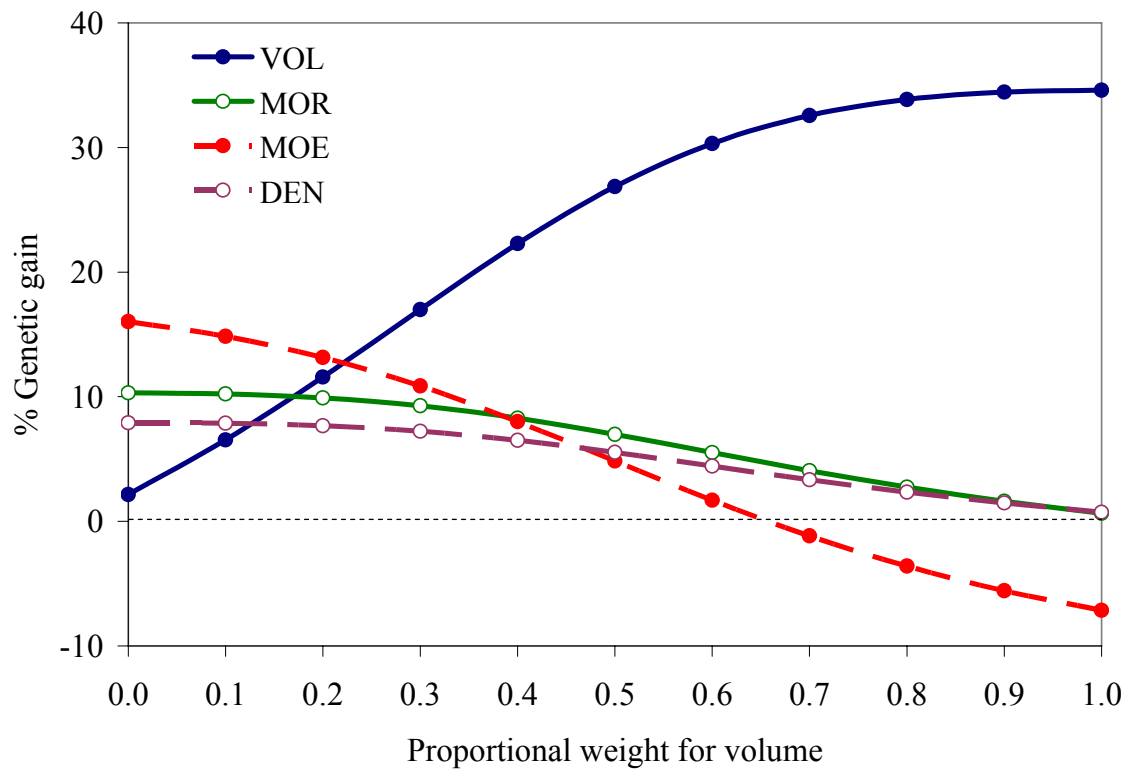


Figure 3.6. Selection response surfaces for individual traits resulting from selection on I_{SWS_AMP} to improve H_{MOR} . Dashed lines indicate correlated responses of non-target wood traits.

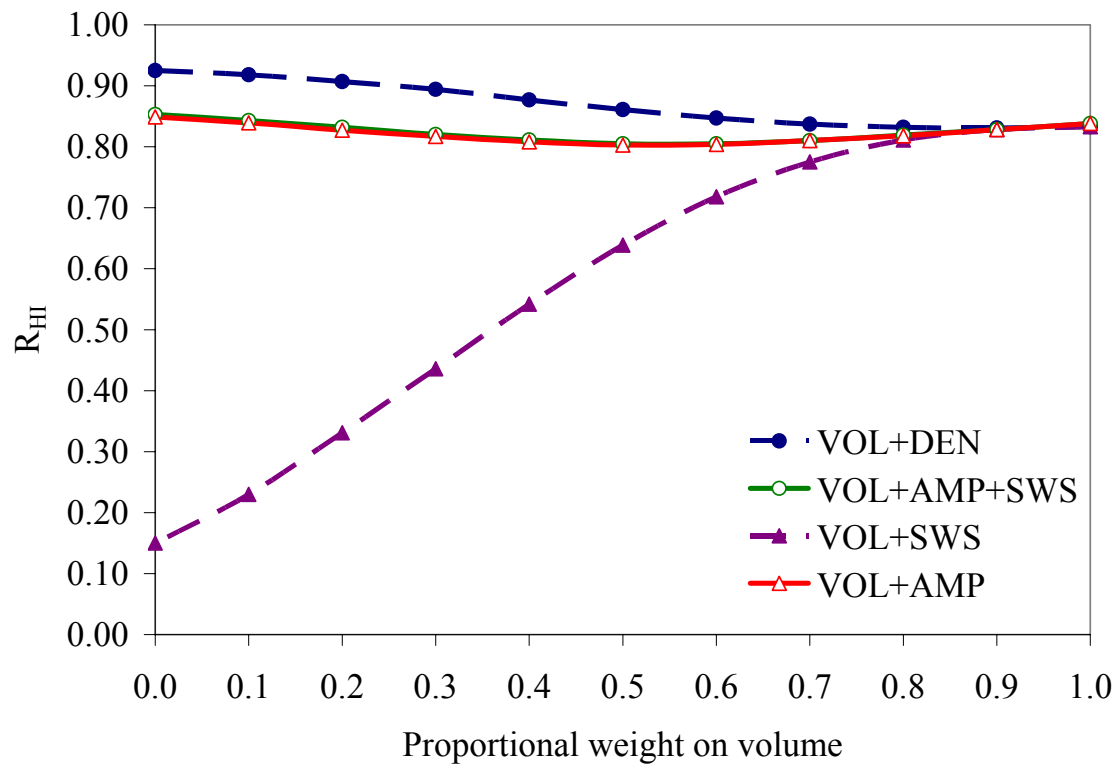


Figure 3.7. Correlations between the aggregate genotypic value H_{DEN} and fitted selection indices over a range of economic weights.

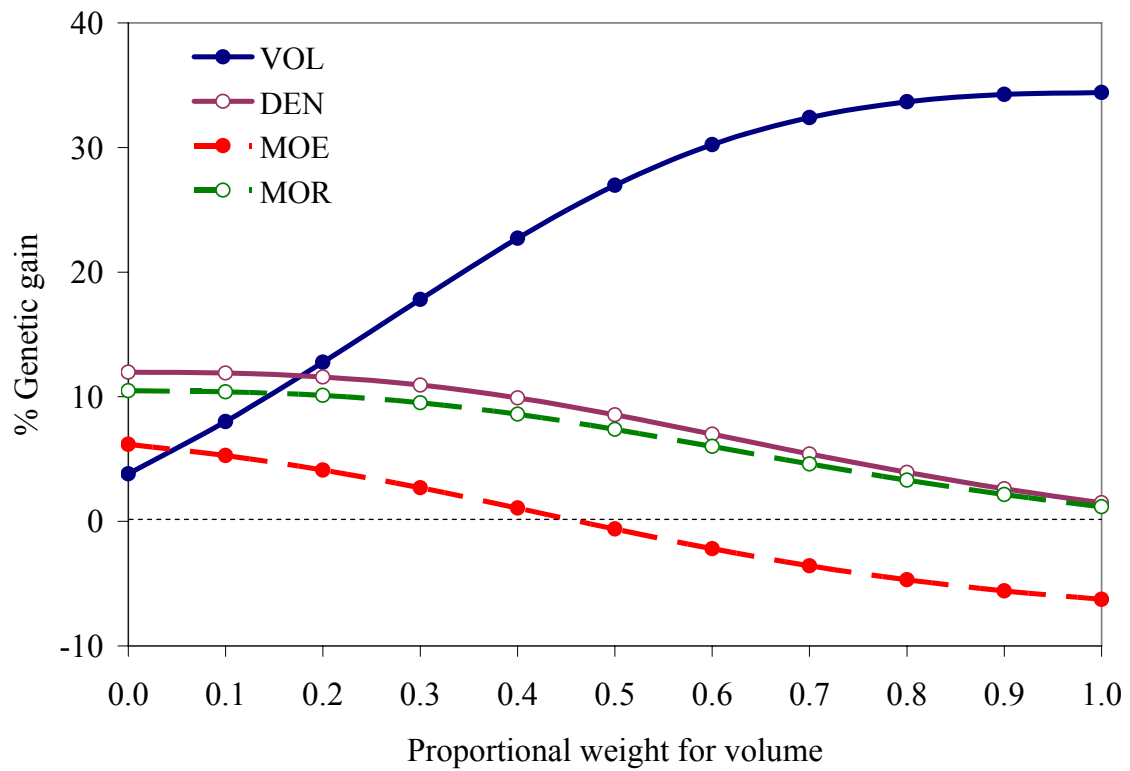


Figure 3.8. Selection response surfaces for individual traits resulting from selection on I_{DEN} to improve H_{DEN} . Dashed lines indicate correlated responses of non-target wood traits.

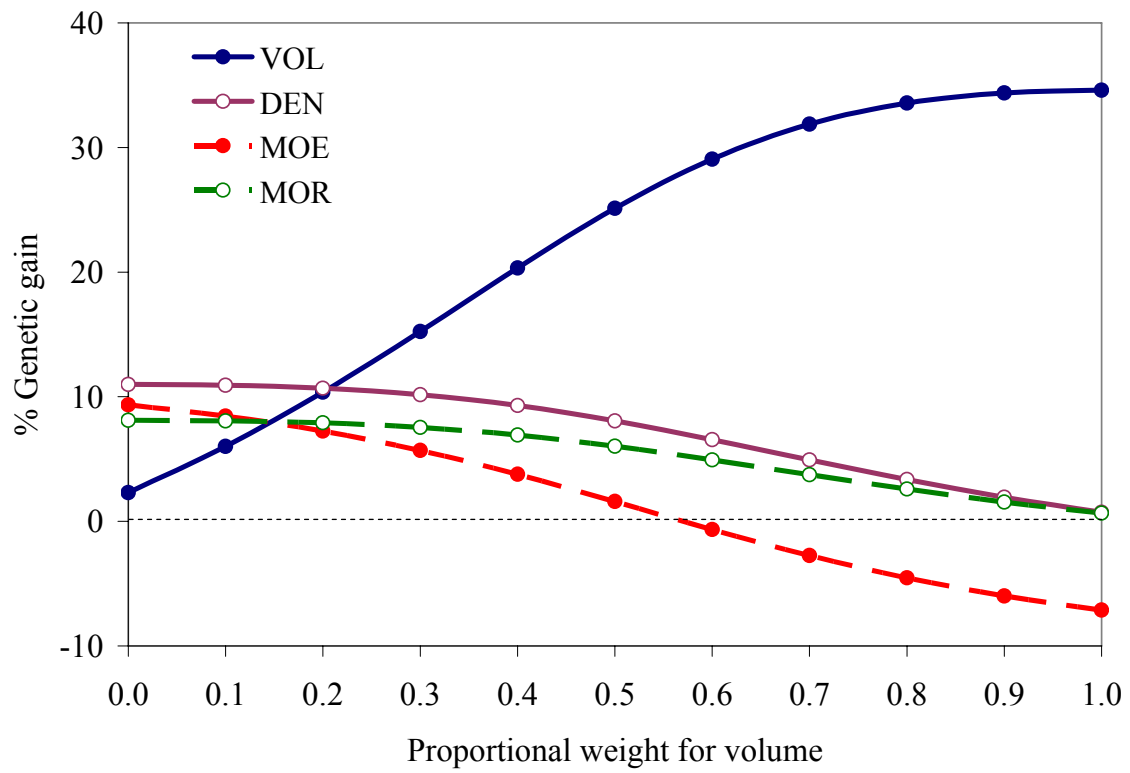


Figure 3.9. Selection response surfaces for individual traits resulting from selection on I_{AMP} to improve H_{DEN} . Dashed lines indicate correlated responses of non-target wood traits.

Table 3.1. Summary of the results from fitting the univariate mixed models

Trait	$\sigma_{C(F)}^2$	σ_E^2	$CV_{\bar{C}(F)}$	$H_{\bar{C}(F)}^2$
VOL (dm ³)	245 (63.3)	649 (56.8)	18.6%	0.69 (0.06)
THT (m)	0.25 (0.07)	0.78 (0.07)	4.7%	0.66 (0.07)
DBH (cm)	1.12 (0.27)	3.40 (0.23)	8.1%	0.68 (0.05)
MOE (GPa)	0.22 (0.08)	0.29 (0.05)	13.2%	0.82 (0.10)
MOR (MPa)	9.12 (3.95)	16.8 (3.15)	8.7%	0.77 (0.12)
DEN (kg/m ³)	357 (125)	375 (71)	4.0%	0.85 (0.08)
SWS (km ² /s ²)	1.13 (0.20)	1.18 (0.08)	13.6%	0.85 (0.07)
AMP (%)	6.4 (1.65)	26.1 (1.77)	11.9%	0.60 (0.03)

Note: parameter estimates are followed by their standard errors in parentheses.

Table 3.2. Clone mean phenotypic (above diagonal) and genetic (below diagonal) correlations among growth and wood quality traits.

	Growth Traits			Wood Quality Traits				
	HT	DBH	VOL	MOE	MOR	DEN	AMP	SWS
HT	-	0.68 (<.0001)	0.79 (<.0001)	-0.14 (0.42)	-0.02 (0.91)	0.18 (0.29)	0.40 (0.02)	0.09 (0.60)
DBH	0.72 (0.09)	-	0.91 (<.0001)	-0.28 (0.10)	-0.19 (0.26)	0.04 (0.82)	0.47 (<.01)	-0.29 (0.09)
VOL	0.83 (0.06)	0.98 (0.07)	-	-0.27 (0.11)	-0.14 (0.42)	0.09 (0.60)	0.16 (0.42)	-0.19 (0.25)
MOE	-0.21 (0.31)	-0.48 (0.30)	-0.35 (0.31)	-	0.74 (<.0001)	0.20 (0.25)	0.05 (0.87)	0.67 (<.0001)
MOR	0.10 (0.35)	0.04 (0.37)	0.14 (0.37)	0.70 (0.16)	-	0.54 (<.001)	0.33 (0.04)	0.50 (<.01)
DEN	0.29 (0.28)	0.18 (0.30)	0.21 (0.30)	0.25 (0.25)	0.77 (0.17)	-	0.70 (<.0001)	0.08 (0.63)
AMP	0.16 (0.22)	0.09 (0.17)	0.10 (0.21)	0.45 (0.30)	0.68 (0.31)	0.99 (0.21)	-	-0.03 (0.84)
SWS	0.35 (0.15)	-0.11 (0.14)	-0.04 (0.19)	0.73 (0.19)	0.50 (0.29)	-0.03 (0.28)	0.01 (0.15)	-

HT = total height, DBH = diameter at breast height, VOL = inside bark stem volume, MOE = modulus of elasticity, MOR = modulus of rupture, DEN = wood density, AMP = amplitude, SWS = stress wave speed.

Note: Genetic correlations are followed by their approximate standard errors and phenotypic correlations are followed by corresponding p-values.

Table 3.3. Direct responses (on diagonal) and correlated responses (off diagonal) from single trait selection ($i = 2.665$).

Selection Criteria	Response			
	VOL	MOE	MOR	DEN
VOL	+34.5%	-6.03%	+1.51%	+1.89%
MOE	-11.2%	+21.7%	+9.42%	+2.94%
MOR	+4.56%	+15.3%	+12.6%	+8.93%
DEN	+6.67%	+5.58%	+10.4%	+11.9%

Table 3.4. Correlated responses from single trait selection based on indirect criteria for wood quality traits ($i = 2.665$).

Selection Criteria	Response			
	VOL	MOE	MOR	DEN
SWS	-1.52%	+16.2%	+6.63%	-0.36%
AMP	+3.19%	+8.35%	+7.59%	+9.89%

Table 3.5. Aggregate genotypic values and clonal selection indices for various proportional economic weights on volume ($i = 2.665$).

a) $w_{VOL} = 0.7$

Genotypic Value (H)	Selection Index (I)	Percent Gain			
		VOL	MOE	MOR	DEN
$0.045VOL + 0.638MOE$	$0.027VOL + 0.186SWS + 0.017AMP$	31.8	0.3	4.5	2.8
$0.045VOL + 0.099MOR$	$0.032VOL + 0.139SWS + 0.029AMP$	32.6	-1.2	4.1	3.3
$0.045VOL + 0.016DEN$	$0.031VOL + 0.059AMP$	31.9	-2.7	3.7	4.9

b) $w_{VOL} = 0.5$

Genotypic Value (H)	Selection Index (I)	Percent Gain			
		VOL	MOE	MOR	DEN
$0.032VOL + 1.064MOE$	$0.015VOL + 0.311SWS + 0.046AMP$	20.8	9.5	8.2	4.7
$0.032VOL + 0.166MOR$	$0.023VOL + 0.234SWS + 0.067AMP$	26.9	4.8	7.0	5.5
$0.032VOL + 0.021DEN$	$0.026VOL + 0.088AMP$	25.1	1.6	6.0	8.1

a) $w_{VOL} = 0.3$

Genotypic Value (H)	Selection Index (I)	Percent Gain			
		VOL	MOE	MOR	DEN
$0.019VOL + 1.489MOE$	$0.003VOL + 0.436SWS + 0.076AMP$	3.0	16.6	9.9	5.3
$0.019VOL + 0.232MOR$	$0.014VOL + 0.328SWS + 0.105AMP$	17.0	10.9	9.3	7.2
$0.19VOL + 0.021DEN$	$0.026VOL + 0.088AMP$	15.2	5.7	7.5	10.2

Chapter 4

Comparison of Uniformity within Open Pollinated, Full-Sibling, and Clonal Groups of Loblolly Pine:

A Research Note

4.1 Introduction

Economic returns from genetic gains produced in tree breeding programs are realized through the plantation deployment of genetically improved seedlings. Different deployment strategies can be used, which will result in different levels of genetic uniformity. Listed in order from the least uniform populations to the most uniform populations, these deployment strategies include (as listed by McKeand et al., 2003): 1) bulk mixes of seed collected from seed orchards, 2) single open-pollinated families collected from wind pollinated seed orchards, 3) single full-sib families created by controlled breeding, and 4) single clones created through vegetative propagation. For southern pine species, deployment populations have traditionally consisted of open pollinated progeny collected from tested parent clones in production orchards as indicated by the vast majority of company lands planted as open-pollinated family blocks (McKeand et al., 2003).

Methodological improvements in mass controlled pollination have resulted in a move towards operational deployment of single full-sib families, while advancements in rooted cuttings, biotechnology, and tissue culture are opening the way for operational deployment of superior clones. Using mass controlled pollination, some forest products companies have either partially or completely converted their deployment strategy to full-sib family block plantings and have even begun providing seedlings from single well tested full-sib families to the open market. Forestry biotech companies are currently using somatic embryogenesis to mass produce clones such that nurseries can now provide large numbers of seedlings from well tested clones. It is well recognized in theory that deploying such populations of greater genetic homogeneity will provide increased plantation uniformity for economically important

traits. Referring to clonal forestry, Zobel (1992) stated that “of key importance, [is] greater uniformity of trees and of the wood produced.” However, there are few research results quantifying the degree of this uniformity.

Thus far in this thesis, the advantage of clonal forestry has been focused on increased genetic gain in deployment populations. However, this research note utilizes the same clonal field trial to provide an initial evaluation of the uniformity of volume and wood density at different levels of genetic homogeneity. The specific objective were to 1) observe the variation for volume and wood density among open-pollinated progeny, full-siblings, and clonal replicates all derived from one common parent and 2) produce empirical histograms for visual comparison of uniformity. If variation for volume and wood density is substantially reduced at each level of increased genetic uniformity, then deployment programs focused on single full-sib family or single clone block plantings should aid forest companies in producing desirable wood products more efficiently on less land.

4.2 Materials and Methods

4.2.1 *Field Design and Sampling*

A clonally replicated field test was established by rooted cuttings in Berkley County, South Carolina in December 1997. The test contained three full-sib families of loblolly pine that were selected from the North Carolina State University Cooperative Tree Improvement Program on the basis of superior volume production and rust resistance. The field design was a randomized complete block with full-sib families planted as block plots within each replication and a single replicate of each clone planted in each replication as single tree plots

(Figure 4.1). Two of the full-sib families in the test were related by a single parent (Parent A). Parent A is a common orchard clone from which open-pollinated progeny have been widely deployed in operation plantations in the Southeast. The third unrelated full-sib family was not used in this analysis. Open-pollinated progeny of this same parent (Parent A) were also planted as an improved check lot in the field test and were included in this analysis. Therefore, one open-pollinated family, two full-sib families, and 230 clones from these full-sib families were used in this analysis and were all derived from Parent A (see Figure 4.2).

At age 4, total heights and breast height diameters were measured for all trees. After account for mortality, this resulted in 97 progeny being measured from the open-pollinated family, 230 clones being measured from the full-sib families, and 10 ramets being measured for each clone (e.g. the sample size to calculate the pooled variance within clones was 2300). At age 8, total height and breast height diameter measurements were repeated on a sample of 37 progeny from the open-pollinated family, 230 clones from the full-sib families, and 6 ramets from each clone. These diameter and height measurements were used to estimate total inside bark stem volume at age 4 (VOL4) and age 8 (VOL8) based on the volume equation of Geobel and Warner (1966). The form of this equation was shown in Chapter 3. A smaller sample of trees consisting of 18 open-pollinated progeny, 60 full-sib progeny, and 6 replicates of each clones were sampled at age 8 for 12 mm bark-to-bark increment cores at breast height (1.4 m) using gas powered drills. Increment cores were processed into 2 mm radial wood strips and basic density (DEN8) was measured from the radial wood strips using X-ray densitometry methods (see Chapter 1 for details).

4.2.2 Statistical Analysis

For each trait (VOL4, VOL8, and DEN8), fixed effects ANOVA models were fit to remove large scale replication effects and estimate the variance within the open-pollinated (OP) family, fill-sib (FS) families, and clones. The form of this model for FS families and clones is shown in Equation 4.1 below:

$$[\text{Eq. 4.1}] \quad y_{ijk} = \mu + b_i + f_j + bf_{ij} + c(f)_{k(j)} + \varepsilon_{ijk}$$

where y_{ijk} is the individual phenotypic observation, μ is the grand mean, b_i is the effect of the i th block, f_j is the effect of the j th family, bf_{ij} is the interaction effect between the i th block and j th family, $c(f)_{jk}$ is the effect of the k th clone within the j th family, and ε_{ijk} is the environmental error. The variance of ramets within clones was estimated by the error variance of this model, while the variance of progeny within full-sib families was estimated as shown in Equation 4.2:

$$[\text{Eq. 4.2}] \quad (SS_{C(F)} + SS_E) / (df_{C(F)} + df_E)$$

where SS is the sum of squares for clones and error and df is the degrees of freedom. The fixed effect ANOVA model used to estimate the variance of progeny within the OP family is shown in Equation 4.3:

$$[\text{Eq. 4.3}] \quad y_{ij} = \mu + b_i + \varepsilon_{ij}$$

where y_{ij} is the individual phenotypic observation, μ is the grand mean, b_i is the effect of the i th block, and ε_{ij} is the environmental error. The family effect was dropped since the analysis was only concerned with a single OP family. The variance within the OP family was estimated by the error variance of this model.

Estimated variances for each trait within the OP family, FS families, and clones were used as defining parameters to construct normal distributions using PROC CAPABILITY in SAS (SAS Institute Inc. 1999). These normal distributions approximated the underlying empirical distributions of phenotypic observations and were used for graphical comparisons of uniformity among genetic groups. The approximated normal distributions were overlaid with the empirical data to examine the fit of the distributions and assess the validity of the assumption of normality. To create the pooled empirical distributions needed for comparison with the approximated normal distributions, the individual tree observations were converted to deviations from group means.

4.3 Results and Discussion

Variances estimated from fitting the fixed effect ANOVA models are shown in Table 4.1. Approximated normal distributions defined by these parameters provided an excellent fit to the empirical distributions of VOL4, VOL8, and DEN8. An example of the fit between the approximated normal and empirical distributions is shown in Figure 4.3. The only discrepancy between the approximated and empirical distributions was found for DEN8 of progeny within the OP family. This distribution was non-normal due to the small sample size (18 progeny), but it was assumed in this analysis that the population of OP progeny

from Family A was normally distributed with the variance estimated from fitting the ANOVA.

Large increases in uniformity were obtained for basic wood density at age 8 with increasing levels of genetic homogeneity (Figure 4.4). For the open-pollinated progeny of Parent A, 95% of the progeny were within 56.2 kg/m³ of the family mean (Table 4.2). Given that the mean density of this OP family was 382 kg/m³, wood density within a block planting of the OP progeny of Parent A would be expected to range from 325.8 to 438.2. For the superior full-sib families derived from Parent A, 95% of the progeny were within 42.7 kg/m³ of the respective family mean. The mean density of these FS families was 399 kg/m³, such that wood density in a single full-sib family block planting would be expected to range from 356.3 to 441.7 kg/m³. For ramets within clones, 95% of the phenotypic observations were within 28.6 kg/m³ of the respective clone mean. The single best clones in this test had a best linear unbiased prediction of 438.0 (from fitting the linear mixed model in Chapter 1). If this genotype was selected, replicated by vegetative propagation and planted in a clonal block, density in this planting at age 8 would be expected to range from 409.4 to 466.8 kg/m³. Such a vast improvement in both the level and uniformity of wood density resulting from clonal deployment is certainly economically significant, as it would provide wood processors with a consistent high quality wood resource as opposed to the highly variable resource that currently provides the majority of the wood furnish.

Increasing in uniformity with increasing levels of genetic homogeneity was present but more subtle for volume (Figure 4.5 and Figure 4.6). At age 4, there were distinct differences in the

distributions of volume among genetic groups, but by age 8 these differences had been reduced (Figure 4.6). These results suggest that increased uniformity for volume at the end of the rotation resulting from clonal deployment is small compared to increased uniformity for wood properties.

The reason that clones provided much greater uniformity for wood density at age 8 while providing only a slight greater in uniformity for volume at age 8 is related to the heritability of these traits. It is well established in the literature that density is a highly heritable trait, while growth traits such as volume are affected to a greater extent by environmental factors (Zobel and Van Buijtenen, 1989). In general, heritability estimates for wood density average around 0.5 in the literature, while estimates for volume average around 0.2 (Zobel, B. J. and J. P. Van Buijtenen 1989). A similar disparity in the level of genetic control for these traits was seen in Chapter 3. Thus, the majority of the observed variance for volume at age 8 was a result of random environmental factors. The larger difference in uniformity among genetic groups for volume at age 4 is likely a function of competition effects. Since inter-tree competition is less significant at age 4, the heritability for volume should be greater. However, by age 8 crown closure has occurred and trees are competing heavily for both light and soil resources, thereby inflating the micro-site variation. Slightly greater levels of genetic control were indeed found for volume at age 8 in this study.

One drawback of this study is the experimental design. Optimal comparisons of uniformity would be made between block plantings of the OP family, FS families, and clones. Although FS families were planted in blocks in this study, clones were planted as individual tree plots

and were subject to competition with their full-siblings rather than with ramets of the same clone. Differential competition effects on the clone in different replications could have inflated variance within clones, thereby underestimating gains in uniformity from clonal deployment. Similarly, the OP family was planted in individual tree plots randomly distributed throughout the test. While progeny of the OP family were surrounded by their half-siblings, these surrounding trees were related to each other as full-sibs, such that competition effects were more uniform than would be expected in a pure half-sib block. The greater uniformity of competition could have the effect of inflating the covariance among the OP progeny.

4.4 Conclusion

In all cases, observed uniformity increased with increasing levels of genetic homogeneity and the differences in uniformity were quantified. However, the extent of the difference in uniformity varied by trait and by age. Much greater uniformity was observed within clones at age 8 for wood density compared to progeny within either full-sib and open-pollinated families. However, differences in uniformity between clones, full-sib families, or the open-pollinated family were comparatively small for volume, and diminished greatly between age 4 and age 8 as inter-tree competition increased. These results suggest that, while both increased genetic gain and uniformity can be expected as benefits of clonal deployment for highly heritable traits such as wood density, increased genetic gains will likely be the main benefit for low heritability traits such as volume that are greatly effected by the environment.

4.5 Literature Cited

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- SAS Institute Inc. 1999. SAS/STAT Users's Guide Version 8. Cary, N.C.: SAS Institute Inc.
- Zobel, B. J. 1992. Vegetative propagation in production forestry. *Journal of Forestry* 90 , no. 4:29-33.
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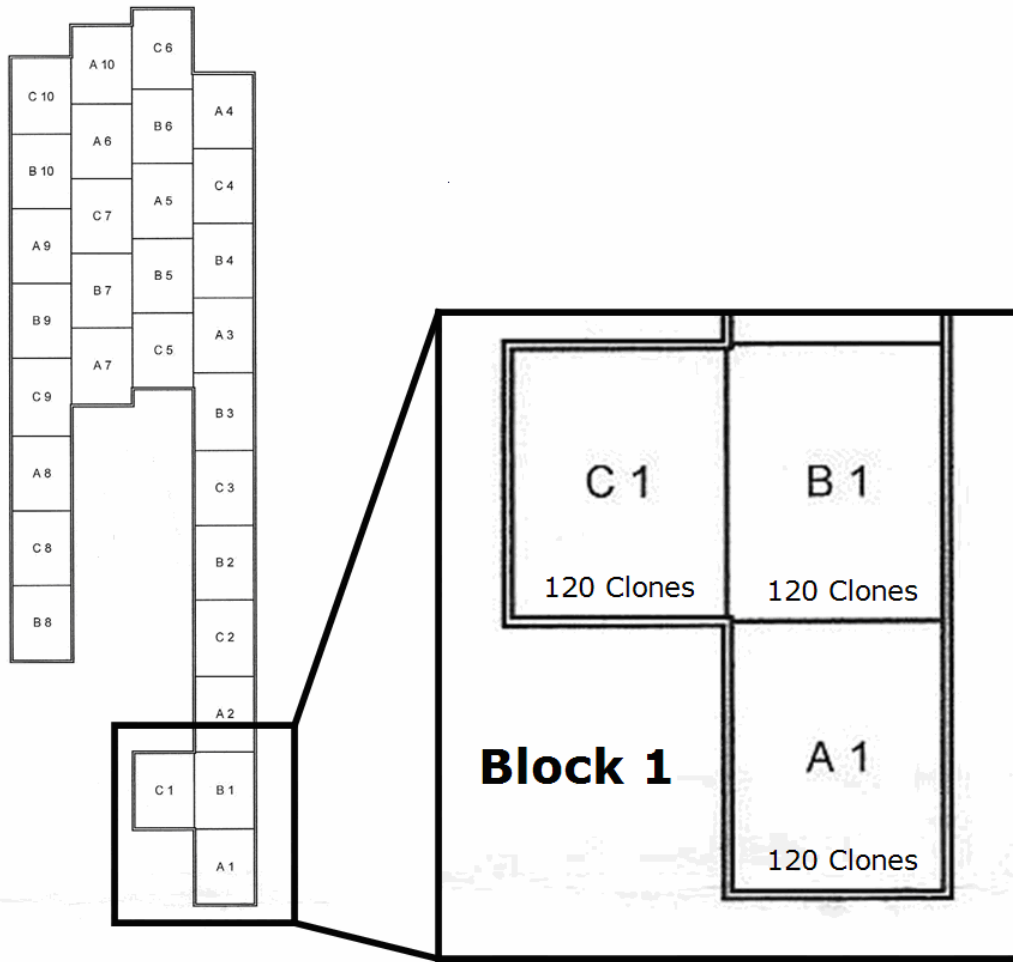


Figure 4.1. Experimental design of the clonal field trial showing an enlarged block 1. The three full-sib families are arbitrarily labeled as A, B, and C.

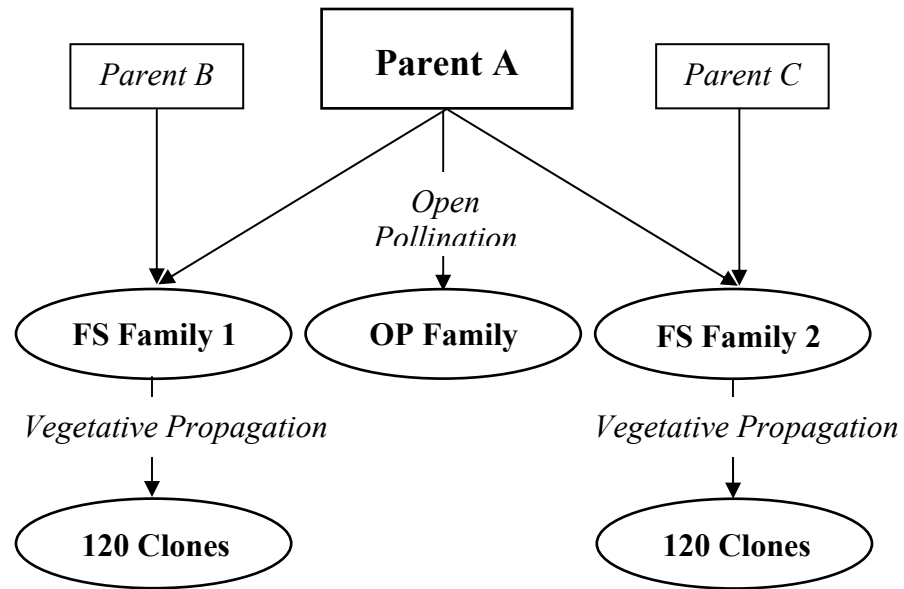


Figure 4.2. Diagram depicting the relationship among genetic groups.

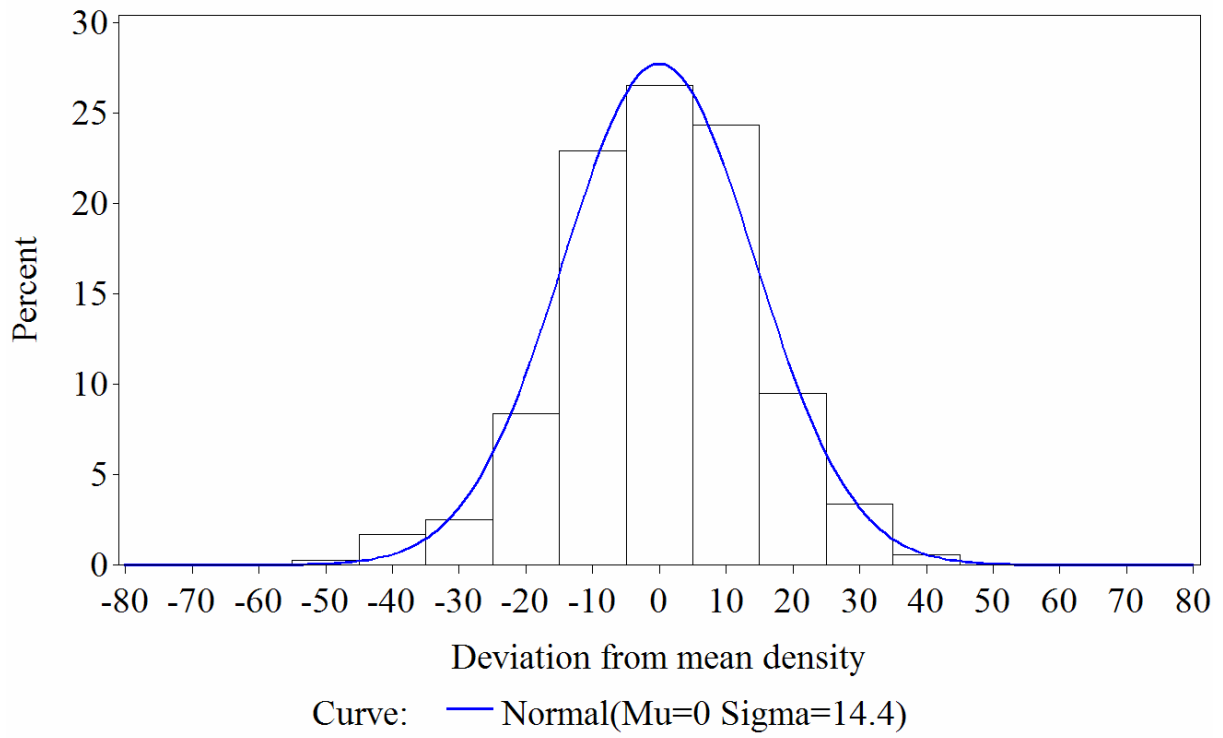


Figure 4.3. Approximate normal distribution of density for ramets within clones (blue line) overlaid with the empirical distribution showing excellent fit.

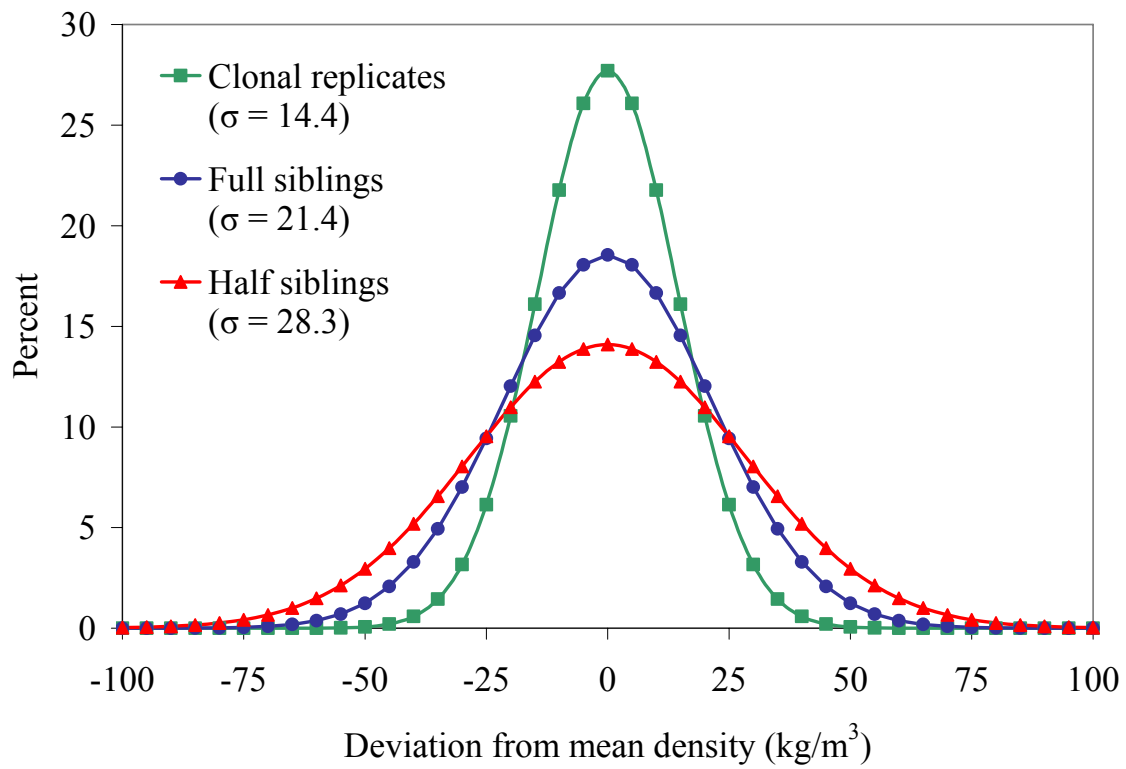


Figure 4.4. Normal distributions representing the observed variance within clones, full-sib-families, and the open-pollinated family (approximately half-siblings) for basic wood density at age 8.

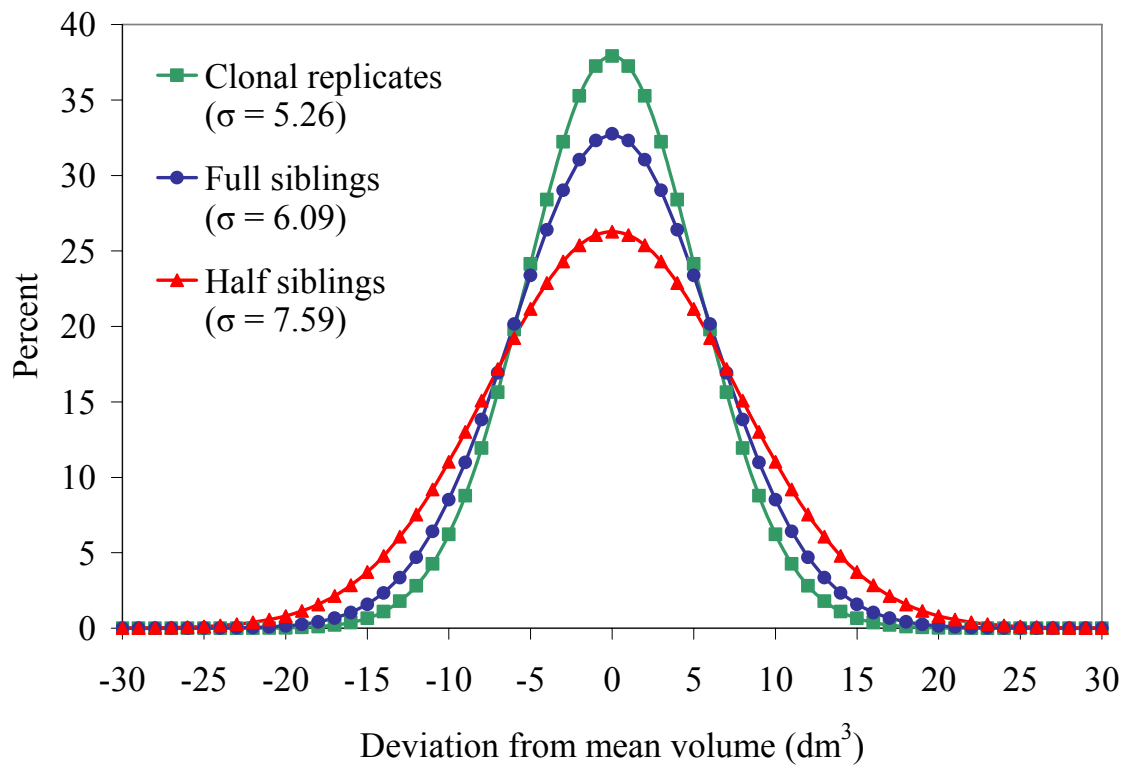


Figure 4.5. Normal distributions representing the observed variance within clones, full-sib-families, and the open-pollinated family (approximately half-siblings) for volume at age 4.

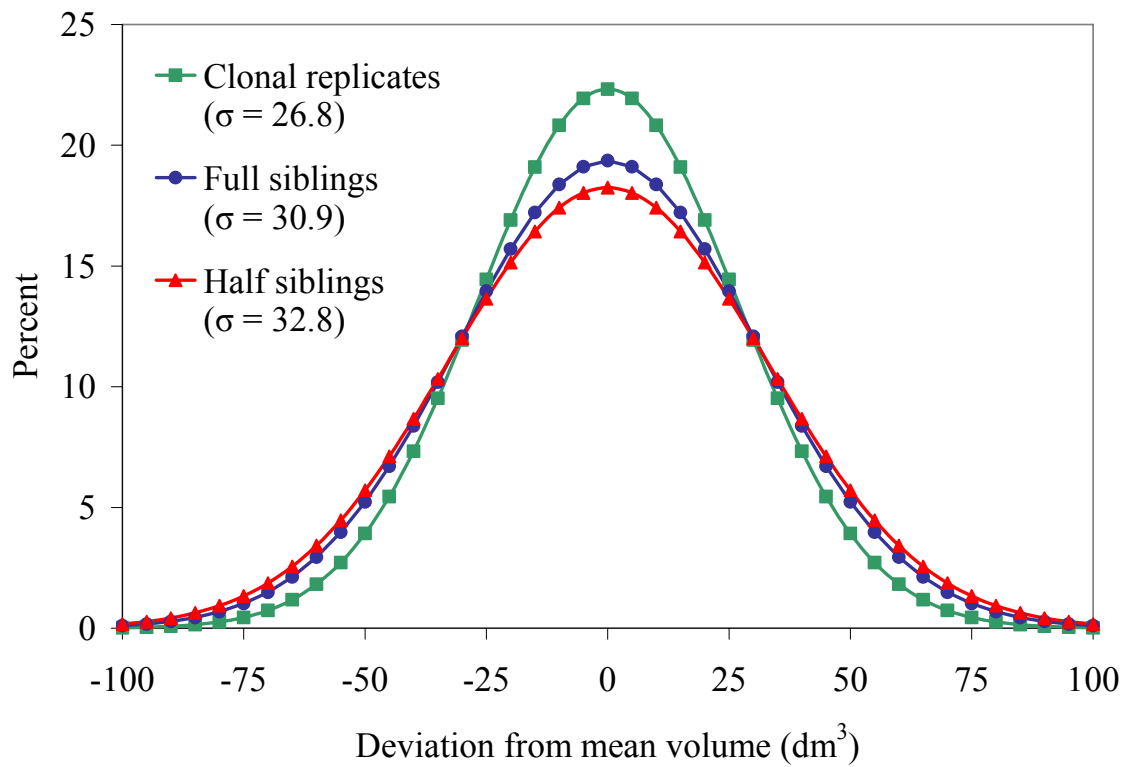


Figure 4.6. Normal distributions representing the observed variance within clones, full-sib-families, and the open-pollinated family (approximately half-siblings) for volume at age 8.

Table 4.1. Phenotypic standard deviations within the open-pollinated family (σ_{WOP}), full-sib families (σ_{WFS}), and clones (σ_{WCL}) and corresponding coefficients of variance.

Parameter	VOL4 (dm³)	VOL8 (dm³)	DEN8 (kg/m³)
σ_{WOP}	7.59	32.8	28.3
σ_{WFS}	6.09	30.9	21.5
σ_{WCL}	5.26	26.8	14.4
CV_{WOP}	37.0%	33.1%	7.1%
CV_{WFS}	35.0%	31.2%	5.4%
CV_{WCL}	27.8%	27.0%	3.6%

Table 4.2. Half-widths of the 95% confidence intervals for distributions of wood density and volume among open-pollinated progeny (approximately half-sib), full-sib progeny, and clonal replicates.

Genetic Relationship	95% Confidence Intervals		
	Age 8 Density ($\pm\text{kg/m}^3$)	Age 4 Volume ($\pm\text{dm}^3$)	Age 8 Volume ($\pm\text{dm}^3$)
Half-Siblings	± 56.2	± 15.1	± 65.1
Full-Siblings	± 42.7	± 12.1	± 61.3
Clonal Replicates	± 28.6	± 10.4	± 53.2

General Summary

This study examined the efficiency of clonal selection for solid wood properties in loblolly pine when selection is based on rapid indirect measurements of these wood properties. Basic wood density, modulus of elasticity (MOE), and modulus of rupture (MOR) are under strong genetic control in this clonal trial as indicated by the high repeatability of clone means. Therefore, it should be expected that selection from such clonally replicated tests would result in genetic improvement of wood quality in deployment populations. This is especially true for MOE, which was found to be the most responsive wood trait to clonal selection as a result of the considerable variance among clone means.

It appears that Resistograph amplitude values can be used successfully to select superior clones for wood density as suggested by high selection efficiencies. This research, in addition to previous research, indicates that amplitude is strongly related with wood density at the genetic level. Drilling with the Resistograph to obtain indirect measurements of wood density is also much cheaper compared to processing and analysis of wood cores. However, numerous sources of environmental variation result in an undesirably low clonal repeatability for amplitude. Friction along the drilling needle was one source of error examined in this study. Further research should focus on identifying and accounting for other possible sources of environmental error in order to improve the value of amplitude as an indirect wood quality selection criterion. If these sources of error can be resolved, Resistograph amplitude values should provide a more efficient method of large scale clonal screening for wood density compared to breast height increment coring. Improvement of drill resistance tools should also focus on greater ruggedness for extensive field use.

TreeSonic stress wave speed measurements also seem to be reliable at selecting superior clones for MOE as indicated by the high selection efficiency. This resulted from a moderately strong genetic relationship between stress wave speed and MOE in addition to highly repeatable clone means for stress wave speed. In fact, stress wave speed was under greater genetic control than those direct measurements of mechanical wood traits that it was intended to predict. Stress wave speed measurements are also very rapidly obtained, reaching a rate of approximately 800 per day with a two person crew. Given the reliability and speed of these stress wave measurements, it appears that acoustic tools can already provided a more efficient method of mass screening clones for mechanical wood properties compared to time consuming and expensive bending measurements. However, stress wave speed provides little value for predicting clone means wood density.

Amplitude and stress wave speed are uncorrelated measurements that provide independent information regarding the variation in wood quality. Therefore, combining amplitude and stress wave speed into a single index for clonal selection will result in greater selection efficiencies for mechanical wood properties, especially MOR. The variance-covariance structure required to derive index weights can be constructed using parameter estimates presented in this research and can assume zero covariance between amplitude and stress wave speed.

While it is clear that wood quality can be improved by selecting clones based on these indirect measurements, clonal selection also needs to consider other economically important traits such as growth. However, it appears that antagonistic selection resulting from negative

genetic correlations between MOE and growth may present a challenge for simultaneous improvement. An additional challenge to simultaneous selection is that clone means for MOR and density are less variable and therefore less responsive to selection compared to volume. Considerable gains for wood quality may result in a fairly large reduction from maximum volume gain, and such a loss in volume gain may be hard to rationalize if there continues to be a large value differential favoring growth.

Large potential genetic gain for wood quality is not the only advantage of clonal forestry. This study confirms that much greater uniformity of wood quality is likely to be achieved in plantations as the level of genetic homogeneity increases. However, increased uniformity for less heritable growth traits may be quite small in comparison.

With continued research and development, indirect wood quality measures should provide genetic gain from clonal selection that surpasses the gain achievable when based on traditional methods of wood sampling and laboratory analysis. This will occur due to increased selection intensity from measuring more clones and greater accuracy of clone means resulting from measuring more ramets per clone. These indirect wood quality measurements can also be used on younger clones due to their non-destructive nature, thereby reducing deployment times. The ability to quickly measure wood quality on such a large scale should provide foresters with information needed to make informed decisions regarding selection and deployment of clones to target specific end product objectives.

APPENDIX

SAS Code for Adjusting Amplitude Profiles

*** Set up blank dataset before running the macro;**

```
DATA data_out;  
  input rep$ clone$ adjamp;  
  datalines;  
RUN;
```

```
%MACRO resi(infile=);
```

*** Step 1: Scan the drilling file for the block and clone identifications and read into new datasets;**

```
DATA rep;  
  infile "&infile"  
  truncover scanover firstobs = 5 obs = 5;  
  input @":" rep $3.;  
RUN;
```

```
DATA clone;  
  infile "&infile"  
  truncover scanover firstobs = 5 obs = 5;  
  input @":" clone $10.;  
RUN;
```

*** Step 2: Read in and modify the raw drilling file to create a dataset with depth and amplitude variables*;**

```
DATA full_resi_profile;  
  infile "&infile"  
  *starts reading file at 8mm to avoid "wood jams"  
  firstobs = 119 delimiter = ",";  
  informat depth amp ;  
  input depth:comma5.1 amp:comma4.1;  
  *removes "air" measurements before drill contacts wood material;  
  if amp < 2 then delete;  
  _TYPE_ = 0;  
RUN;
```

*** Step 3: Use Expand procedure to calculate centered moving means and centered moving minimums*;**

```
PROC EXPAND data=full_resi_profile out=moving_values1 method=none;  
  convert amp = smamp / transform=( cmovave 10 );  
  convert amp = minamp / transform=( cmovmin 100 );  
RUN;
```

*** Step 4: Calculate the mean of the moving minimums for the first half of profile for re-establishing scale*;**

```
DATA depth;
  infile "&infile"
  firstobs = 3 obs = 3 delimiter = ":" missover;
  input trait$ depth $6.;
  ddepth = translate(depth, '.', ',');
  maxdepth = ddepth*10;
  halfdepth = maxdepth/2;
  call symput('halfdepth',halfdepth);
RUN;

DATA half_resi_profile;
  set full_resi_profile;
  if depth > &halfdepth then delete;
RUN;

PROC EXPAND data=half_resi_profile out=moving_values2 method=none;
  convert amp = minamp / transform=( cmovmin 100 );
RUN;

PROC MEANS data = moving_values2 mean;
  var minamp;
  output out = mean_minamp mean = mean_minamp;
RUN;

DATA adjusted_resi_profile;
  merge moving_values1 mean_minamp;
  by _TYPE_;
  diff = smamp - minamp;
  adjamp = diff + mean_minamp;
RUN;

PROC MEANS data = adjusted_resi_profile mean;
  var adjamp;
  output out = adjamp mean = adjamp;
RUN;

* Step 5: Read the new mean adjusted amplitude and associated clone id into a dataset for output;

DATA adjusted_amplitude;
  set rep;
  set clone;
  set adjamp;
  keep rep clone adjamp;
RUN;

DATA data_out;
  set data_out adjusted_amplitude;
RUN;

%MEND;
```

- Macro statement that calls the files and runs the program;

```
%MACRO autorun;  
  %let rep = 6;  
  %do id = 1 %to 93;  
    %resi(infile = C:\Documents and Settings\Jon Tyler\My Documents\School  
Files\Grad Project\Pigeon Pond Data\Breast Height Data\RESI Profiles\Text  
Files\&rep.\&id..txt);  
  %end;  
%MEND;  
  
%autorun;  
  
PROC SORT data = data_out;  
  by rep clone;  
RUN;
```