ABSTRACT

SYKES, ROBERT WAYNE. Genetic Variation and Parameter Estimation of Juvenile Wood Properties in a Diallel Loblolly Pine (*Pinus taeda* L.) Population. (Under the direction of Dr. Bailian Li)

Genetic tree improvement has made substantial gains in productivity, stem straightness, and rust resistance for loblolly pine (*Pinus taeda* L.) in the southern U.S. Improved growth has reduced rotation ages to 20 to 25 years for intensively managed plantations, resulting in a higher percentage of juvenile wood from plantations. Juvenile wood, with low density, shorter tracheid length and higher lignin content, has been shown to reduce yields and increase pulping costs. However, wood properties of juvenile wood can be improved if there is sufficient genetic variation within the breeding population. This study examined the genetic variation and genotype by environment interaction for several important wood properties in loblolly pine, and investigated the rapid assessment of these wood traits by Near Infrared spectroscopy.

Increment cores were collected from fourteen 11-year-old full-sib families from one progeny test. Earlywood and latewood of ring 3 (juvenile wood) and ring 8 (transition wood) for each increment core was analyzed for α-cellulose content (ACY), average fiber length (FLW), coarseness (COA) and lignin content (LIG). Transition wood had significantly higher ACY, FLW and COA and lower LIG than juvenile wood. Latewood of both rings had higher ACY, FLW and COA than earlywood. Loblolly pine families differed significantly for ACY, FLW and COA, but not for LIG. In general, additive genetic effects explained greater percentages of family variation than dominance genetic effects in these traits. For all traits, genetic variation increased from juvenile to transition wood. While weak individual heritabilities

were found for ACY, FLW and COA for juvenile wood, individual and family heritability estimates for transition wood were moderate.

Genetic variation and genotype by environment (GxE) interaction were examined for these juvenile wood properties by combining the data from an additional test site. Families differed significantly for all the chemical and morphological wood properties on both sites. Genetic variation due to general combining ability and specific combining ability was greater in transition wood than juvenile wood. Noticeable family rank changes were observed between two sites for these traits, which were largely due to a significant site by specific combining ability interaction. The family heritability estimates from the combined analysis showed that ACY, FLW, and COA in transition wood were under moderate degrees of genetic control. Favorable genetic correlations with stem straightness were found for ACY and FLW.

Near infrared (NIR) spectroscopy was examined for the rapid estimation of ACY, FLW, and COA. Transmittance measurements of NIR spectra from thin wood wafers cut from increment cores were used to develop calibration models for ACY, FLW, LIG, and COA measured in the laboratory. Calibrations based on one site were generally reliable with coefficients of determination (R^2) ranging from 0.55 to 0.86 for FLW and ACY, respectively. Predicting ring 8 spectra using ring 3 calibration equations may be possible for ACY and COA with R^2 values around 0.60. Predicting the wood properties from one site to the other may be possible for ACY and COA but not for FLW.

Significant genetic variation among and within families and moderate heritabilities from this study suggest that it may be possible to improve wood properties of juvenile wood through tree improvement programs in loblolly pine. Positive genetic correlations of wood density with ACY, FLW and COA indicate that genetic improvement of wood density may improve these important wood and traits. While NIR spectroscopy showed feasibility as a rapid method to predict wood properties for many trees, sampling techniques need to be refined before using NIR to assess wood properties for breeding programs.

GENETIC VARIATION AND PARAMETER ESTIMATION OF JUVENILE WOOD PROPERTIES IN A DIALLEL LOBLOLLY PINE (*PINUS TAEDA* L.) POPULATION

by
ROBERT WAYNE SYKES

A thesis submitted to the Graduate Faculty of
North Carolina State University
In partial fulfillment of the
Requirements for the Degree of
Master of Science

FORESTRY

Raleigh

2004

APPROVED BY:

Dr. Barry Goldfarb	Dr. Gary Hodge
Dr. John Kadla	Dr. Bailian Li Chair of Advisory Committee

DEDICATION

This work is dedicated to my parents, Ronnie and Mollie Sykes, who raised me to work hard and never give up, and to my sister Melissa Sykes for providing encouragement when I needed it most.

BIOGRAPHY

The author is a native North Carolinian born in Durham, North Carolina, in 1979. He attended public schools in Orange County where he played tennis on the Orange High School junior varsity squad and was a member of the National Honor Society. After graduating from Orange High School with honors, he attended North Carolina State University on academic scholarship. Completing his undergraduate work in 2001, he earned a Bachelor of Science in Forest Management with a minor in Environmental Science, graduating Cum Laude. Combining his interests in natural resources and genetics, he entered the Master of Science in Forestry program with the NCSU Tree Improvement Cooperative. As a graduate research assistant, he worked on diverse projects including analyzing the genetic variation of juvenile wood properties in loblolly pine and estimation of wood properties using Near-Infrared spectroscopy. This document is the summation of his graduate research work.

ACKNOWLEDGEMENTS

Many people have been a part of my graduate education, as friends, advisors, and colleagues. I am indebted to my Master's thesis committee: Dr. Bailian Li, Dr. Barry Goldfarb, Dr. Gary Hodge, and Dr. John Kadla. Thank you for your support, guidance and encouragement, for help in the design and implementation of my research project, for answering my questions, and for the necessary motivation when needed.

Patrick Cumbie, Jamie Dunbar, and Dr. Fikret Isik: Thank you for your hard work in material collection for this project. In addition, Dr. Fikret Isik provided invaluable assistance with my analysis and was always willing to help in any way possible. I would also like to acknowledge Bowater Incorporated for their cooperation in allowing us to take samples from the progeny tests on their timberlands.

This study was supported by a grant from the Department of Energy (Grant DE-FC36-01GO10624), NCSU-Industry Cooperative Tree Improvement Program, Department of Forestry, and Department of Wood and Paper Sciences at N. C. State University.

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General Introduction

Forest plantations must be managed efficiently to increase productivity through intensive silvicultural practices and genetic improvement in order to meet the large demand for wood in the United States. Significant improvements in productivity, stem form and disease resistance for loblolly pine (*Pinus taeda* L.) in southeastern U.S have been made in recent years. Improved growth due to intensive management and genetics advancements have allowed rotation ages to be reduced to 20 to 25 years for loblolly pine plantations, compared to 40 to 50 years in natural stands. Consequently, the juvenile wood ratio of plantation trees has greatly increased. When compared to mature wood, juvenile wood has lower wood density, shorter tracheid length, and higher lignin content. Thus, the increased use of juvenile wood has resulted in reduced yields and higher pulping costs for the pulp and paper industry.

Genetic improvement of wood quality has not been a major focus for loblolly pine breeding programs, partly because of the limited genetic information on important wood properties, limited information on genotype by environmental (GxE) interaction, and lack of rapid methods for measuring progeny trees on a large scale. This study examined the genetic variation and genotype by environment interaction for several important wood properties and the rapid assessment of these wood traits by using near infrared spectroscopy for loblolly pine.

Genetic Variation of Wood Properties

Most studies concerning wood properties of loblolly pine have concentrated on the genetic control of wood density. Extensive genetic variation and moderate heritabilities found for specific gravity of loblolly pine suggests that specific gravity of juvenile wood can be improved through tree breeding. Research on the age of transition from juvenile to mature wood for loblolly pine showed that juvenile wood is produced through age six, had a transition period of during ages 6 to 14, and produced mature wood beyond age 14. Wood properties continually change from pith to bark as trees shift from producing juvenile wood through the production of transition wood. Specific gravity and tracheid length generally increase with age, while lignin content decreases. In mature wood, wood properties became more stable from year to year. Although several studies found weak inheritance of cellulose yield of loblolly pine and found that most of the variation was inherited in a non-additive manner, these studies had a limited number of samples. In one study, tracheid length varied widely in 15-year-old loblolly pine, but weak individual tree and moderate family heritabilities were observed.

Genetic variation in juvenile wood properties of loblolly pine will be essential to improve the quality and uniformity of solid and chemical wood products. Specific gravity, cellulose content, lignin content, average fiber length, and coarseness are several key wood properties that affect the quality and quantity of wood produced in plantations or natural stands. Favorable juvenile wood properties may be obtained through a breeding program, if significant genetic variation among families and populations can be found. Extensive genetic variation in holo-cellulose content, α-cellulose content, average fiber length, and lignin

content have been reported in juvenile wood for loblolly pine and other species. This genetic variation allows breeders to select trees with desirable juvenile wood properties to increase yield, improve product properties, and lower pulping costs. Currently there is insufficient information on genetic variation of these important juvenile wood and chemical properties for loblolly pine.

This study consists of three separate chapters to address different questions about wood properties of loblolly pine. The first chapter focused on quantifying genetic variation of these important juvenile wood quality traits in a loblolly pine population. Specific objectives were to determine among and within-family genetic variation in α-cellulose content (ACY), average fiber length (FLW), coarseness (COA), and lignin content (LIG); estimate genetic parameters of earlywood and latewood; and examine correlations among these chemical and morphological wood traits. This information would benefit tree improvement programs and the pulp and paper industry by allowing selection and planting of trees with desirable traits. Genetically improved trees with desirable juvenile wood properties would also allow mills to process wood more efficiently and economically.

GxE Interactions in Wood Properties

The environment in which a tree is grown may have significant impacts on the wood properties in loblolly pine. A population of families must be studied under different environmental conditions to determine the impact of the environmental conditions on wood properties. In order to improve wood properties in a breeding program, tree breeders need to know if the wood properties of families are predictable in different environments.

Determining the magnitude of GxE would help tree breeders and practical foresters decide how to design breeding programs and decide how genotypes should be planted in different environments. If there are no noticeable rank changes or genetic variance changes over different environmental conditions (no important GxE interactions), then family performance can be predicted across a range of environments. On the other hand, if there are significant GxE interactions, e.g., changes in family rank and variance, family performance in wood properties cannot be predicted across environments. Depending on the nature and magnitude of the GxE interactions, different breeding populations may need to be developed. This would increase costs and decrease the efficiency of breeding programs. Although many studies analyzed GxE for growth and other traits, little information has been reported about how chemical and morphological traits interact with the environment for loblolly pine.

The second chapter of this study was to determine the genetic variation and GxE interactions of the chemical and morphological juvenile wood properties in loblolly pine on two test sites. Specific objectives were to compare genetic variation in ACY, FLW, COA, and LIG on two testing sites, and examine GxE interactions of these chemical and morphological wood traits. This information would benefit tree improvement programs and the pulp and paper industry by aiding the design of breeding and testing activities and learning how to planting desirable trees on suitable sites.

Rapid Assessment of Wood Properties

Laboratory measurements of chemical and morphological wood properties involve expensive and time-consuming wet chemistry. In order to assess wood properties of a breeding

population, large numbers of samples must be analyzed. Although a microanalytical method may reduce the tissue required for chemical analysis, the laboratory process is still too labor intensive for screening the large number of progenies for wood properties in a breeding program. If a rapid screening method can be used for these wood property measurements, large populations can be screened for improving those traits in a breeding program.

Near infrared (NIR) reflectance spectroscopy has been used for measuring wood properties in forest trees. This technology allows rapid estimation of wood properties from non-destructive wood samples from trees with minimal expense. The NIR data must be calibrated using wet chemistry to determine chemical wood properties. Once the appropriate calibration equation is established, the wood properties of a sample are determined in a matter of minutes, compared to days using classical methods. In combination with non-destructive sampling, NIR may be used to screen many progeny quickly and at a reasonable cost.

NIR reflectance calibrations for wood and pulping properties have been reported in several studies on a range of species. Calibration involves measuring the NIR spectra of a number samples whose wood properties were known, and then linking the spectra to their individual wood property values. Once the calibration equation was obtained, the model can be used to predict the chemical wood properties for a new sample of wood. In some cases calibration equations may be used to predict wood properties for trees on different sites. Most studies have used NIR reflectance to predict wood properties of wood meal or solid wood.

When NIR reflectance measurements for wood meal were used, the uniformity of particle size becomes very important. Once a sample is milled to sawdust, the sample has to be screened to remove the larger particles to ensure uniformity of the sample. Usually a large wood sample is required and, thus, no detailed measurements of earlywood and latewood were possible for each sample.

There has been little information published regarding the use of transmittance NIR to measure wood properties. One recent study was published using transmittance NIR to successfully predict the lignin content of 12mm increment cores. Several studies using reflectance NIR on solid wood have shown promising results in eliminating the particle size problem. The transmittance NIR allows a smaller sample to be used and, therefore, it may be possible to study individual rings of each sample to examine variation within a tree. NIR reflectance may produce higher prediction standard errors because only the surface of the sample is scanned.

The third chapter was to use transmittance NIR spectroscopy to predict α-cellulose content, average fiber length, and coarseness of juvenile wood and transition wood in loblolly pine. Earlywood and latewood of juvenile and transition wood were examined for NIR predictions of wood properties using 12 mm increment cores of 11-year loblolly pine trees. An accurate calibration with wet chemistry data would allow NIR spectroscopy to be used to screen large samples with non-destructive wood core samples. The ability to assess chemical wood traits rapidly and efficiently would allow tree breeders to improve trees to have high cellulose content, low coarseness and long fibers.

Chapter 1

Genetic Variation of Juvenile Wood Properties in a Loblolly Pine (*Pinus taeda* L.) Progeny Test

1.1 Introduction

The demand for timber production and pulp in the southeast of the United States has increased greatly, as harvesting has decreased on public lands in other areas of the United States [*1*]. As urban sprawl increases and rural land is converted into urban areas, the land available for wood production has decreased. In order to meet the demand for wood in the United States, the remaining timberland must be managed efficiently to increase productivity through intensive silvicultural practices and genetic improvement [*2*].

Intensive silvicultural practices and genetic improvement of trees have increased forest plantation productivity significantly in the southeast U.S. for loblolly pine (*Pinus taeda L.*) [*3*]. Improved growth has allowed rotation ages to be reduced to about 20 to 25 years for intensively managed loblolly pine plantations, compared with 40 to 50 years in natural stands. Consequently, the percent of juvenile wood from plantations has increased [*4*]. Compared to mature wood, juvenile wood has low wood density, shorter tracheid length and higher lignin content [*5, 6*]. The pulp and paper industry has experienced reduced yields and higher pulping costs due to the increased use of juvenile wood [*7*].

Most studies on wood properties have concentrated on the genetic control of wood density [*8-10*]. Zobel and Sprague [*11*] compiled a list of narrow-sense heritabilities for loblolly pine specific gravity that ranged from 0.20 to 1.00. Genetic control concerning transition age from juvenile to mature wood for loblolly pine has been studied. Loo *et al*. [*12*] reported that loblolly pine families produce juvenile wood through age six, with a transition period of ages 6 to 14, and mature wood production beyond age 14. Szymanski and Tauer [*7*] reported

similar results [*12*] for transition age, and Hodge and Purnell [*9*] reported the average transition age for slash pine as 9.4 rings from the pith.

Genetic variation in juvenile wood properties in loblolly pine is very important in improving the quality and uniformity of solid and chemical wood products. Specific gravity, cellulose content, lignin content, average fiber length, and coarseness are several key wood properties that affect the quality and quantity of wood produced either in plantations or in natural stands. Favorable juvenile wood properties may be obtained through a breeding program, if there is significant genetic variation among families and populations. Genetic variation in holo-cellulose content, α-cellulose content, average fiber length, and lignin content have been reported in juvenile wood for loblolly pine and other species [*13-18*]. This genetic variation allows the breeder to select trees with desirable and uniform juvenile wood properties, that will increase yield, improve product properties and lower pulping costs [*19*].

Studies of chemical wood properties have been completed on other species such as the study by Yu *et al*. [*16*], where the physiochemical wood properties of hybrid aspen (*P. tremula x P. tremuloides*) clones were measured. Those authors tested two sites and found the family variance for alkali-soluble lignin accounted for 0.0 % and 48.6% of the total variance on sites. Jett *et al*. [*20*] reported weak inheritance of cellulose yield using a limited number of samples with most of the variance inherited in a non-additive manner. Zobel *et al*. [*21*] found similar results for inheritance of cellulose yield. Loo *et al*. [*12*] studied the inheritance of tracheid length of 15-year-old loblolly pine and found weak individual tree and moderate family heritabilities.

This study was to quantify genetic variation of important juvenile wood quality traits of loblolly pine. Specific objectives were to determine among and within family genetic variation in α-cellulose content, average fiber length, coarseness, and lignin content, study genetic variation of earlywood and latewood and estimate genetic parameters, and examine correlations among these chemical and morphological wood traits. This information would benefit the tree improvement program and the pulp and paper industry by allowing selection and planting of trees with desirable traits. Genetically improved trees with uniform and desired juvenile wood properties would also allow mills to process wood more efficiently and economically [*19*].

1.2 Materials and Methods

1.2.1 Material and data collection

Fourteen full-sib families generated by a six parent half-diallel mating design were tested in the Piedmont of South Carolina. A randomized complete block design with six replications was used in the field. Each full-sib family was laid out in six-tree row-plots for each replication. Wood core samples from one site in Florence Co. (South Carolina) were collected from 11-year-old trees. Wood samples of 12 mm increment cores were taken from each tree at breast height (about 1.30 m) using generator-powered drills. Wood cores having visible limbs, curves, resin pockets, compression wood, or rust infections were avoided. The samples were placed into sealed plastic storage bags and stored on ice in coolers to retain moisture during the material collection.

The bark and cambium were removed from the wood cores, and the cores were split at the pith into two halves. Chemical analysis was done using microanalytical techniques developed by Yokoyama *et al*. [*22*], which allow the rapid characterization of fiber components and morphology of loblolly pine in a large number of samples. Briefly, the techniques involved are extractive removal, holocellulose preparation, α-cellulose and lignin content determination, and average fiber length and coarseness analyses. Nonvolatile extractives greater than 95% were removed from the increment core by four successive two-day acetone extractions [*22*]. Extractives were removed from all cores using the successive acetone method. The increment cores were then soaked in water overnight before ring samples were taken.

Within-core samples were taken from ring three and ring eight to study chemical properties of juvenile wood (ring 3) and transition wood (ring 8), respectively. Thin wafers from ring three earlywood, ring three latewood, ring eight earlywood, and ring eight latewood were taken using a microtome. At least 300-500 mg of each sample were taken from the earlywood and latewood of each. Each sample was oven-dried 12 hours.

1.2.2 Statistical analyses

Earlywood and latewood means of wood traits within each ring were compared using T-tests. Analysis of variance was conducted to compare full-sib families using the GLM procedure of SAS [*23*]. A standard general linear mixed model for diallel analysis [*25*] was used to analyze the data, which included fixed effect of replication, and random effects of general combining ability (GCA), specific combing ability (SCA), their interactions with replication,

plot, and experimental error. Variance components of random effects, which measure the magnitude of variation, were estimated using the SAS PROC MIXED procedure developed for diallel analysis [*25*].

The coefficients of variation for GCA and SCA were estimated by dividing the square roots of the estimates with their means. Family least squares means with 95% confidence intervals were estimated. Earlywood and latewood of ring three (juvenile wood) and ring eight (transition wood) were compared for each wood trait. Using variance components from the mixed model, individual (h^2_i), half-sib family (h^2_{hs}), full-sib family (h^2_{fs}), and within full-sib family (h^2_{wfs}) heritabilities were estimated.

$$h_i^2 = \frac{4\sigma_{GCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2 + \sigma_{plot}^2 + \sigma_e^2} \quad \text{[Eq. 1]}$$

$$h_{hs}^2 = \frac{\sigma_{GCA}^2}{\left(p\sigma_{GCA}^2 + \sigma_{SCA}^2 + \frac{\sigma_{plot}^2}{b} + \frac{\sigma_e^2}{bn}\right)\left(\frac{1}{p-1}\right)} \quad \text{[Eq. 2]}$$

$$h_{fs}^2 = \frac{2\sigma_{GCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2 + \frac{\sigma_{plot}^2}{b} + \frac{\sigma_e^2}{bn}} \quad \text{[Eq. 3]}$$

$$h_{wfs}^2 = \frac{2\sigma_{GCA}^2}{\left(\frac{b-1}{b}\right)\sigma_{plot}^2 + \left(\frac{bn-1}{bn}\right)\sigma_e^2} \quad \text{[Eq. 4]}$$

where σ^2_{GCA} is genetic variance, σ^2_{SCA} is dominance variance, σ^2_{plot} is SCA x replication variance, σ^2_e is error variance, p is # of parents in the diallel, b is # of blocks, and n is # of

trees per family per replication. Product-moment correlations and genetic correlations were estimated among these traits and with wood density [*26*] that was determined by the standard volumetric method. Standard errors of heritabilities and genetic correlations were calculated using the Delta Method [*27*] and ASREML software [*28*].

1.3 Results

1.3.1 Differences of earlywood and latewood, rings and families

Latewood had significant greater ACY, FLW and COA than earlywood in ring three (juvenile wood) (Figure 1.1). The differences between latewood and earlywood for ACY, FLW, and COA were more pronounced in ring eight (transition wood) compared to ring three. Latewood of ring eight had greater ACY, longer average fiber length and greater coarseness than earlywood. FLW for latewood of ring eight was 13.4% longer than earlywood of the same ring. Earlywood and latewood of ring eight were not different for LIG.

Families differed significantly for all the chemical and morphological wood properties at the probability of <0.001 level (data not presented). Family means for FLW ranged from 1.42 (family I) to 1.81 mm (family G) (Figure 1.2). There was considerable variation among families for COA. Family I had the greatest COA (0.336), whereas family G had the lowest value (0.256). Families I and G were consistent in ranking for FLW and COA. Family means for α-cellulose content ranged from 38.8 to 43.3 %, with family D having the highest value and family N the lowest. Very little variation was found among families for lignin.

Family E had the lowest lignin content (29.1 %), while family K had the highest lignin content (30.7 %) (Figure 1.2).

1.3.2 Phenotypic and genetic parameters

Coefficients of genetic variation (CV) for fiber length, cellulose content, coarseness and lignin content were generally large for these traits (Figure 1.3). In general, additive genetic effects (GCA variance) explained greater variation for fiber length, coarseness and cellulose content than dominance genetic effects (SCA variance). The CV of SCA for fiber length was essentially zero for earlywood and latewood of juvenile and transition wood. The CV of GCA was clearly greater than that of SCA for FLW, ACY, and COA for latewood or ring three and ring eight. There was no genetic variance for lignin in juvenile wood, but the CV of SCA for transition wood was higher that of GCA for both earlywood and latewood. In general, GCA variance increased from earlywood to latewood of the same ring and from ring three to ring eight. However, the CV of SCA was inconsistent from one ring to another.

Moderately high family heritabilities were estimated for ACY, FLW and COA (Table 1.1). Weak individual and within full-sib family heritabilities were observed for early wood of ring three. Heritabilities for LIG for earlywood and latewood of ring three were zero due to lack of GCA variance. Latewood heritabilities for ring three were higher than earlywood heritabilities. In contrast to ring three, heritabilities for earlywood and latewood of ring eight were similar but higher for all traits. Among all the traits studied, COA and ACY had the highest heritabilities, whereas lignin was more controlled by non-genetic effects in this study.

The chemical and morphological wood properties had higher heritabilities for transition wood (ring 8) than for juvenile wood (ring 3) (Table 1.2). For example, the family heritability for ACY was 0.38 for juvenile wood, whereas it was 0.55 for transition wood. Heritabilities for ACY and COA were similar and higher than those of FLW, both for ring three and ring eight. Heritabilities for lignin were essentially zero for ring three, but moderate for ring eight. When data from both rings were combined, lower heritabilities were observed for all traits compared to the individual ring data.

Average fiber length had a high genetic correlation with wood density (r_g=0.95) (Table 1.3). Positive and moderate genetic correlations were observed between wood density, cellulose content, lignin content and coarseness. ACY had a high negative genetic correlation with lignin content. Lignin content was also negatively correlated with fiber length. Genetic correlation between FLW and COA was positive and high (r_g = 0.99). Genetic correlations could not be estimated for ring three due to lack of genetic variation.

Wood density of the wood core was phenotypically correlated with cellulose content but not with other traits. Phenotypic correlations between FLW, COA and ACY were significant. None of the traits had significant phenotypic correlation with lignin content.

1.4 Discussion

The chemical and morphological wood properties evaluated in this study are very important for pulp and paper production. Alpha cellulose content relates to the amount of pulp that is obtained from wood. The higher the α-cellulose content in a tree, the more pulp the tree will produce [*29*]. Increasing the amount of cellulose content of wood will reduce pulping costs

and increase the efficiency of the pulp and paper mill. Average fiber length also plays an important role in the pulp and paper industry. Long fibers or short fibers can be favored depending on the product being produced. Long fibers give paper greater tensile and tear strength, for products such as cardboard and paper bags [*30*]. Short fibers are favored for products such as fine printing paper, where surface smoothness is of importance. Coarseness is related to thickness of fiber wall, with thicker fibers corresponding to higher the coarseness values. Lower coarseness values result in better fiber collapse, tighter fiber bonds, and therefore formation of dense paper with a smooth surface. Trees with higher coarseness values yield pulp and paper products with higher bulk, which is beneficial to products requiring higher absorbance and/or higher bending stiffness. One of the most important properties in wood is lignin content. In order to separate the wood fibers, lignin must be removed [*31*]. This requires chemical breakdown of lignin, which is a very expensive process. Reducing lignin content in wood could save processing costs for the pulp and paper industry.

Results from this study showed that latewood had greater cellulose content, longer average fiber length and higher coarseness compared to earlywood of the same rings. Juvenile wood at ring three appeared to be less desirable for these chemical wood traits compared to ring eight, which is considered transition wood rather than mature wood for loblolly pine [*7*]. There was a trend of increasing cellulose content, fiber length and coarseness from juvenile wood to transition wood. Genetic selection based on latewood of the growth ring could be an effective way to manipulate chemical and morphological wood properties.

Considerable genetic variation in the chemical and morphological wood properties was found in both earlywood and latewood, except for lignin. A considerable portion of the genetic variance for cellulose content, fiber length and coarseness was explained by additive genetic effects (CV of GCA, Figure 1.3). Additive genetic variation (GCA) in cellulose content was higher than that found by Jett *et al*. [*20*]. There was little to no GCA variance for lignin for juvenile and transition wood (Figure 1.3). In order to make genetic improvement on lignin, controlled crosses or vegetative propagation techniques should be used to capture non-additive genetic variance [*6*]. Dominance genetic effects (SCA) explained about 50% of total genetic variance for these two traits in transition wood. Lowering lignin content through breeding will involve considerable effort and cost. In this study with limited sample size, selecting the family with the lowest lignin content would cause less than a two percent reduction in lignin when compared to the family with the highest lignin content (Figure 1.2).

Cellulose content had a high genetic correlation with wood density, suggesting that increasing wood density would increase cellulose content (Table 1.3). Similarly, coarseness and fiber length had positive genetic relationships with density. Because wood density can be measured relatively easy and less expensive, it may be possible to use it to improve other traits indirectly. However, wood density was positively correlated with lignin in this study, and selecting for higher wood density might also increase lignin content. Latewood fibers are thicker and longer than earlywood fibers and result in higher α-cellulose content and coarseness values [*32*]. In this study, a high and positive genetic correlation (0.99) was observed between fiber length and coarseness, indicating that long fiber is associated with thicker fiber wall.

Family heritabilities were moderate to high for all chemical and morphological traits, except for lignin content of juvenile wood. Genetic parameters estimated from this study may be imprecise due to a small population size (6 parents and 14 full-sib families). These estimates may also be biased upward because only one test was measured and the genotype by environmental interaction may be confounded in the genetic variance. Nevertheless, these moderate estimates suggested that genetic improvement for cellulose content and fiber length could be realized through genetic improvement. Heritabilities for fiber length from this study were similar to those found by Loo *et al*. [*12*] for loblolly pine. Loo *et al*. [*12*] reported 0.31 and 0.37 for individual heritabilities, and 0.45 and 0.51 for family heritabilities for transition wood at two sites compared to 0.28 in this study (Table 1.1). Increasing cellulose content will result in the production of more paper per cubic meter of wood.

Transition wood heritabilities may be more meaningful than those of juvenile wood, as they are closer to the age (age six) where most selections are made for the North Carolina State University-Industry Tree Improvement Coop [*3*]. As transition wood is formed, the wood properties become more uniform, which may contribute to more accurate estimates of genetic parameters. Juvenile wood (ring 3) seems to be more affected by environmental conditions such as moisture content and resin. For example, expression of genetic differences for lignin content was observed in transition wood, but not in juvenile wood. At ring eight, genetic differences for lignin appeared to be more important in this study.

The combined measure of a whole wood core may not provide meaningful information on genetic variation on these traits. By averaging over rings, the genetic variation of different components of rings, earlywood and latewood cannot be determined. This information is important for selection if one component has higher genetic variation, e. g., wood trait in latewood at ring eight. Considerations should be taken into account before using chemical and morphological wood properties in a tree-breeding program. Costs for sampling each tree from a progeny test and processing wood samples for chemical analysis in the lab can be prohibitively expensive. New methods for measuring chemical traits that are cheaper and less time consuming need to be developed. One such method is using a Near Infrared Reflectance (NIR) instrument to scan the sample to predict the wood properties. The technology has been used on other species, such as eucalyptus, but not for loblolly pine.

Operational use of trees with genetically improved wood properties also poses a problem. Many forest industry companies are selling their land bases for various reasons. The largest gains can be obtained by using the best families with the desired wood properties, if family information in plantations are well documented. While this may be simple on industry land, that is not where all the wood is harvested. In the southeast U.S., private landowners currently provide much of the wood that is used in pulp and paper mills. Genetic differences among families for chemical wood traits provide an opportunity for family forestry. Planting in family blocks will allow the industry to grow individual families for the specific chemical properties needed, but company land bases will need to be maintained.

1.5 Conclusions

In conclusion, considerable genetic variation was detected for all chemical and morphological traits in this loblolly pine progeny test. Heritabilities were moderate to high for all traits, indicating potential for genetic improvement. High positive genetic correlations between cellulose content, fiber length and wood density suggested simultaneous improvement of chemical wood properties and wood density may be possible. Further studies with large population size are on the way to confirm the genetic variation and develop breeding strategies for improving these wood quality traits.

1.6 Acknowledgements

This study was supported a grant from the Department of Energy (Grant DE-FC36-01GO10624), NCSU-Industry Cooperative Tree Improvement Program, Department of Forestry, and Department of Wood and Paper Sciences at N. C. State University. We thank anonymous reviewers for invaluable suggestions on revising the manuscript.

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Table 1.1. Heritabilities and standard errors for fiber length (FLW), α-cellulose content (ACY), coarseness (COA) and lignin content (LIG) traits for earlywood and latewood of ring 3 and ring 8 (3E, 3L, 8E and 8L).

		Ring # and Wood Type			
Heritability[1]	Trait	3E	3L	8E	8L
h^2_i	FLW	0.05 ± 0.08	0.09 ± 0.10	0.23 ± 0.22	0.25 ± 0.20
	ACY	0.07 ± 0.14	0.14 ± 0.17	0.32 ± 0.23	0.36 ± 0.31
	COA	0.08 ± 0.11	0.31 ± 0.22	0.23 ± 0.19	0.22 ± 0.18
	LIG	0	0	0.04 ± 0.11	0.16 ± 0.21
h^2_{HS}	FLW	0.04 ± 0.31	0.52 ± 0.27	0.61 ± 0.20	0.67 ± 0.13
	ACY	0.37 ± 0.44	0.53 ± 0.29	0.72 ± 0.09	0.66 ± 0.15
	COA	0.44 ± 0.34	0.71 ± 0.10	0.66 ± 0.14	0.66 ± 0.14
	LIG	0	0	0.26 ± 0.56	0.54 ± 0.31
h^2_{FS}	FLW	0.26 ± 0.29	0.35 ± 0.31	0.47 ± 0.30	0.58 ± 0.24
	ACY	0.21 ± 0.36	0.37 ± 0.34	0.68 ± 0.20	0.57 ± 0.27
	COA	0.27 ± 0.32	0.66 ± 0.21	0.55 ± 0.25	0.55 ± 0.26
	LIG	0	0	0.13 ± 0.36	0.38 ± 0.38
h^2_{WFS}	FLW	0.03 ± 0.04	0.05 ± 0.06	0.14 ± 0.14	0.15 ± 0.13
	ACY	0.04 ± 0.08	0.08 ± 0.10	0.19 ± 0.14	0.23 ± 0.21
	COA	0.05 ± 0.07	0.18 ± 0.14	0.13 ± 0.12	0.13 ± 0.11
	LIG	0	0	0.02 ± 0.06	0.10 ± 0.13

[1] h^2_i individual, h^2_{HS} half-sib, h^2_{FS} full-sib and h^2_{WFS} within full-sib heritability

Table 1.2. Heritabilities and standard errors for fiber length (FLW), α-cellulose content (ACY), coarseness (COA) and lignin content (LIG) traits for earlywood and latewood of ring 3 and ring 8 (3E, 3L, 8E and 8L) for juvenile wood (ring 3), transition wood (ring 8), and for whole core.

		Traits			
	Heritability[1]	FLW	ACY	COA	LIG
Ring 3 (Juvenile)	h^2_i	0.09 ± 0.10	0.13 ± 0.18	0.32 ± 0.25	0
	h^2_{HS}	0.54 ± 0.21	0.48 ± 0.33	0.69 ± 0.12	0
	h^2_{FS}	0.38 ± 0.26	0.32 ± 0.35	0.61 ± 0.23	0
	h^2_{WFS}	0.05 ± 0.06	0.08 ± 0.11	0.20 ± 0.16	0
Ring 8 (Transition)	h^2_i	0.28 ± 0.23	0.43 ± 0.32	0.37 ± 0.29	0.13 ± 0.19
	h^2_{HS}	0.65 ± 0.15	0.70 ± 0.11	0.68 ± 0.13	0.48 ± 0.35
	h^2_{FS}	0.55 ± 0.26	0.64 ± 0.23	0.60 ± 0.25	0.31 ± 0.37
	h^2_{WFS}	0.17 ± 0.15	0.28 ± 0.23	0.24 ± 0.20	0.08 ± 0.12
Whole Core	h^2_i	0.08 ± 0.06	0.12 ± 0.10	0.16 ± 0.12	0.00 ± 0.08
	h^2_{HS}	0.52 ± 0.18	0.58 ± 0.16	0.61 ± 0.15	0.01 ± 0.78
	h^2_{FS}	0.35 ± 0.21	0.43 ± 0.22	0.48 ± 0.22	0.00 ± 0.32
	h^2_{WFS}	0.04 ± 0.04	0.07 ± 0.06	0.09± 0.07	0.00 ± 0.04

[1] h^2_i individual, h^2_{HS} half-sib , h^2_{FS} full-sib and h^2_{WFS} within full-sib heritability

Table 1.3. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) among fiber length (FLW), α-cellulose content (ACY), coarseness (COA), lignin content (LIG) and wood density for transition wood (ring 8).

		Genetic Correlations			
	FLW	ACY	COA	LIG	Density
FLW		0.37 ± 0.48	0.99 ± 0.01	-0.39 ± 0.66	0.95 ± 0.25
ACY	0.52 ***		0.40 ± 0.45	-0.99 ± 0.01	0.56 ± 0.51
COA	0.13 *	0.33 ***		0.57 ± 0.51	0.32 ± 0.55
LIG	-0.03	-0.10	-0.11		-[1]
Density	0.10	0.13 *	0.12	-0.04	

Phenotypic Correlations

*, ***: Coefficients are significant at 0.05 and 0.001 level respectively. Number of observations used ranged from 183 to 242.

[1] Genetic correlation was not estimable

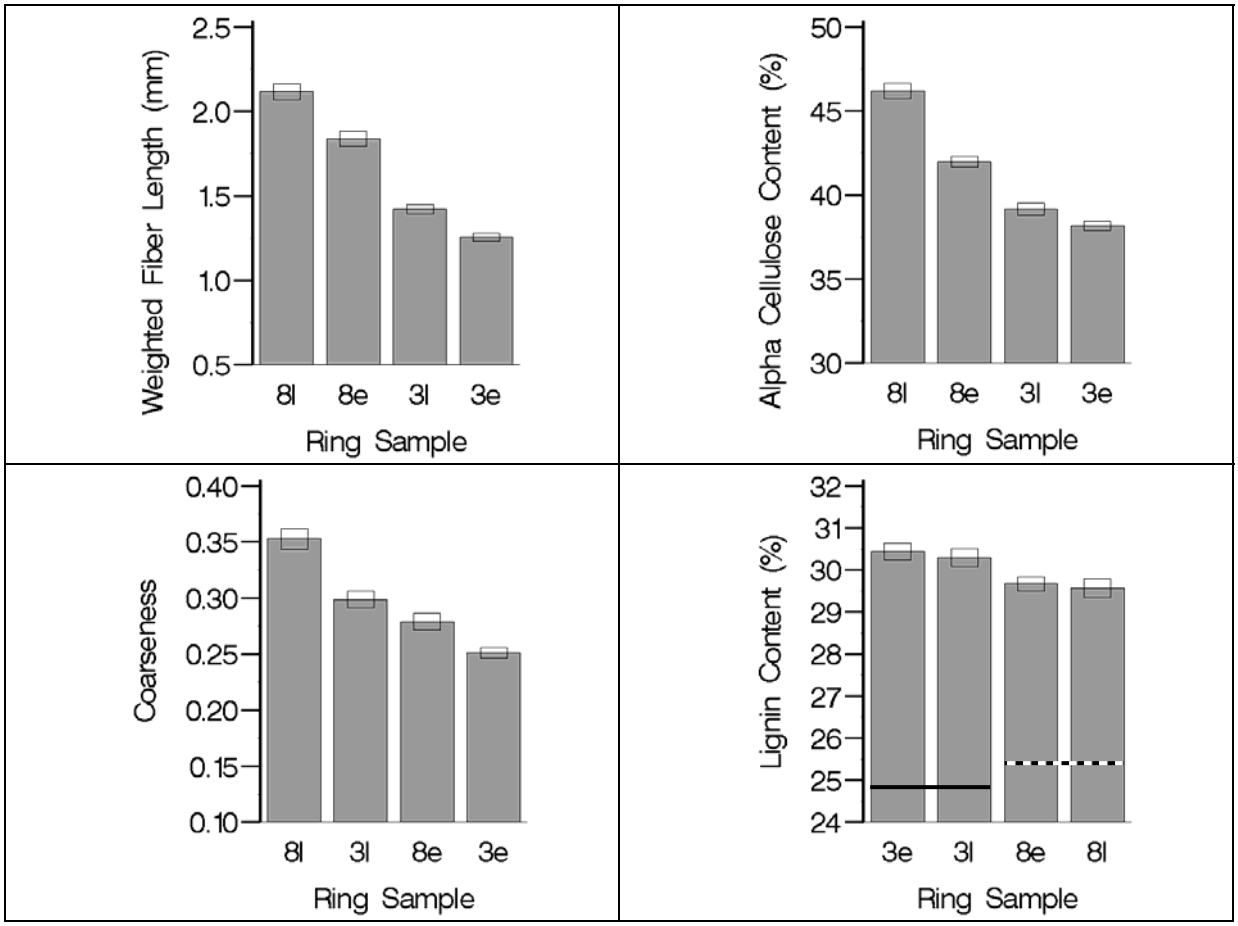

Figure 1.1. Least squares means and 95% confidence intervals of average fiber length, α-cellulose content, coarseness, and lignin content of juvenile wood (ring 3) for earlywood and latewood (3E and 3L) and transition wood (ring 8, 8E and 8L). (Note: Ring segments within the same line were not significantly different at 5% significance level.)

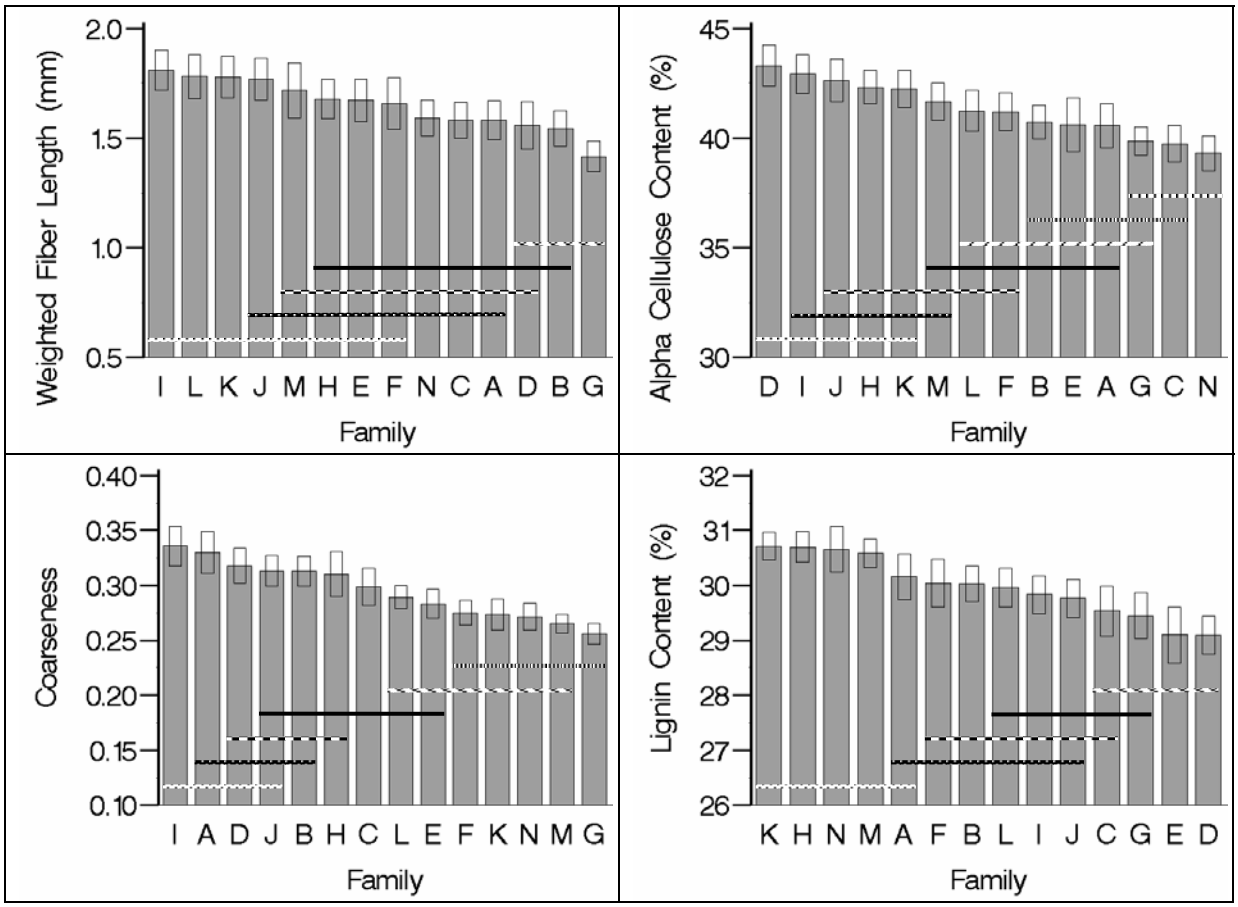

Figure 1.2. Rankings of 14 full-sib family means (A - N) and 95% confidence intervals for average fiber length, α-cellulose content, coarseness, and lignin content. (Note: Families within the same line were not significantly different at 5% significance level.)

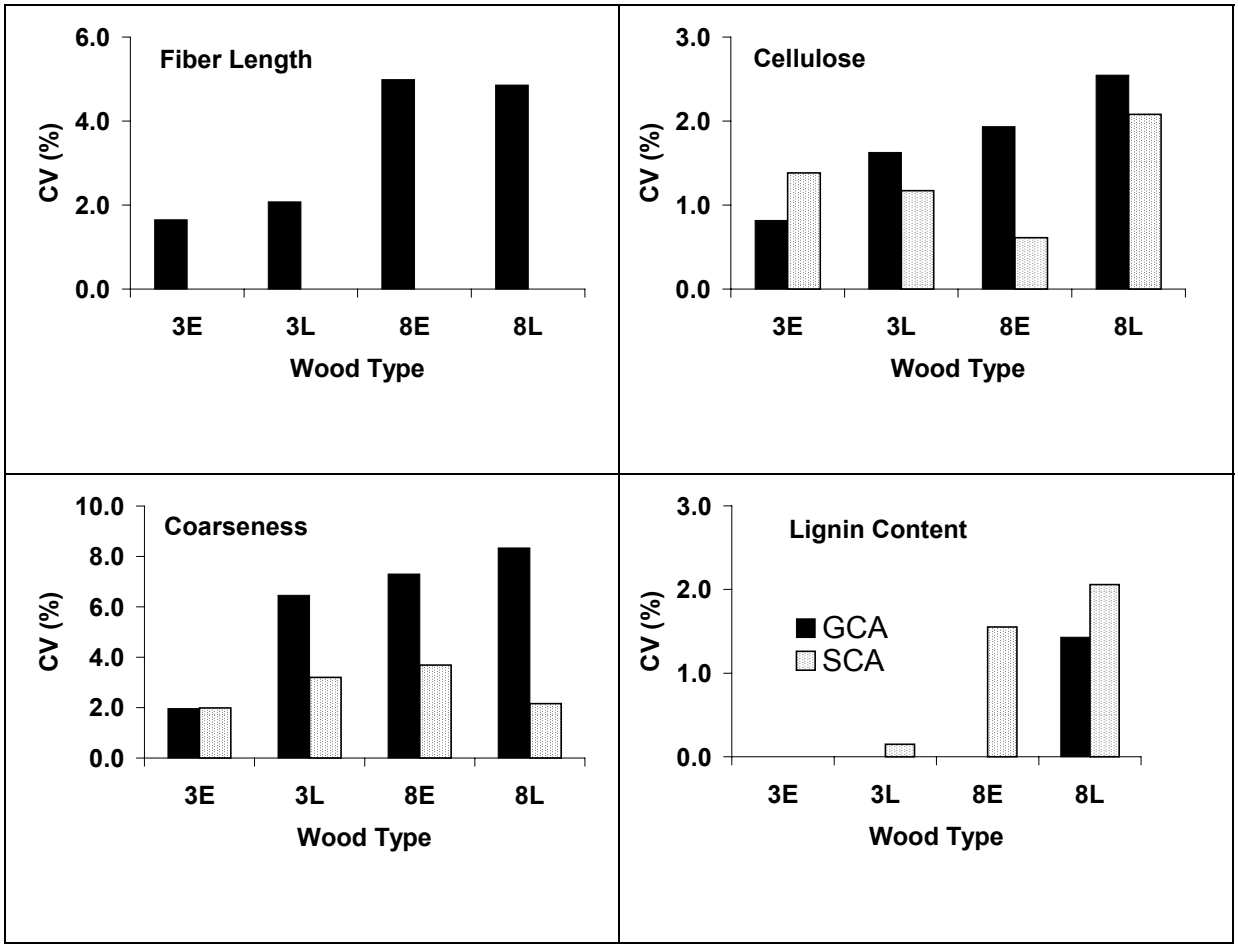

Figure 1.3. Coefficients of variation (CV) for general combining ability (GCA) and specific combining ability (SCA) estimates for fiber length (FLW), α-cellulose content (ACY), coarseness (COA) and lignin content (LIG) at early and latewood of rings three and eight (3E, 3L, 8E and 8L).

Chapter 2

Genetic Variation and Genotype by Environment Interaction of Juvenile Wood Properties in Loblolly Pine

2.1 Introduction

Intensive silviculture and genetic improvement have increased forest plantation productivity significantly in the southeast U.S. for loblolly pine (*Pinus taeda* L.) [*1*]. With improved growth, rotation ages have been reduced to about 20 to 25 years for intensively managed loblolly pine plantations, compared with 40 to 50 years in natural stands. Consequently, the percent of juvenile wood from plantations has increased [*2*]. Juvenile wood typically has less desirable wood properties than mature wood, e.g., low wood density, shorter tracheid length and higher lignin content and, thus, results in lower pulp yield and high pulping costs [*3-5*]. However, if there is genetic variation in these juvenile wood properties in loblolly pine, it may be possible to improve the juvenile wood for solid and chemical wood products through a breeding program.

Genetic variation has been found in some wood properties in loblolly pine by Sykes *et al.* [*3*]. Variation in α-cellulose content, average fiber length, and lignin content have been reported in juvenile wood for loblolly pine and several other tree species [*4-8*]. However, there is little information on how those chemical wood properties interact with the environment, e.g., genotype by environmental interactions (GxE). Determining the magnitude of the GxE would help tree breeders and practical foresters to decide how to design breeding programs and how genotypes with desirable wood traits should be planted over different environments. If GxE is deemed to be negligible, then selected genotypes could be used for plantations that would produce wood under different environmental conditions. One single breeding population may be sufficient to breed for desirable wood properties across environments. This would increase yield, improve product properties and lower pulping costs [*9*]. On the

other hand, if there is significant GxE interaction, i.e., changes in family rank and variance, family performance in wood properties cannot be predicted across environments. Different breeding populations may need to be developed depending on the nature and magnitude of the GxE interaction. This would increase costs and decrease efficiency of the breeding program.

The goal of this study was to determine genetic variation of key juvenile wood properties in loblolly pine on two test sites and examine GxE interaction. Specific objectives were to compare genetic variation in α-cellulose content (ACY), average fiber length (FLW), coarseness (COA), and lignin content (LIG) on two testing sites and examine GxE interaction of these chemical and morphological wood traits. This information would benefit the tree improvement program and the pulp and paper industry by allowing selection and planting suitable trees on suitable sites.

2.2 Materials and Methods

2.2.1 Material and Data Collection

Fourteen full-sib families generated by a 6-parent half-diallel mating design were tested on four sites in the Piedmont of South Carolina. Site 1 was established on a Piedmont site, and had relatively smaller trees that were less uniform in size, while the site 2 was established near a river bottom and had larger trees. A randomized complete block design with six replications was used in the field. Each full-sib family was laid out in 6-tree row-plots in each replication. Wood core samples were collected when the trees were 11-year-old. Increment cores (12 mm) were taken from each tree at breast height (about 1.30 m above

ground) using generator-powered drills. Wood cores having visible limbs, curves, resin pockets, compression wood, or rust infections were avoided. The samples were placed into sealed plastic storage bags and stored in coolers to retain moisture during the material collection.

The bark and cambium were removed from the wood cores, and the cores were split at the pith into two halves. Chemical analysis was done using microanalytical techniques developed by Yokoyama *et al*. [*10*], which allow the rapid characterization of fiber components and morphology of loblolly pine in a large number of samples. Briefly, the techniques involved are extractive removal, holocellulose preparation, α-cellulose and lignin content determination, and average fiber length and coarseness analyses. More than 95% of the nonvolatile extractives were removed from increment cores by four successive two-day acetone extractions [*10*]. The increment cores were then soaked in water overnight before ring samples were taken.

Within-core samples were taken from ring 3 and ring 8 to study chemical properties of juvenile wood (ring 3) and transition wood (ring 8), respectively. Thin wafers from ring 3 earlywood, ring 3 latewood, ring 8 earlywood, and ring 8 latewood were taken using a microtome. At least 300-500 mg of each sample were taken from the earlywood and latewood of each ring. Each sample was oven-dried for 12 hours [*3*].

2.2.2 Statistical Analyses

Analysis of variance was conducted using the GLM procedure of SAS [*11, 12*]. Juvenile wood and transition wood were compared for micro wood traits using paired T-tests. A general linear mixed model was used to estimate variance components for each site and for combined sites using the method developed for diallel analysis [*13*]. The mixed model used for combined sites analysis was an extension of the model given by Sykes *et al*. [*3*] with additions of site, site by general combining ability and site by specific combining ability. Using variance components from the mixed model, individual-tree (h^2_i), half-sib family (h^2_{hs}), full-sib family (h^2_{fs}), and within full-sib family (h^2_{wfs}) heritabilities were estimated following Sykes *et al* [*3*] with the addition of the test and interaction terms.

$$h_i^2 = \frac{4\sigma_{GCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2 + 2\sigma_{GCAxT}^2 + \sigma_{SCAxT}^2 + \sigma_{Plot}^2 + \sigma_E^2} \qquad \text{[Eq. 1]}$$

$$h_{hs}^2 = \frac{\sigma_{GCA}^2}{\left(p\sigma_{GCA}^2 + \sigma_{SCA}^2 + \frac{p\sigma_{GCAxT}^2}{t} + \frac{\sigma_{SCAxT}^2}{t} + \frac{\sigma_{Plot}^2}{tb} + \frac{\sigma_E^2}{tbn} \right) \frac{1}{(p-1)}} \qquad \text{[Eq. 2]}$$

$$h_{fs}^2 = \frac{2\sigma_{GCA}^2}{2\sigma_{GCA}^2 + \sigma_{SCA}^2 + \frac{2\sigma_{GCAxT}^2}{t} + \frac{\sigma_{SCAxT}^2}{t} + \frac{\sigma_{Plot}^2}{tb} + \frac{\sigma_E^2}{tbn}} \qquad \text{[Eq. 3]}$$

$$h_{wf}^2 = \frac{2\sigma_{GCA}^2}{\frac{(t-1)}{t}\left(2\sigma_{GCAxT}^2 + \sigma_{SCAxT}^2 + \frac{(b-1)\sigma_{Plot}^2}{b} + \frac{(bn-1)\sigma_E^2}{bn} \right)} \qquad \text{[Eq. 4]}$$

where σ^2_{GCA} is genetic variance, σ^2_{SCA} is dominance variance, σ^2_{GCAxT} is GCA by test interaction, σ^2_{SCAxT} is SCA by test interaction, σ^2_{plot} is among plot variance, σ^2_e is error variance, p is # of parents in the diallel, b is # of blocks, and n is # of trees per family per replication. Product-moment correlations were estimated among the morphological wood traits and their relationships with growth traits. Approximate genetic correlations among traits were calculated using individual-tree breeding values of each trait that were estimated according to Xiang and Li [*13, 14*].

2.3 Results

2.3.1 Comparison of Wood Types

Latewood and earlywood of ring 3 and 8 showed similar differences on both sites (Figure 2.1). Latewood had significantly greater ACY, FLW and COA than earlywood in ring 3 (juvenile wood) on both sites. The differences between latewood and earlywood for ACY, FLW, and COA were generally greater in ring 8 than in ring 3. Earlywood and latewood of ring 3 were not different for LIG on site 1, but they differed on site 2. Earlywood and latewood of ring 8 were not significantly different for LIG on both sites. Site 1 had considerably greater COA in latewood of ring 8 than site 2.

Earlywood and latewood of each ring were averaged for juvenile wood (ring 3) and transition wood (ring 8), and T-tests for these means showed that juvenile wood (ring 3) and transition wood (ring 8) were significantly different for all traits (Figure 2.2). Transition wood had higher ACY, FLW, COA, and a lower LIG content than juvenile wood.

2.3.2 Differences among Families

Families differed significantly for all the chemical and morphological wood properties on both sites (Figure 2.3). Variation among families for all the traits was greater on site 2 than on site 1. Families had generally greater ACY, COA, FLW and lower LIG means on site 2 than on site 1. Although there was significant variation among families for lignin, the range of families was small.

2.3.3 GxE Interaction

Families showed considerable rank changes for all four chemical wood traits between two sites, especially for COA and FLW (Figure 2.3). For example, family A had high COA and low FLW on site 1, but the reverse was true on site 2. With the mixed model for diallel analysis [*13*], two types of GxE interactions were estimated, i.e., site by general combining ability interaction and site by specific combing ability interaction. No significant site by general combining ability interaction was found for any of the traits, but site by specific combing ability interaction was significant for most traits (Table 2.1). Variance components for site by general combining ability ranged from 0 to 12.5% of the total phenotypic variation, while site by specific combining ability interaction variance ranged from 0 to 22.4% of the total phenotypic variance (Table 2.1). The site by specific combining ability interaction variances were greater for ring 3 than ring 8.

2.3.4 Heritabilities and Genetic Correlations

Heritabilities of earlywood and latewood of ring 3 and 8 are presented in Table 2.3. In general, heritabilities were higher for earlywood and latewood of ring 8 than those of ring 3. Weak or near zero individual-tree, half-sib, full-sib family and within full-sib family heritabilities were observed for earlywood and latewood of ring 3, except half-sib family heritabilities. Heritabilities for earlywood of ring 8 were higher than latewood for most traits. FLW had higher heritabilities at the transition wood (ring 8) than at juvenile wood (ring 3). Heritabilities for LIG at earlywood and latewood of ring 3 was zero, due to lack of genetic variation. Similarly, heritabilities were zero for COA and FLW in ring 3, due to no GCA variances. Individual heritabilities for site 1 and site 2 are reported in Table 2.2. In general, little additive genetic variation was found on site 2 and, therefore, heritability estimates were weak or zero for most traits.

Moderate genetic correlations were observed among the chemical and morphological wood traits, stem straightness, volume, height and rust infection (Table 2.4). Stem straightness had favorable correlations with ACY, COA and FLW in the combined analysis. ACY was positively correlated with FLW and COA. There was a slightly negative correlation of rust infection with ACY, COA and FLW. There was a negative correlation of FLW with volume and height. There was a moderate negative correlation between volume and LIG.

2.4 Discussion

Results from two sites showed that latewood had greater cellulose content, longer average fiber length and higher coarseness compared to earlywood of the same rings. Juvenile wood

at ring 3 appeared to be less desirable for chemical wood traits compared to ring 8, which is considered transition wood rather than mature wood for loblolly pine [*15*]. There was an increasing trend of cellulose content, fiber length and coarseness from juvenile wood to transition wood. The results based on combined two sites from this study were parallel to results by Sykes *et al.* [*3*] that were based on one site.

Considerable differences in the chemical wood properties were found between earlywood and latewood of both rings, except for lignin content (Figure 2.1). Genetic variation in cellulose content was higher than that found by Jett *et al*. [*16*], with both additive and dominance components. Lignin had little to zero GCA variance for both juvenile and transition wood. Lowering lignin content through breeding and selection may not be successful due to weak heritabilities.

Juvenile wood was significantly different from transition wood for all traits. Transition wood had lower lignin content than juvenile wood, although the difference was small at less than 1% (Figure 2.2). Alpha cellulose yield, coarseness, and weighted fiber length all had larger means values for transition wood compared to those of juvenile wood.

Although genetic variances and heritabilities were generally weak, selection based on full-sib family means may help to improve certain chemical wood traits. Significant differences among families for morphological wood traits imply efficiency of family selection. These results suggest that planting individual families may help to improve certain wood traits. Family I appeared to have high pulp or cellulose yields and low lignin. However, before

considering one or two wood traits for breeding and selection, more efforts are needed. Tree improvement programs must decide which traits will be of most importance in the future before incorporating them into their breeding programs.

Full-sib family heritabilities ranged from weak to moderate for most of the chemical wood properties. The results suggested that genetic improvement for cellulose content and coarseness may be realized based on transition wood because heritabilities were higher for transition wood than juvenile wood. Transition wood heritabilities may be more meaningful than those of juvenile wood, as they are closer to the age (age 6) where most selections are made for the North Carolina State University-Industry Tree Improvement Cooperative [*1*]. The unbiased heritabilities based on combined sites were lower than those from Sykes *et al*. [*3*], due to removing the GCA by test interaction term from the numerator of the heritability equations. Heritabilities for fiber length were lower than those individual heritabilities of Loo *et al*. [*4*] for loblolly pine. Standard errors of all the heritabilities were high due to the limited number of parents in the experiment. Measurement of chemical wood traits is costly and time consuming. Unless laboratory measurement techniques are improved, it will be costly to increase sample size for more reliable estimation of genetic parameters.

Lignin had moderately negative genetic correlations with both height and volume (≈ -0.50), and positive genetic correlations with rust infection and straightness (≈ 0.30). The favorable correlations of stem straightness with ACY and FLW indicated that selection for straight trees could improve these wood traits in a breeding program. Since volume and height can be measured more effectively, and has already been incorporated into the tree improvement

programs, improving height or volume may decrease lignin content. Increasing cellulose content may result in the production of more paper per cubic meter of wood.

The noticeable GxE interaction for these traits was mainly due to site by specific combining ability, which was caused by non-additive genetic effects by environment interaction. This may explain the family rank changes between two sites (Figure 2.3). There was essentially no site by general combining ability interactions, suggesting that additive genetic effect by environment interaction was not important for these traits (Table 2.1). However, these results are preliminary and based on the limited samples from only two test sites. There were differences in magnitude of genetic variances between two sites. Site 2 had much higher SCA variation than site 1, and resulted in lower heritability estimates (Table 2.2). Site 2 was established near a river bottom and had larger trees, while site 1 was established on a Piedmont site, and had relatively smaller trees than site 2 and was less uniform. Due to cost restraints, the sample size on site 2 was approximately 60% of the sample size on site 1. Imprecision due to smaller sample size may have influenced estimates of GxE interaction.

Genotype by environment interactions may play a larger role as the trees mature, and need to be investigated further [*17,18*]. Our preliminary results suggested that loblolly pine families, and particularly full-sib families, might not be stable across different site conditions for some of the wood properties. Two additional sites will be analyzed in this study to more accurately assess the GxE component for these wood properties. If site by specific combining ability continues to be an important interaction, as shown in these two tests,

breeding for full-sib families along with a family deployment strategy may be considered for word quality improvement.

2.5 Conclusions

Significant genetic variation was detected for all chemical wood traits for loblolly pine, except for lignin content. Heritabilities were generally moderate for cellulose content and coarseness, and weak for lignin content and fiber length. There were family rank changes between the two sites for these traits, which were caused by large site by specific combining ability interaction. Tree straightness appeared to have favorable correlations with several wood traits, suggesting that selection for straightness may lead to the increase of cellulose, fiber length and coarseness, while decreasing lignin content. There was a weak negative correlation of these traits with growth, indicating it may be possible to improve these traits simultaneously in a tree improvement program by developing a selection index for multiple traits. Additional sites need to be analyzed in order to better estimate genetic parameters and GxE for wood traits.

2.6 Acknowledgements

This study was supported a grant from the Department of Energy (Grant DE-FC36-01GO10624), NCSU-Industry Cooperative Tree Improvement Program, Department of Forestry, and Department of Wood and Paper Sciences at N. C. State University.

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Table 2.1. Variance components of two rings for general combining ability by site (GxE) interaction and specific combining ability by site (SxE) interaction expressed as a percentage of total phenotipic variation for average fiber length (FLW), coarseness (COA), α-cellulose content (ACY), and lignin content (LIG)

Wood types	Interaction	FLW	COA	ACY	LIG
Ring 3 earlywood	GxE	0.075	3.702	0.000	0.000
	SxE	3.081	0.000	18.521 **	0.254
Ring 3 latewood	GxE	0.000	6.567	0.000	0.000
	SxE	10.423	11.708	16.133 *	6.095
Ring 3 Mean	GxE	0.000	8.292	0.000	0.000
	SxE	6.202	7.895	22.437 *	4.999
Ring 8 earlywood	GxE	2.412	5.195	0.000	0.000
	SxE	12.355 *	7.443	7.920	8.241 *
Ring 8 latewood	GxE	1.497	9.906	2.625	0.000
	SxE	8.294	6.561	19.145 *	7.838
Ring 8 Mean	GxE	3.317	12.470	0.000	0.000
	SxE	9.342	13.531*	22.106 *	12.499 *
Whole Core	GxE	0.569	4.348	0.000	0.000
	SxE	3.892 *	3.950	8.611 *	5.605 *

*,** are significant at the 0.05 and 0.01 significance levels, respectively

Table 2.2. Individual heritability (± standard error) estimates for site 1 and site 2 for fiber length (FLW), α-cellulose content (ACY), coarseness (COA) and lignin content (LIG) for earlywood and latewood of ring 3 (3E, 3L) and ring 8 (8E, 8L).

Traits	Ring Segment	Site 1	Site 2
FLW	3E	0.06 ± 0.10	0
	3L	0.12 ± 0.11	0
	8E	0.24 ± 0.23	0.23 ± 0.22
	8L	0.24 ± 0.19	0
ACY	3E	0.08 ± 0.14	0
	3L	0.16 ± 0.17	0
	8E	0.32 ± 0.23	0.32 ± 0.23
	8L	0.28 ± 0.25	0.05 ± 0.51
COA	3E	0.08 ± 0.11	0.70 ± 0.48
	3L	0.32 ± 0.23	0.13 ± 0.43
	8E	0.22 ± 0.19	0.23 ± 0.19
	8L	0.20 ± 0.18	0.74 ± 0.53
LIG	3E	0	0
	3L	0	0
	8E	0.01 ± 0.11	0.04 ± 0.11
	8L	0.17 ± 0.21	0

Table 2.3. Heritabilities (± standard error) for fiber length (FLW), α-cellulose content (ACY), coarseness (COA) and lignin content (LIG) traits for combined sites for earlywood and latewood of ring 3 (3E, 3L) and ring 8 (8E, 8L).

		Ring # and Wood Type			
Heritability[1]	Trait	3E	3L	8E	8L
h^2_i	FLW	0.00 ± 0.06	0	0.02 ± 0.14	0.08 ± 0.14
	ACY	0.05 ± 0.16	0.06 ± 0.17	0.28 ± 0.25	0.04 ± 0.28
	COA	0.07 ± 0.16	0	0.14 ± 0.22	0
	LIG	0	0	0	0.10 ± 0.16
h^2_{HS}	FLW	0.08 ± 1.16	0	0.20 ± 1.02	0.49 ± 0.40
	ACY	0.25 ± 0.64	0.30 ± 0.57	0.65 ± 0.17	0.15 ± 0.95
	COA	0.40 ± 0.56	0	0.58 ± 0.33	0
	LIG	0	0	0	0.51 ± 0.38
h^2_{FS}	FLW	0.03 ± 0.53	0	0.09 ± 0.54	0.30 ± 0.44
	ACY	0.13 ± 0.41	0.16 ± 0.40	0.54 ± 0.29	0.07 ± 0.48
	COA	0.21 ± 0.45	0	0.37 ± 0.45	0
	LIG	0	0	0	0.35 ± 0.43
h^2_{WFS}	FLW	0.00 ± 0.03	0	0.01 ± 0.08	0.04 ± 0.08
	ACY	0.03 ± 0.10	0.04 ± 0.11	0.18 ± 0.17	0.03 ± 0.19
	COA	0.04 ± 0.09	0	0.08 ± 0.13	0
	LIG	0	0	0	0.06 ± 0.10

Table 2.4. Genetic correlations based on individual-tree breeding values for juvenile wood (above diagonal) and transition wood (below diagonal) among fiber length (FLW), α-cellulose content (ACY), coarseness (COA), lignin content (LIG), height at age 6 (HT6), volume at age 6 (VOL6), fusiform rust infection at age 6 (RUST6), and straightness at age 6 (STRT6) for combined sites.

		Juvenile Wood						
	ACY	COA	FLW	LIG	HT6	VOL6	RUST6	STRT6
ACY		0.54***	0.58***	-[1]	0.13**	-0.07	-0.19***	-0.34***
COA	-[1]		0.70***	-	0.16***	-0.01	-0.38***	-0.53***
FLW	0.26***	-		-	-0.19***	-0.44***	-0.30***	-0.69***
LIG	-0.14**	-	-0.23***		-	-	-	-
HT6	0.11*	-	-0.37***	-0.56***		0.92***	0.11*	-0.24***
VOL6	-0.12**	-	-0.53***	-0.52***	0.92***		0.22***	-0.03
RUST6	-0.30***	-	-0.53***	0.37***	0.11*	0.22***		0.07
STRT6	-0.48***	-	-0.43***	0.31***	-0.24***	-0.03	0.07	

Transition Wood

*, **, ***: Correlations are significant at 0.05, 0.01, and 0.001 level, respectively. Number of observations used ranged from 515 to 550

[1] Genetic correlation was not estimable due to zero GCA variance

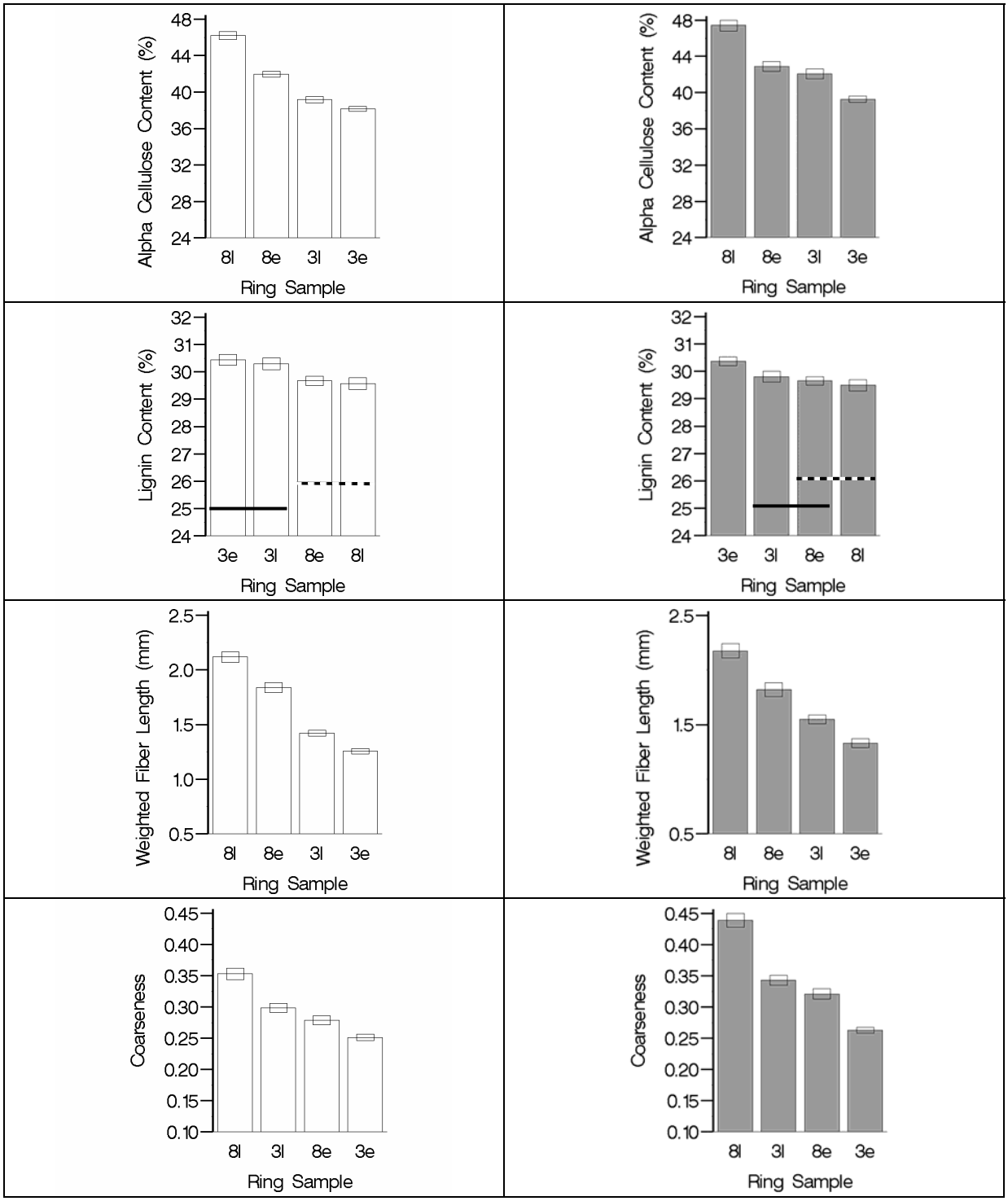

Figure 2.1. Site 1 (left) and Site 2 (right) means and 95% confidence intervals of α-cellulose content, lignin content, weighted fiber length, and coarseness for ring 3 earlywood (3e), ring 3 latewood (3l), ring 8 earlywood (8e), and ring 8 latewood (8l). (Note: Ring segments within the same line were not significantly different at 5% significance level.)

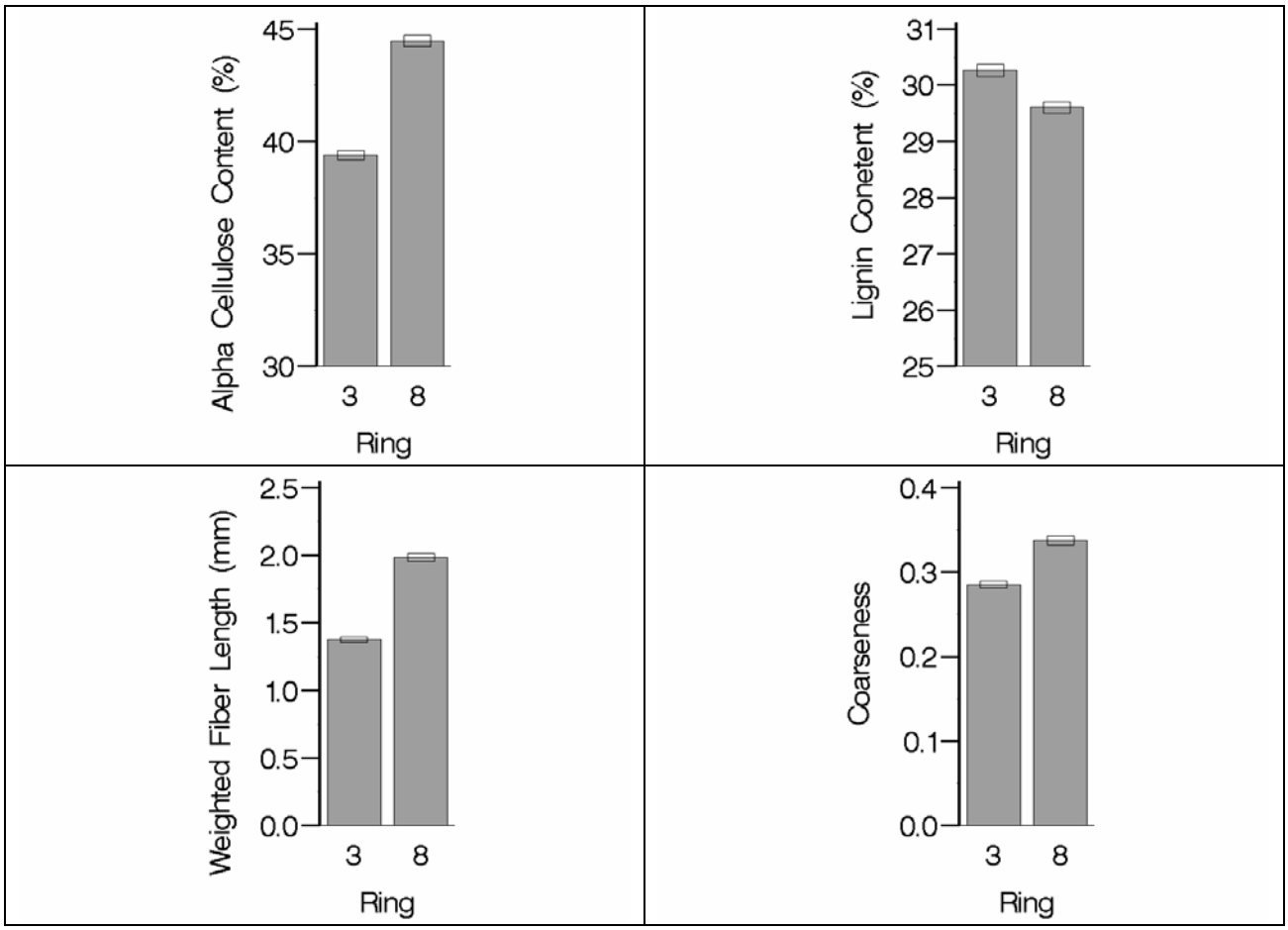

Figure 2.2. Combined sites juvenile wood (ring 3) and transition wood (ring 8) means and 95% confidence intervals for α-cellulose content, coarseness, weighted fiber length, and lignin content

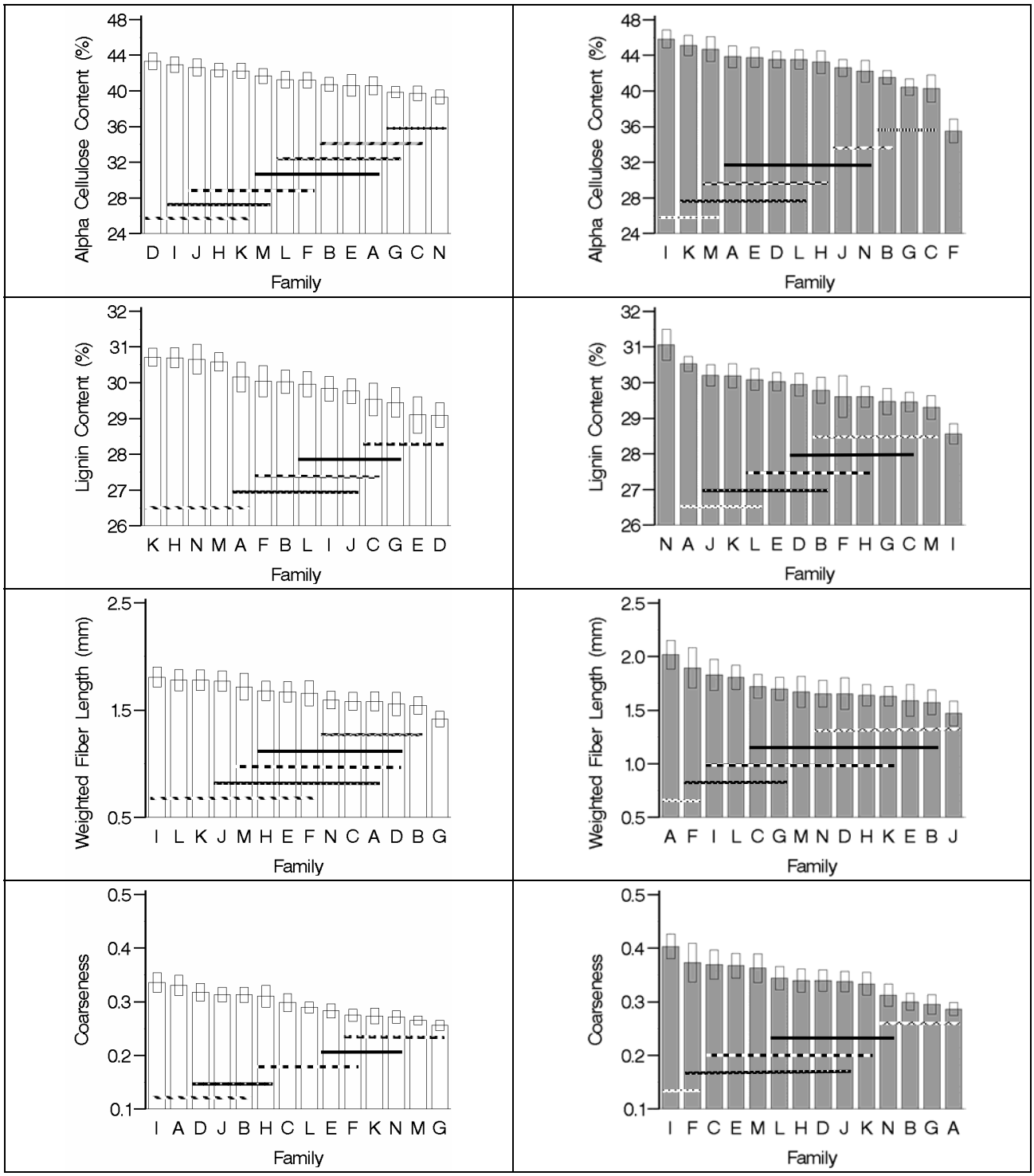

Figure 2.3. Family means and 95% confidence intervals for α-cellulose content, lignin content, weighted fiber length, and coarseness at Site 1 (left) and Site 2 (right). (Note: Families within the same line were not significantly different at 5% significance level.)

Chapter 3

Rapid Prediction of Wood Properties of Loblolly Pine using Transmittance Near Infrared Spectroscopy

3.1 Introduction

Chemical and morphological wood properties, including fiber length, α-cellulose content and coarseness can have major impacts on pulp and paper products [*1-3*]. The α-cellulose content relates to the amount of pulp that is obtained from wood, and is highly correlated with pulp yield [*1*]. Increasing the cellulose content in wood would reduce pulping costs and increase the efficiency of the pulp and paper mill [*3*]. Fiber length also plays an important role in the pulp and paper industry. Depending on the paper product being produced from the pulp, long fibers or short fibers can be favored. Long fibers provide paper with greater tensile and tear strength for products such as cardboard and paper bags [*4*]. Short fibers are preferred for products such as fine printing paper where surface smoothness and ink bleeding is important. Coarseness is the measurement of the total mass of a sample of fibers divided by the total length of the sample, and reflects the thickness of fiber walls. However, coarser fibers also tend to have large lumens and, therefore, it is difficult to separate the effects of lumen size and wall thickness [*2*]. Lower coarseness results in easier fiber collapse causing better bonding of fibers and for formation of dense paper with smooth surface. Wood with higher coarseness values yield pulp and paper products with higher bulk, which is beneficial to products requiring higher absorbance and/or higher bending stiffness.

Improvement for growth, stem straightness, disease resistance and specific gravity have been the primary focus of tree breeding due to their economic importance and the ease of measurement [*3*]. Large gains have been documented for loblolly pine (*Pinus taeda* L.) for growth, stem form and rust infection from two cycles of loblolly pine breeding [*5*], but relatively little work has been done on the improvement of wood properties in this

species. One of the limitations in genetic improvement of wood is the difficulty in measuring these traits. To obtain an accurate assessment of a breeding population, analyses of large numbers of samples are required. Traditional laboratory methods for the measurement of chemical properties of wood are expensive, involving time consuming wet chemistry measurements on large amounts of samples. Recently a microanalytical technique has been developed to more rapidly screen the chemical properties of progeny trees with a small wood sample [*6*]. Using this method, genetic variation for a number of properties has been identified in a large loblolly pine progeny test [*7*]. Although this analysis was substantially faster than traditional chemical analyses, it is still costly and time consuming, particularly for a large progeny test. Therefore, if we are to predict wood properties for the breeding of large populations of trees, a more rapid screening technique is needed.

Near infrared (NIR) spectroscopy is a rapid non-destructive technique that has been utilized in the characterization of everything from pharmaceuticals to wood. NIR spectroscopy is a *relative* spectroscopic technique wherein its utility in analyzing large data sets is only as accurate as the samples set used to calibrate it. Therefore, the determination of wood chemical properties using wet chemistry methods is critical. Once the appropriate calibration equation is established, the wood properties of a sample can be predicted in a matter of minutes, compared to days using classical methods. In combination with non-destructive sampling, the NIR may be used to screen many progeny tests quickly at a reasonable cost. Calibration equations may also be used to predict wood properties for trees on different sites.

NIR spectroscopy has been used to characterize wood and fiber properties [*8-11*]. Most NIR studies have utilized reflectance NIR spectroscopy to predict wood properties of wood meal or solid wood [*12*]. Reflectance measurements suffer from several limitations, the most serious being the small penetration depth (1-4 mm) into the sample. Thus, when using reflectance measurements the uniformity of particle size becomes very important [*10, 13-15*]). The milled wood must be screened to remove the larger particles to ensure the uniformity of the sample. For non-homogeneous samples such as wood, this limited penetration leads to a large variation in results and a strong dependence on sample size and preparation technique. Thus, large sample sizes are typically utilized to better represent the sample of interest. Therefore, detailed measurements of earlywood and latewood are not possible without large destructive sampling.

By contrast, transmittance techniques, which penetrate fully through the sample, are less sensitive to sample preparation and homogeneity and permit analysis of smaller quantity samples. In fact, Yeh et al. [*16*] have recently reported the use of transmittance NIR spectroscopy to study lignin content in earlywood and latewood from 12 mm increment wood cores. In this paper we examined the use of transmittance NIR spectroscopy to predict α-cellulose content, average fiber length, and coarseness of juvenile wood and transition wood in loblolly pine. Using 12-mm increment cores of 11-year loblolly pine trees, earlywood and latewood of juvenile and transition rings are evaluated by transmittance NIR spectroscopy and their relationships with laboratory data are used for predictions in wood properties.

3.2 Materials and Methods

3.2.1 Wood Sample Collection

Wood samples of loblolly pine trees from field progeny tests were collected and processed as described previously [*7*]. Briefly summarized, fourteen full-sib families generated by a six-parent half-diallel mating design were tested in the Piedmont of South Carolina. A randomized, complete-block design with six replications was used in the field. Each full-sib family was laid out in six-tree row-plots for each replication. Wood core samples from 11-year-old trees were collected from two sites in South Carolina. Increment cores (12 mm) were taken from each tree at breast height (about 1.30 m) using generator-powered drills. Wood cores having visible limbs, curves, resin pockets, compression wood, or rust infections were avoided. The samples were placed into plastic storage bags, labeled, and placed in coolers to retain moisture during the material collection and transportation. In this study, 30 trees from two full-sib families were sampled on site 4, and another 40 trees from three full-sib families were sampled on site 3. Families were different on each site.

3.2.2 Measurements of Wood Properties

The bark and cambium were removed from the wood cores, and the cores were split at the pith into two halves. Chemical analysis was done using microanalytical techniques developed by Yokoyama et al. [*6*], which allows the rapid characterization of fiber components and morphology of loblolly pine on small quantities of wood tissue. Techniques involved include removal of more than 95% of non-volatile extractives, α-cellulose content determination, average fiber length measurement, and coarseness analyses [*6*]. The

increment cores were then soaked in deionized water overnight before ring samples were taken.

Thin wafers (200 μm) from ring 3 earlywood (3E), ring 3 latewood (3L), ring 8 earlywood (8E), and ring 8 latewood (8L) were taken from each wood core. Using a micotome, at least 300-500 mg of each sample was taken from the earlywood and latewood of each ring. The total number of measurements was 70 increment cores with 4 ring segments sampled per core, resulting in approximately 280 total measurements. Sixteen completely round wood wafers were taken from earlywood and latewood of ring 3 and ring 8 of each increment core for NIR measurement, with extra wafers were saved for cellulose measurement. The wafers were pressed between glass microscope slides with small weights on top of them to ensure flatness during the drying process. Wafers with noticeable thickness variation were discarded. Each sample was placed in a vacuum desiccator with P_2O_5 for two days prior to NIR analysis to remove moisture.

Wood wafers were analyzed using a Foss NIR spectrometer, Model 6500. A single wafer was inserted into a machined aluminum sample holder, and scanned using the Foss Intact Tablet Analyzer module. This module measures the amount of NIR light (600-1900 nm) that is transmitted through the sample. To ensure uniformity, fiber orientation was kept constant for all samples. Samples were analyzed in a dry condition, and were only removed from desiccators during analysis. The 16 spectra were averaged to create one spectrum, which was used for the calibration equations for α-cellulose content (ACY), average fiber length (FLW),

and coarseness (COA). Increment cores from site 3 and site 4 were used to calibrate the NIR spectra. Each site was analyzed separately, and then the data were combined and analyzed.

3.2.3 Calibration development

The Intact Tablet Analyzer module for the Foss NIR spectrometer produced spectra from 600-1900 nm in 2 nm increments. The spectra were converted to the second derivative using VISION software (Version 2.51provided with the instrument) to remove baseline offset and sloping effects that are common in NIR spectra [*17*]. Development of all calibration equations was done based on second derivative data. Upon examining the second derivative spectra, noise was evident at both ends of the spectra (Figure 3.1). Michell and Schimleck [*18*] reported that one of the major bands for cellulose prediction occurs at 1477 nm. Using this information, the scan was reduced to 800-1600 nm for coarseness, fiber length, and α-cellulose content analysis (Figure 3.1). Several wider wavelength ranges were tested, but 800-1600 nm resulted in the highest coefficients of determination for the calibration sets.

The coefficient of determination (R^2) was estimated between the wet chemistry data and the NIR predicted values using partial least squares (PLS) regression models. In addition to R^2, the standard error of calibration (SEC) was used to evaluate how precise the regression line fit the data [*19*]. SEC is the standard deviation for the residuals due to the difference between wet chemistry data from the lab and the NIR predicted values within the calibration set [*20-21*]. The standard error of cross validation (SECV) was also determined to measure how well the calculated equations predicted the samples that were not used to create the calibration set [*22*]. A prediction function was used to predict a set of unknown data using

the previously established calibration equations to produce the standard error of prediction (SEP) as well as the R^2 for the prediction set.

To minimize the thickness variation of wood wafers and variation within the ring, several mathematical options were applied. The PLS analysis was completed using the multiplicative scatter correction option on second derivative transformed data. This option standardizes the data and eliminates some of the variation in NIR intensity due to thickness differences. Although several other regression options were examined, these did not result in improved calibration equations and, thus, were not employed.

Data were analyzed with all samples combined into one data set including both sites, and subsequently divided into smaller sets. Each site was analyzed independently and each ring sample (3E, 3L, 8E, and 8L) was analyzed separately to test whether earlywood or latewood produced better results and each site was analyzed individually. Ring 3 equations were used to predict ring 8 data for determining age-age correlations. Calibration equations based on site 3 were used to predict data collected from site 4. All datasets were split into a calibration set and a validation set using VISION software. The default software percentages of 75% of the data used for calibration and 25% of the data for validation were maintained for all analyses. The validation set was not used in creating the calibration set and provides a measure of how well the calibration equation can predict a similar set of wood samples.

3.3 Results

3.3.1 Wood Property Measurements in Laboratory

Descriptive statistics of ACY, COA and FLW for the individual sites and for the combined sites are presented in Table 3.1. Trees on site 3 had higher FLW, with lower ACY and COA than trees on site 4. Standard deviations were slightly higher on site 4 for COA and FLW, but lower for ACY. The range for ACY (35-55%) was similar on both sites. COA values had a higher range on site 4 (0.24-0.69) compared to site 3 (0.22-0.55). Although the overall range for FLW was similar in magnitude, the mean FLW on site 3 was approximately 0.33 mm longer than site 4.

3.3.2 Variation of NIR Spectra

There were noticeable differences in the spectra for the different ring samples (Figure 3.1). The peaks were located at the same wavelengths for the four different ring samples, while the intensities varied. Latewood (3L and 8L) had greater peak intensities than earlywood (3E and 8E) peaks, and transition wood had greater intensities than juvenile wood. Certain wavelengths (1300-1500 nm) within the spectrum exhibited larger differences among the peaks than other wavelengths. Although the intensities varied, the relative ranking stayed the same throughout the peaks.

3.3.3 NIR Calibrations with all Data

The fitted calibration models of ACY, COA and FLW obtained from NIR to predict laboratory results are presented in Figure 3.2 and in Table 3.2. The relationships between

NIR and laboratory measurements of three traits were linear. For the combined site analysis, regression models based on the whole core data set resulted in higher R^2 values than subsets broken out by earlywood/latewood or ring 3/ring 8 (Table 3.2). This was true for all traits. For example, the range of the R^2 value for α-cellulose content varied between 0.56 to 0.63 for the two wood types and the two rings, whereas the coefficient increased to 0.75 for thw whole core data set (Figure 3.2). Results from NIR spectroscopy explained 75% of the variation in laboratory ACY; however, relationships between two measurements (NIR and laboratory) were lower for COA ($R^2 = 0.64$) and for FLW (R^2=0.43). Regression models fitted to separate data for wood types (earlywood and latewood) and for single rings explained smaller percentages of variation for all three variables (Table 3.2).

3.3.4 NIR Calibrations by Sites

Regression models based on site 3 data were more successful in explaining laboratory measurements than the whole core data set (Table 3.3). Relationships between the NIR and laboratory ACY were the highest for all the individual datasets and ranged from R^2=0.68 to R^2=0.88. Relationships between NIR and laboratory measurements types for COA and FLW were moderately high for site 3. The latewood and ring 8 datasets yielded highest coefficients of variation, but had slightly higher standard errors than the earlywood and ring 3 datasets on site 3.

Regression models based on site 4 data were less successful as shown by smaller R^2 values for all the traits. The best calibration equation for ACY had a R^2 value of 0.70 for the earlywood data on site 4, compared to $R^2 = 0.73$ for site 3 (Table 3.3). In general, all

standard errors for site 4 were higher than those from site 3. Standard errors (SEC and SECV) for COA on site 4 were approximately twice those reported from site 3. Similarly, standard errors for FLW on site 4 were generally 1.5 times higher than those from site 3.

In general, combining data into a whole core data set (3E, 3L, 8E, and 8L) for calibrations resulted in more reliable calibration equations for both sites. R^2 based on the whole core data set ranged from 0.72 to 0.86 on site 3 and 0.55 to 0.73 on site 4 (Table 3.3).

3.3.5 NIR Predictions Based One Site Calibration

Calibration equations based on site 3 resulted in higher R^2 values and were used to predict the wood properties of the trees on site 4. Using site 3 ACY and COA to predict site 4 ACY and COA yielded $R^2 = 0.64$ and $R^2 = 0.63$, respectively (Figure 3.3). The SEP for ACY was approximately 1.5 times greater than the SEC found on site 3, and the SEP of COA was twice the SEC (Table 3.3). FLW did not predict well between sites. The correlation for FLW was low ($R^2 = 0.43$) and the SEP was nearly twice the SEC from site 3.

To test the NIR calibrations to calculate age-age correlations, ring 3 calibration equations were used to predict the values of the ring 8 NIR spectra. ACY predictions resulted in a positive correlation with an $R^2 = 0.60$ and a SEP of 3.53% (Figure 3.4). Site 3 prediction equations for COA on site 4 resulted in a higher R^2 (0.68) than using the site 4 data independently with a SEP of 0.08. A noticeable separation of earlywood and latewood was apparent on the COA distribution (Figure 3.4). Latewood COA values were higher than

those of earlywood and separated into clusters on the graph. FLW predictions were not made due to the lower correlations with the original site data.

3.4 Discussion

NIR analysis using transmittance provided relatively reliable calibrations for predicting α-cellulose content and coarseness in Loblolly pine (Figure 3.2). The standard errors of calibration and prediction ranged from 1.43% to 3.55%, depending on the dataset that was used (Table 3.3, Figure 3.4). The standard errors for ACY were higher and R^2 values were slightly lower than those reported by Raymond and Schimleck [*19*] using *Eucalyptus globulus* and Schimleck et al. [*23*] using *Eucalyptus nitens*. Different methods for determining cellulose values may lead to the higher errors in this study. Another difference in this study's NIR methods was that the wood samples were maintained separately (3E, 3L, 8E, and 8L), whereas other studies ground the whole core and use the resulting wood meal for cellulose determination.

Cellulose determination and prediction can be crucial to the pulp and paper industry because of the impact on pulp yields and lignin content since cellulose content has been shown to be highly correlated with pulp yield [*1, 11, 24-27*]. Tree breeders can use NIR spectroscopy to rapidly and cost effectively screen progeny tests to select trees with high COA. The selected trees will have higher pulp yields due to favorable correlations between the two traits. Recently, we [*7*] reported a very high genetic correlation (-0.99) between α-cellulose yield and lignin content. Using NIR spectroscopy to select trees with high cellulose content will reduce lignin content of the wood, and therefore reduce pulping costs at the mill.

Coarseness calibration equations were the most accurate when all four-ring samples (3E, 3L, 8E, and 8L) were combined into a whole core data set (Table 3.2 and 3.3). The complete dataset produced a wider range but did not reveal evident clustering according to analysis of residuals. However, when subsets of the data were used, clusters became evident as shown in Figure 3.4. The individual ring data sets ($R^2 \approx 0.40$ to 0.70) had higher R^2 values than those of the earlywood and latewood ($R^2 \approx 0.2$ to 0.6) datasets.

Fiber length calibration equations varied in accuracy from $R^2 \approx 0.40$ (site 4) to $R^2 \approx 0.70$ (site 3) (Table 3.3). The results suggested that environmental factors such as site differences may affect fiber length more than the other two traits. This was evident when data from both sites were used to create calibration equations. Correlations based on the whole core data set were lower than those from individual sites and the corresponding SEC was higher (Table 3.2).

NIR spectroscopy is mainly used to measure overtone and combination bands of the fundamental stretching vibrations of O-H, N-H, and C-H functional groups and contain chemical and physical information about a sample [*28*]. However, in this study two physical properties of wood, coarseness and average fiber length, were predicted moderately well using transmittance NIR. This may be due to their genetic correlation with α-cellulose yield (Table 2.4). The predictability of COA and FLW may be due to pleotrophic effects, where the same gene has effects on more than one trait simultaneously. Although calibration equations for FLW and COA produced lower R^2 values than ACY equations, there was a

moderate association which may be useful for using NIR spectra in predicting COA and FLW.

In general, using linear regressions calibration equations from site 3 to predict site 4 characteristics was successful. Prediction of ACY and COA ($R^2 \approx 0.63$) for NIR spectra was moderate. Although, these R^2 values were lower than the individual site predictions, calibration equations may be employed to screen large number of trees for high or low ACY and COA for ranking and selection purposes. On the other hand, the model for FLW from site 3 was not successful in predicting the NIR spectra from site 4, with a low R^2 (0.43) and a high SEP (0.437 mm) values.

Selecting trees earlier will allow breeders to decrease breeding cycle intervals and, therefore, increase greater gain per tear [*3*]. Age-age correlations calculated between ring 3 and ring 8 were moderately high for ACY (r = 0.78) and for COA (r = 0.82). Ring 3 explained 61% of the variation in ring 8 for ACY. Similarly, 68% of the variation in ring 8 for COA was explained by ring 3. However, the decisions to select for ring 8 at ring 3 should be based on correlated response between two traits.

When the results of this study are interpreted, several factors should be considered. Although the sites were within 15 miles distance, site 3 was located on a river bottom site with faster growth and greater uniformity than site 4, which was established on an upland piedmont site. These environmental factors may have impacted the predictions. Due to time constraints, analysis of wood cores of the two sites were completed by different technicians

in the lab, which may have introduced variations or bias between sites. The families sampled for this study also differed between sites. Genetic differences among families may have created lower predictions in site 4. Finally, wet chemistry methods dictated the thickness of the wood wafer that was used and apparent differences in thickness of the wood wafers were noted. In general, latewood of both rings was difficult to cut in 200 micrometer slices since the latewood fibers tend to separate causing a thicker wafer. These slices were avoided when visibly noted and, therefore, latewood data sets were derived from slightly fewer wafers than earlywood data sets. Wood sampling methods may have introduced bias in comparing earlywood and latewood relationships.

3.5 Conclusions

This study demonstrated that calibrations for ACY, COA, and FLW can be developed using NIR spectra from the transmittance measurements using thin wood wafers cut from increment cores. Predicting the wood properties between two sites may be possible with moderate success for ACY and COA, but predicting for FLW were poor. Calibrations based on individual sites using combined data were successful with R^2 ranging from 0.55 to 0.86. Predicting ring 8 spectra using ring 3 calibration equations is possible for ACY and COA with R^2 values around 0.60. To ensure higher R^2 values, accuracy of the lab data is critical.

3.6 Acknowledgements

This study was supported a grant from the Department of Energy (Grant DE-FC36-01GO10624), NCSU-Industry Cooperative Tree Improvement Program, Department of Forestry, and Department of Wood and Paper Sciences at N. C. State University.

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Table 3.1. Descriptive statistics of chemical wood traits obtained by NIR spectra for sub data sets 3E, 3L, 8E, and 8L at individual test sites and for the combined sites.

	Wood Property[1]	Minimum	Maximum	Mean	SD
Combined site n=240	ACY (%)	35.6	55.2	44.7	4.3
	COA	0.223	0.699	0.369	0.101
	FLW (mm)	0.49	3.04	1.60	0.53
Site 3 n=128	ACY (%)	35.6	54.3	43.3	4.5
	COA	0.223	0.547	0.337	0.071
	FLW (mm)	0.85	3.04	1.75	0.49
Site 4 n=112	ACY (%)	36.6	55.2	46.3	3.6
	COA	0.242	0.699	0.406	0.116
	FLW (mm)	0.49	2.66	1.42	0.52

[1] ACY is α-cellulose content, COA is coarseness, and FLW is average fiber length, SD is standard deviation.

Table 3.2. Linear regression models fitted to predict laboratory measured wood chemical traits using NIR spectra as the independent variable for the combined sites and for latewood (3L and 8L), earlywood (3E and 8E), ring 3 (3E and 3L), ring 8 (8E and 8L), and for the whole core (3E, 3L, 8E, and 8L).

		ACY (%)[1]			COA			FLW (mm)		
Dataset	n	R^2	SEC	SECV	R^2	SEC	SECV	R^2	SEC	SECV
Latewood	130	0.63	2.718	2.882	0.36	0.076	0.068	0.38	0.390	0.453
Earlywood	126	0.60	2.341	1.890	0.39	0.041	0.058	0.27	0.367	0.350
Ring 3	136	0.57	2.162	2.232	0.44	0.056	0.053	0.18	0.266	0.303
Ring 8	104	0.56	2.510	3.400	0.52	0.070	0.089	0.27	0.505	0.560
Whole Core	242	0.75	2.417	2.134	0.64	0.059	0.066	0.43	0.387	0.392

[1] R^2 is coefficient of determination, SEC is standard error of calibration, SECV is standard error for cross validation, ACY is α-cellulose content, COA is coarseness, and FLW is average fiber length

Table 3.3. Linear regression models fitted to predict laboratory measured wood chemical traits using NIR spectra as the independent variable for individual sites and for sub data sets latewood (3L and 8L), earlywood (3E and 8E), ring 3 (3E and 3L), ring 8 (8E and 8L), and for the combined data (3E, 3L, 8E, and 8L).

A) Site 3

		ACY (%)[1]			COA			FLW (mm)		
Dataset	n	R^2	SEC	SECV	R^2	SEC	SECV	R^2	SEC	SECV
Latewood	72	0.88	1.882	2.322	0.61	0.053	0.037	0.71	0.231	0.345
Earlywood	65	0.73	1.545	1.305	0.54	0.022	0.026	0.67	0.255	0.275
Ring 3	73	0.68	1.617	1.715	0.74	0.029	0.038	0.60	0.203	0.222
Ring 8	65	0.83	1.811	2.074	0.74	0.042	0.046	0.68	0.283	0.285
Whole Core	141	0.86	1.931	2.212	0.73	0.044	0.045	0.72	0.309	0.354

B) Site 4

		ACY (%)[1]			COA			FLW (mm)		
Dataset	n	R^2	SEC	SECV	R^2	SEC	SECV	R^2	SEC	SECV
Latewood	55	0.63	2.037	3.099	0.24	0.084	0.079	0.42	0.363	0.278
Earlywood	60	0.70	1.435	1.692	0.47	0.054	0.064	0.48	0.282	0.203
Ring 3	51	0.51	2.189	1.853	0.41	0.076	0.098	0.54	0.235	0.292
Ring 8	60	0.46	2.606	3.100	0.35	0.098	0.055	0.55	0.448	0.475
Whole Core	100	0.73	1.992	2.135	0.63	0.067	0.079	0.55	0.297	0.372

[1] R^2 is coefficient of determination, SEC is standard error of calibration, SECV is standard error for cross validation, ACY is α-cellulose content, COA is coarseness, and FLW is average fiber length

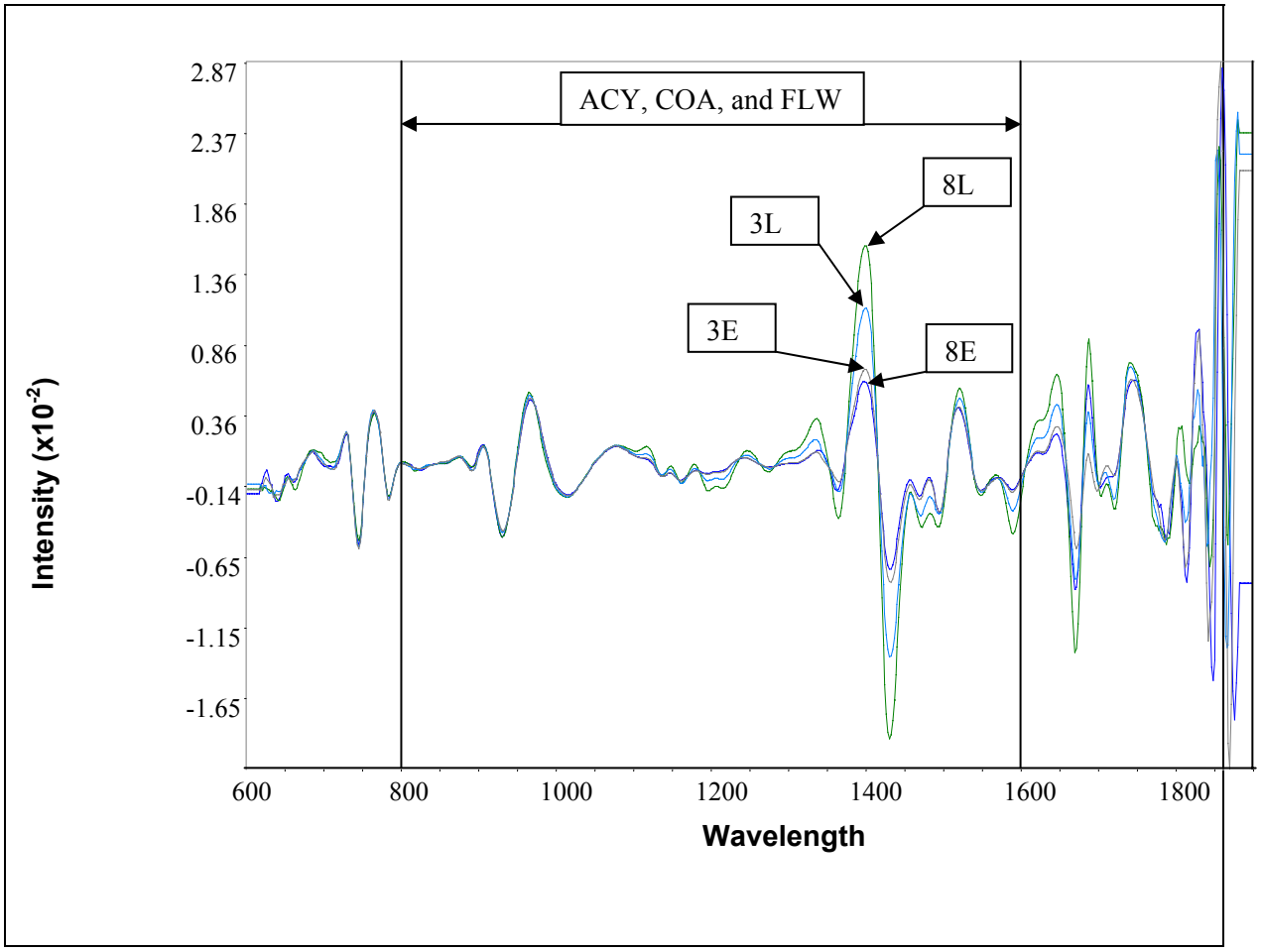

Figure 3.1. Example of a second derivative NIR spectra for ring 3 earlywood (3E), ring 3 latewood (3L), ring 8 earlywood (8E) and ring 8 latewood (8L) for of one sample. Cut off points used for α-cellulose content (ACY), coarseness (COA), and weighted fiber length (FLW) were wavelengths 800-1600nm.

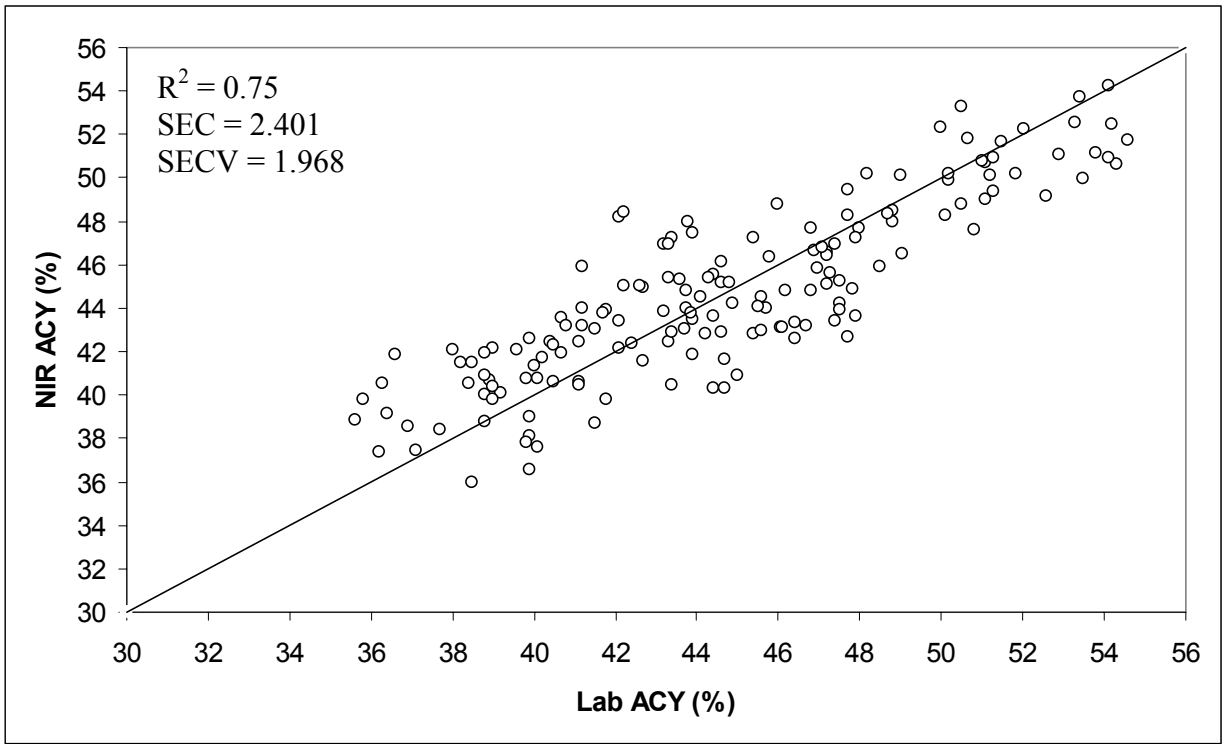

Figure 3.2. Fitted regression equation for combined sites to predict α-cellulose content (ACY) using NIR spectra. Standard error of prediction (SEP) and R^2 of each model were given with the scatter plots (n ≈ 180)

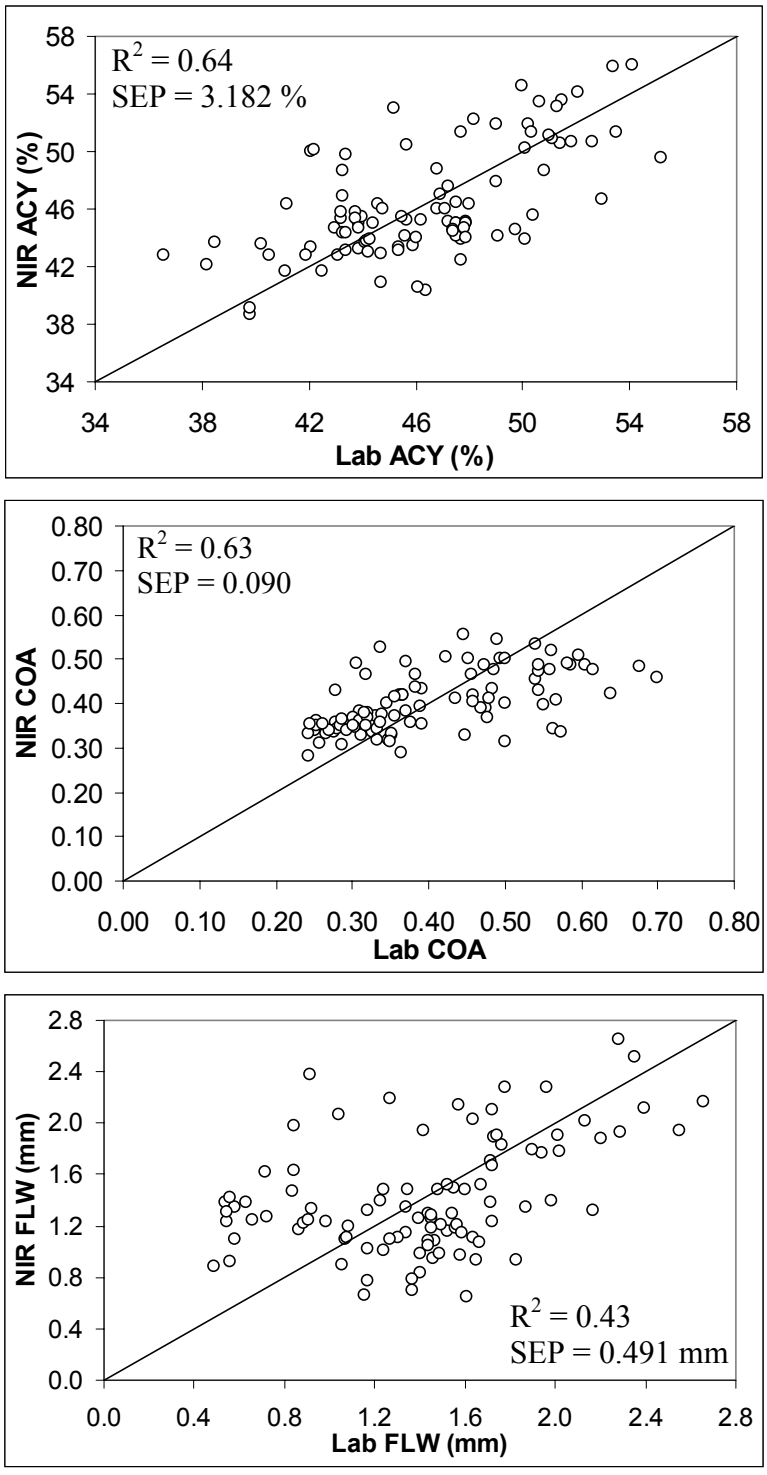

Figure 3.3. Comparison of wood properties predicted using NIR calibration models from site 3 and lab measurements for samples from site 4 for α-cellulose content (ACY), coarseness (COA) and average fiber length (FLW). Standard error of prediction (SEP) and R^2 of each model were given with the scatter plots. (n = 99).

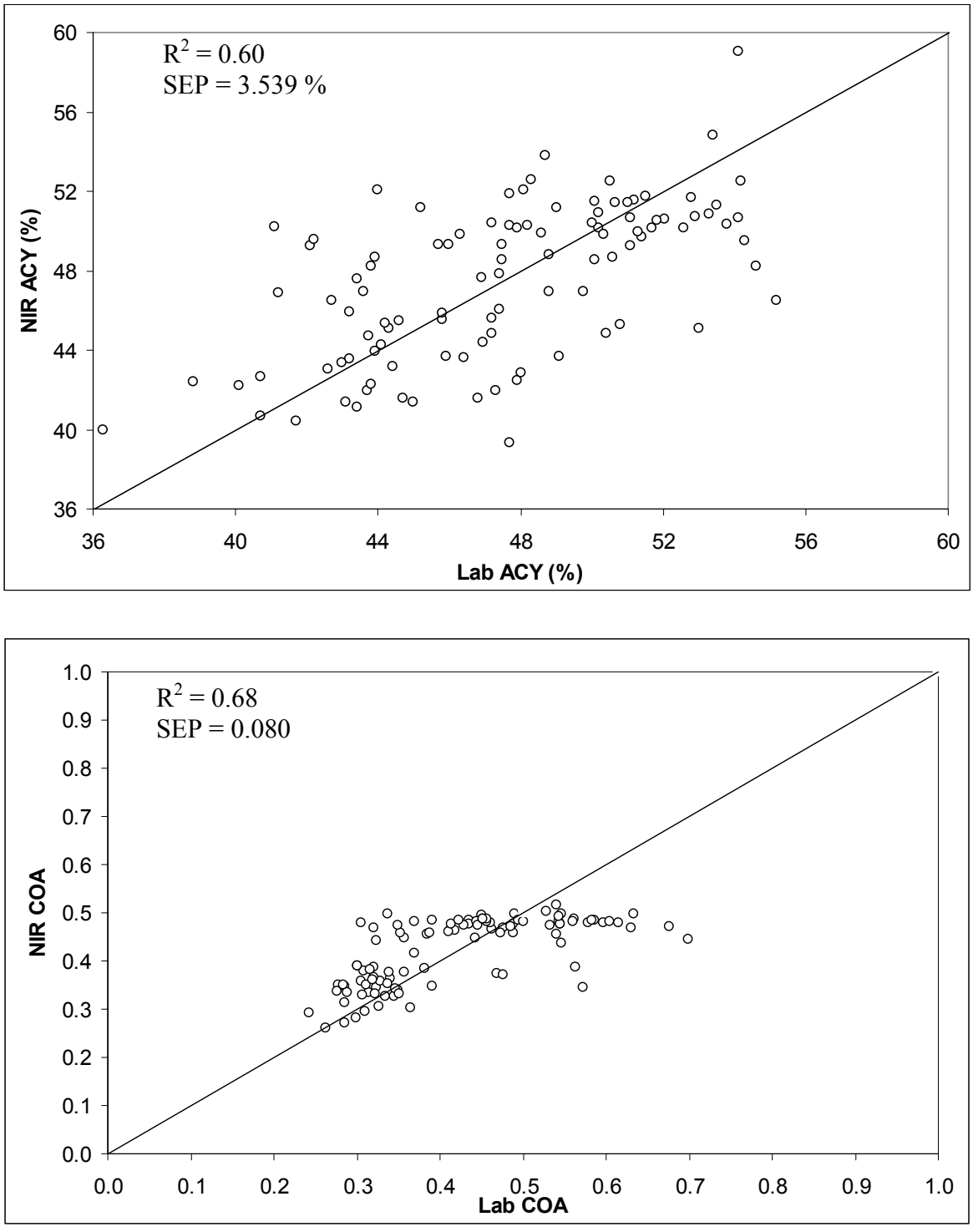

Figure 3.4. Comparison of wood properties predicted using NIR calibration models from juvenile wood (ring 3) and lab measurements for samples from transition wood (ring 8) for α-cellulose content (ACY) and coarseness (COA). Standard error of prediction (SEP) and R^2 of each model were given with the scatter plots. (n = 103).

General Summary

This study examined the genetic variation, genotype by environment interaction (GxE), and rapid assessment by near-infrared spectroscopy for several important wood properties in loblolly pine. Preliminary results from this study showed that there was important genetic variation and noticeable genotype by environmental interaction for these important wood properties. Results also demonstrate the possibility for rapid measurement of these traits using NIR. The potential manipulation of these wood properties through tree breeding could have profound effects on both the forest products and the pulp and paper industries.

Genetic variation was detected for all the chemical and morphological wood traits on one site and combined across two sites. High positive genetic correlations between cellulose content, fiber length and wood density suggested simultaneous improvement of chemical wood properties and wood density may be possible.

Although a genotype by environment interaction for ACY, FLW, and COA was detected, it was primarily caused by large site by specific combining ability interaction. The additive genetic effect by site interaction was not significant. These results were based on samples from only two test sites. Different sample sizes may have contributed to the interaction. In order to confirm the significance of the interaction and better predict the genetic parameters, additional sites need to be analyzed.

Tree straightness appeared to have favorable correlations with several wood traits, suggesting that selection for straightness may lead to increase of cellulose, fiber length and coarseness,

while decreasing lignin content. There was a weak negative correlation of these traits with growth, indicating it may be possible to improve these traits simultaneously in a tree improvement program by developing a selection index for multiple traits.

This study demonstrated that calibrations for ACY, COA, and FLW can be developed using NIR spectra from the transmittance measurements using thin wood wafers cut from increment cores. Predicting the wood properties from one site to another may be possible with moderate success for ACY and COA, but not for FLW. Calibrations based on individual sites using the whole core data set were successful with R^2 ranging from 0.55 to 0.86. Predicting ring 8 spectra using ring 3 calibration equations is possible for ACY and COA with R^2 values around 0.60. In order to ensure higher R^2 values, accuracy of the lab data is critical.

Trees in this study were sampled over a small geographic region in South Carolina, and results may not be applicable for the whole loblolly pine population in the southeast. In order for this research to be applied in breeding programs, more parents will need to be analyzed. This would more accurately sample the total genetic variation that is available in the population for loblolly pine. Including more parents over a wider geographical range would allow the resulting NIR calibration equations to be applicable to larger populations, and possibly be useful in breeding programs for estimating wood properties of trees in large progeny tests. Future research plans include the addition of genetic material from other regions of the southern United States. Analyzing additional sites will increase the precision in measuring genotype by environment interactions.

The outlook for use of NIR spectroscopy to predict chemical and possibly physical wood traits are promising. Time and cost can be reduced dramatically if this technology can be used on a wide scale. Calibrations must be accurate, and include individuals that represent the environments of the trees that will be measured to ensure accurate estimation. Tree breeding programs may be able to use NIR to quickly assess wood properties of progeny tests and base selection on these in addition to growth characteristics, as techniques are refined.