

PHYSIOLOGICAL GENETICS OF CONTRASTING LOBLOLLY AND SLASH PINE
FAMILIES AND CLONES

By

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by

Veronica I. Emhart

To my parents and brothers, who loved and supported me along my journey.

To my friends, whose company filled my heart with happiness all five years.

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Abstract of Dissertation Presented to the Graduate School
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My study focused on the biology and genetic structure of 300 clones from five different full-sib loblolly and slash pine families. The study was divided into three main areas of research: (1) detailed quantification of crown structure and estimation of annual absorbed photosynthetically active radiation (APAR); (2) seasonal dynamics and phenology of basal area growth and its association with soil water balance; and (3) leaf carbon isotope discrimination and whole-tree sap flow.

Genetic variation in crown structural traits, APAR, stem volume growth, basal area growth phenology, basal area growth rates, leaf carbon isotope discrimination ($\Delta^{13}\text{C}$), and crown conductance were more apparent at the clonal level than at the species and family levels.

The one loblolly pine family we studied tended to grow faster, developed larger crowns with more acute branch angles, had more leaf area and intercepted more radiation than the four slash pine families averaged. Loblolly and slash pine within-family

individual-tree broad-sense heritabilities (H^2_{WF}) ranged from 0.00 to 0.41 for growth and crown structural traits, and most were between 0.10 and 0.25 when estimated from a combined analysis across families. Genetic correlations of crown size, leaf area, and APAR with volume increment were generally positive.

Basal-area growth spanned March through October for both species. In both years, peaks in basal-area increment occurred in short (2-3 week) periods in the early spring for all families, followed by relatively constant rates of basal-area growth until cessation. The H^2_{WF} ranged from 0.01 to 0.37 for basal area growth phenology. Both the strength and direction of correlation estimates of phenological traits with growth rate varied across families and years.

Clonal mean values for $\Delta^{13}C$ ranged from 19‰ to 25.45‰. The H^2_{WF} for $\Delta^{13}C$ and crown conductance parameters ranged from 0.01 to 0.32. Genetic and environmental correlations of stem growth with $\Delta^{13}C$ or with crown conductance were low. There was no evidence of clone-by-year interaction in stem growth, basal-area growth phenology, and $\Delta^{13}C$ for any family.

Understanding the biology of physiological processes and their genetic parameters gives us insight into the key functional and structural traits that determine genotype performance differences in southern pines.

CHAPTER 1 INTRODUCTION

Loblolly pine (*Pinus taeda* L.) and slash pine (*Pinus elliottii* Engelm. var. *elliottii*) are widely planted as commercial timber species in the southeastern United States (Smith *et al.* 2004). From the early 1950s, large-scale tree-breeding programs in both species improved forest productivity by selecting trees for superior growth rate, form, and disease resistance (McKeand *et al.* 2003). The genetically improved material currently being established in commercial plantations is deployed from bulked orchard seed, half-sib families, and full-sib families (with growing interest in the deployment of outstanding clones). Tree breeding has proven to be a very effective tool, and breeding will continue to be the most important mechanism for developing recombinant genotypes to achieve increasing genetic gains (White and Carson 2004).

Management of southern pine plantations in the United States is being transformed from a relatively extensive system of planting coupled with isolated individual treatment, to a much more intensive system in which genetic and site resources are manipulated in concert, to optimize stand productivity (Fox *et al.* 2004). Increased productivity in southern pines create more value for the forest industry, and decreases pressure on native pine forests, considering that the demand for forest products will continue to increase and intensive management will be needed to meet this demand. Increased forest productivity also provides great potential for sequestering atmospheric carbon (Johnsen *et al.* 2004).

Clonal forestry may be an excellent way to increase the productivity of southern pine plantations in the near term (Fox *et al.* 2004). Many potential benefits of clonal

forestry have been previously described (Libby 1982; Libby and Rauter 1984; Carson 1986) and include:

- Gains arising from testing and selection of clones;
- Clone/site matching to increase genetic gains by capturing favorable genotype by environment effects (G x E), and by targeting expression to existing site properties;
- Greater uniformity (little impact on growth and yield traits, but extremely valuable for log and wood quality and disease resistance traits, and for harvesting and processing);
- Greater repeatability (better yield prediction and planning). Specific clones can be identified that are most adapted to different site qualities. Identifying these clones help us take advantage of positive genotype X environment interaction and to optimize silvicultural practices, including spacing, weed control, and fertilizer regimes. To identify superior clones within-family we must understand the biological basis for growth differences and identify key structural and functional attributes at the organ, tree, and stand level.

Growth involves many integrated physiological processes influenced by genetic and environmental factors (Kozlowski and Pallardy 1997). Processes like radiation absorption, carbon gain capacity, crown conductance responses to changes in the environment, growth phenology, nutrient assimilation, and growth regulation may control stem volume growth in contrasting southern pine families and clones.

Better understanding the physiological processes underlying genetic differences in growth performance may allow geneticists to be more deliberate in their selections, focusing specific objectives and allowing for more predictable gains. Martin *et al.* (2005) described several potential obstacles to ecophysiological contributions to tree improvement programs:

- Selecting wrong physiological parameters for screening;
- Making physiological measurements at inappropriate spatial and temporal scales;
- Attempting to use seedlings to predict field performance of adult trees.

My overall goal was to investigate biological traits and their genetic structure in 300 clones from five different full-sib loblolly and slash pine families. My study used measurements that integrated biological information over space and time, with the intent of studying biological traits that correspond more closely to the spatial and temporal scales at which growth was observed (e.g., whole trees over seasons to years).

The study was divided into three main areas of investigation:

- The first phase used detailed crown structural information for each ramet within-clone to parameterize the process model MAESTRA, which was then used to estimate the total amount of radiation intercepted by each ramet over a year;
- Second, repeated basal-area measurements of each ramet was used to estimate seasonal dynamics and phenology of basal-area growth, associated with a soil-water balance calculation to examine relationships between basal area growth and integrated environmental variables;
- Third, leaf carbon isotope discrimination analysis (integrates leaf physiology over the time of leaf formation) and whole-tree sap flow analysis (integrates leaf physiology over the tree crown over long periods of time) was used to analyze integrated gas exchange properties and its relationship with growth.

My study focused on the following specific objectives, organized by main areas of investigation:

- **Specific aim 1a:** quantify growth and crown structural variation among species, families and clones representing a range of growth performance in loblolly and slash pines;
- **Specific aim 1b:** integrate crown structural variables into a radiative transfer model to estimate variation in intercepted radiation for different genotypes for a given period of time and their relationship with growth rate;
- **Specific aim 1c:** estimate within-family genetic control and environmental influence on crown structural attributes and growth;
- **Specific aim 2a:** compare two years basal area growth phenology among species, families and clones;
- **Specific aim 2b:** estimate genetic parameters for basal area growth phenology, its correlation with growth rates, and the genotype interaction with seasonal environmental changes;

- **Specific aim 3a:** determine whether genetic variation for leaf carbon isotope discrimination occurs among slash and loblolly pine genotypes (species, families, or clones);
- **Specific aim 3b:** examine genetic variation in crown-level stomatal conductance (crown conductance) sensitivity to vapor pressure deficit between species, among slash pine families, and among clones within slash and loblolly pine families;
- **Specific aim 3c:** determine broad-sense heritabilities and genetic correlations for leaf carbon isotope discrimination, growth and crown conductance.

Results from my study should positively impact future tree growth modeling and will help in decisions that involve genotype deployment and silvicultural treatments.

Results will also aid in examining genetic and environmental control of several key structural and functional processes that determine productivity in different full-sib families and clones within-family in southern forest plantations.

CHAPTER 2
CLONAL VARIATION IN CROWN STRUCTURE, ABSORBED
PHOTOSYNTHETICALLY ACTIVE RADIATION, AND GROWTH OF LOBLOLLY
AND SLASH PINE

Introduction

Crown structural characteristics (such as crown size, branching frequency, branch diameter, branch angle, and leaf area quantity and spatial distribution) influence the efficiency and magnitude of radiation interception and competitive interactions with other trees (Wang and Jarvis 1990; Stenberg *et al.* 1994; Vose *et al.* 1994; McCrady and Jokela 1996, 1998). As a result, crown architecture is an important determinant of both tree-level and stand-level productivity (Dalla-Tea and Jokela 1991; Stenberg *et al.* 1994; McCrady and Jokela 1996). This linkage is often reflected in ideotypes or conceptual models of desirable tree phenotypes intended to guide plant genetic research and breeding programs (Donald 1968; Dickmann *et al.* 1994). For example, the published ideotypes for *Populus* (Dickmann 1985; Dickmann and Keathley 1996) and Scandinavian conifers (Karki and Tigerstedt 1985) incorporate numerous crown structural variables. Genetic variation in growth has been the subject of much research (White 1996), and forms the basis of most commercial tree improvement programs (White *et al.* 1993; McKeand and Bridwater 1998; Li *et al.* 2000). In contrast, the genetic architecture of crown structure has been much less intensively studied, and is seldom used in tree improvement programs (Martin *et al.* 2001).

Xiao *et al.* (2003) showed that significant differences in growth between loblolly pine (*Pinus taeda* L.) and slash pine (*Pinus elliottii* Engelm. var *elliottii*) were associated

with variation in crown structure and biomass allocation. At age 3 and 4 y, loblolly pine had more branches per tree and allocated more biomass to branches than slash pine. Greater branch/leaf biomass as a growth strategy might develop spacious crowns facilitating faster growth by increasing the leaf-area carrying capacity in the crown.

Knowledge of heritabilities and genetic correlations is needed to understand the genetic structure of breeding populations, and to determine deployment strategies in tree improvement programs (White 1987). Broad-sense heritabilities for a number of structural and growth properties have been estimated for *Populus* and *Eucalyptus* (Wilcox and Farmer 1967; Weber *et al.* 1984; Borralho *et al.* 1992; Lambeth *et al.* 1994; Osorio 1999). Genetic correlations between growth traits and crown structural attributes are scarce, but studies in *Populus*, *Eucalyptus*, loblolly and slash pine have identified positive genetic correlations between growth performance and branching patterns, and growth performance and crown vigor (Wilcox and Farmer 1967; Lambeth *et al.* 1994; Lambeth and Huber 1997; Xiao *et al.* 2003). Incorporating new information on crown structural attributes (such as crown size, crown shape ratio, and arrangement and diameter of branches) would improve our understanding of how canopy structure affects absorbed photosynthetic active radiation and stand development. Crown structural attributes also may prove useful in selection of families or clones for silvicultural programs, and development of new crop tree ideotypes.

Our objectives were as follows:

- Quantify growth and crown structural variation among species, families, and clones, representing a range of growth performance in loblolly and slash pines;
- Integrate crown structural variables into a radiative transfer model to estimate variation in intercepted radiation for different genotypes for a given period of time and to estimate their relationship with growth rate;

- Estimate within-family genetic control and environmental influence on crown structural attributes and growth.

According to Martin *et al.* (2005), one reason ecophysiological research has failed to contribute to southern pine tree improvement programs is that researchers have focused on small spatial and short temporal scales that are too far removed in space and time from growth processes. Accordingly, we hypothesized that tree growth would be genetically correlated with crown structural traits, and that traits which integrated information over space and/or time would be more highly correlated with growth than would less-integrated traits.

Materials and Methods

Site Description and Plant Material

The study area was located on lands managed by Rayonier Inc. in Bradford County, Florida. The climate is humid and subtropical, with a mean annual temperature of 21°C, mean annual rainfall of 1316 mm, and over 50% of the rainfall occurring in June through September. Periods of drought are normal in the spring and fall. Mean annual rainfall during 1999-2001 was 967 mm, in contrast to 1405 mm in year 2002 (NOAA 2002). The soils are classified as Pomona and consist of very deep, somewhat poorly to poorly drained soils that are formed in sandy and loamy marine sediments (sandy, siliceous, hyperthermic Ultic Alaquods). Slopes are 0 to 2 %. In a typical profile, the spodic horizon occurs at 30-60 cm, with an argillic horizon at 90-120 cm. Water table is typically at a depth of 15 to 45 cm for one to three months and a depth of 25 to 100 cm for six months or more, during most years (Soil Survey Staff 1998).

The study took place in an area containing 16 full-sib and half-sib loblolly and slash pine families planted in 337 m² family plots in January 1997. The experiment was

designed as a randomized complete block with four replicates (Appendix A). We used one full-sib loblolly pine family and four full-sib slash pine families. Each family plot contained 60 clones propagated as rooted cuttings from a single family, planted at 1.7 m x 3.4 m spacing (1730 trees ha⁻¹). Cuttings were taken from donor hedges in the spring, and were rooted and grown in a greenhouse for six months before planting. Each of the four plots of the same family contained the same 60 genotypes, but with the ramets planted into different, randomly-determined planting locations in the plot. In total we studied approximately 1,200 trees: 60 trees per family plot x 5 families x 4 replications. Fertilization and weed control were applied periodically to reduce interspecific competition and prevent nutrient deficiency (Appendix B).

Growth and Crown Architectural Traits

Stem volume growth in the 2000, 2001, and 2002 growing seasons (ages 4, 5, and 6 y, respectively) was determined from dormant-season measurements of tree diameter at 1.37 m height (DBH) and total tree height (HT). Outside-bark individual-tree stem volume was calculated with a general equation (Hodge *et al.* 1996) as shown in Equation 2-1, where DBH and HT were entered in m:

$$\text{VOL (dm}^3\text{)} = (0.25 * 3.14 * (\text{DBH})^2 * (1.37 + 0.33 (\text{HT} - 1.37))) * 1000 \quad (2-1)$$

Crown architecture was assessed by measuring length and width of the living crown and basal diameter of all living branches at the end of the 2001 and 2002 growing seasons. Also, branch angle was measured in four branches in the 2000 cohort of each tree, with a protractor. Other traits derived from these records included total number of branches per tree, crown shape ratio (CSR= crown height/ crown width), and branch-free stem height. Individual-tree leaf area at age 5 y was calculated by summing individual branch leaf area estimated from regional allometric equations (McGarvey 2000).

Regression equations were developed between crown size at age 5 y (independent variable) and leaf area at age 5 y (dependent variable) by family; and then leaf area at age 6 y was predicted using crown volume at age 6 y by family.

Estimating Absorbed Photosynthetically Active Radiation (APAR)

Total APAR was simulated for each tree in the study from January 1, 2002 to December 31, 2002, using hourly radiation data from a weather station at the site, input into the process model MAESTRA, a modification of the MAESTRO model (Wang and Jarvis 1990; Medlyn 2004). MAESTRA uses Norman and Welles (1983) method to calculate PAR at grid points within the crown, taking into account the spatial distribution of foliage in the target crown and in adjacent tree crowns. Crown shapes were assumed to be ellipsoidal. Vertical foliage distribution was specified by a Beta function developed for loblolly pine in North Carolina (Luo *et al.* 2001), while horizontal foliage distribution was assumed to be uniform. Simulations were run for each tree, in each of the 20 study plots. For each tree, the location, crown radius in two directions, total tree height, height to the base of the live crown, and leaf area were specified. Tree locations, crown dimensions, and leaf area for a two-tree border surrounding each study plot were also specified. When study plots were near non-study plots, crown dimensions and leaf area of border trees were predicted from measured height and diameter. Crown dimensions were assumed to increase linearly from March 1st to December 1st.

Statistical Analysis

Analysis of variance (ANOVA) was used to analyze growth, crown structural traits, and APAR data. PROC GLM in the SAS® System were used to test for significance of random effects (clone), while PROC MIXED was used to test the fixed effects (species and families). Equation 2-2 shows the ANOVA model for the analyses, where Y_{ijkl} is the

performance of the ramet of the l^{th} clone within the k^{th} family nested in the j^{th} species in the i^{th} replication; $i = 1, 2, 3,$ and 4 for replications; $j = \text{slash}, \text{loblolly}; k = 1, 2, 3, 4,$ and 10 for families; $l = 60$ identification numbers for 60 clones within each of the five families:

$$Y_{ijkl} = \mu + b_i + S_j + F_{k(j)} + c_{l(jk)} + bS_{ij} + bF_{ik(j)} + \varepsilon_{ijkl} \quad (2-2)$$

μ = population mean,

b_i = random variable of replication $\sim \text{NID}(0, \sigma_b^2)$,

S_j = fixed effect of species (slash or loblolly),

$F_{k(j)}$ = fixed effect of family nested within species,

$c_{l(jk)}$ = random variable of clone nested within-family and species $\sim \text{NID}(0, \sigma_c^2)$,

bS_{ij} = random variable for replication x species interaction $\sim \text{NID}(0, \sigma_{bS}^2)$,

$bF_{ik(j)}$ = random variable for replication x family(species) interaction $\sim \text{NID}(0, \sigma_{bF}^2)$, and

ε_{ijkl} = error term $\sim \text{NID}(0, \sigma_\varepsilon^2)$.

Genetic Parameter Estimation

For each species and family, two types of parameters were estimated: within-family heritability for each trait, and genetic and environmental correlations among traits.

Within-family variance and covariance components were obtained using Multiple Trait Derivate-free Restricted Maximum Likelihood (MTDFREML) software (Boldman *et al.* 1995).

Within-family individual-tree broad-sense heritability was calculated as

$$H^2_{WF} = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_\varepsilon^2} \quad (2-3)$$

Theoretically, broad-sense within-family heritability for full-sib families contains $\frac{1}{2}$ the additive genetic variance, $\frac{3}{4}$ of the dominance genetic variance, and most of the epistatic genetic variance (Falconer and Mackay 1996). The standard error for heritability estimates was calculated using a method described by Dickerson (1962). The residual likelihood ratio test (Wolfinger 1996) was used to test heterogeneity of variances among

slash pine families, and heritabilities were estimated separately for each family ($X^2_{(6, 0.05)} = 12.6$), or pooled, as appropriate. We estimated all genetic parameters from data collected from only one experimental site; therefore, the clonal genetic variance contains the clone-environment interaction variance in the above results, and the estimated genetic parameters are biased upward if the interaction is non-zero (Hodge and White 1992).

Within-family genetic and environmental correlations among growth traits and crown structural variables (Falconer and Mackay 1996) were calculated as shown in Equation 2-4, where σ_{xy} is the covariance (clonal or residual) between two traits, and σ_x σ_y corresponds to the square root of the product of the clonal or residual variance within-family of each trait:

$$r_{xy} = \frac{\sigma_{xy}}{\sigma_x \sigma_y} \quad (2-4)$$

Results

Genetic Variation in Stem and Crown Traits

We examined variation in cumulative stem volume, annual stem volume growth, and crown architectural and functional traits. Comparisons were made between species (loblolly vs. slash pine), among families within species (four full-sib slash pine families), and among clones within species (60 clones within each of the four slash pine families and one loblolly pine family).

By age 6 y, loblolly pine stem volume was almost 25% larger than mean slash pine stem volume ($p=0.0727$, 31.42 dm^3 vs. 25.47 dm^3 , respectively), reflecting fairly consistent species-level differences in annual stem volume increment (Table 2-1). Within slash pine, there were consistent differences among families ($p<0.10$) in stem volume and stem volume increment, with the exception of age 5-6 yr increment (Table 2-1, Figure 2-

1). Within-family clonal variation in stem volume and stem volume increment was highly significant for all years for slash pine ($p < 0.0001$, Table 2-1).

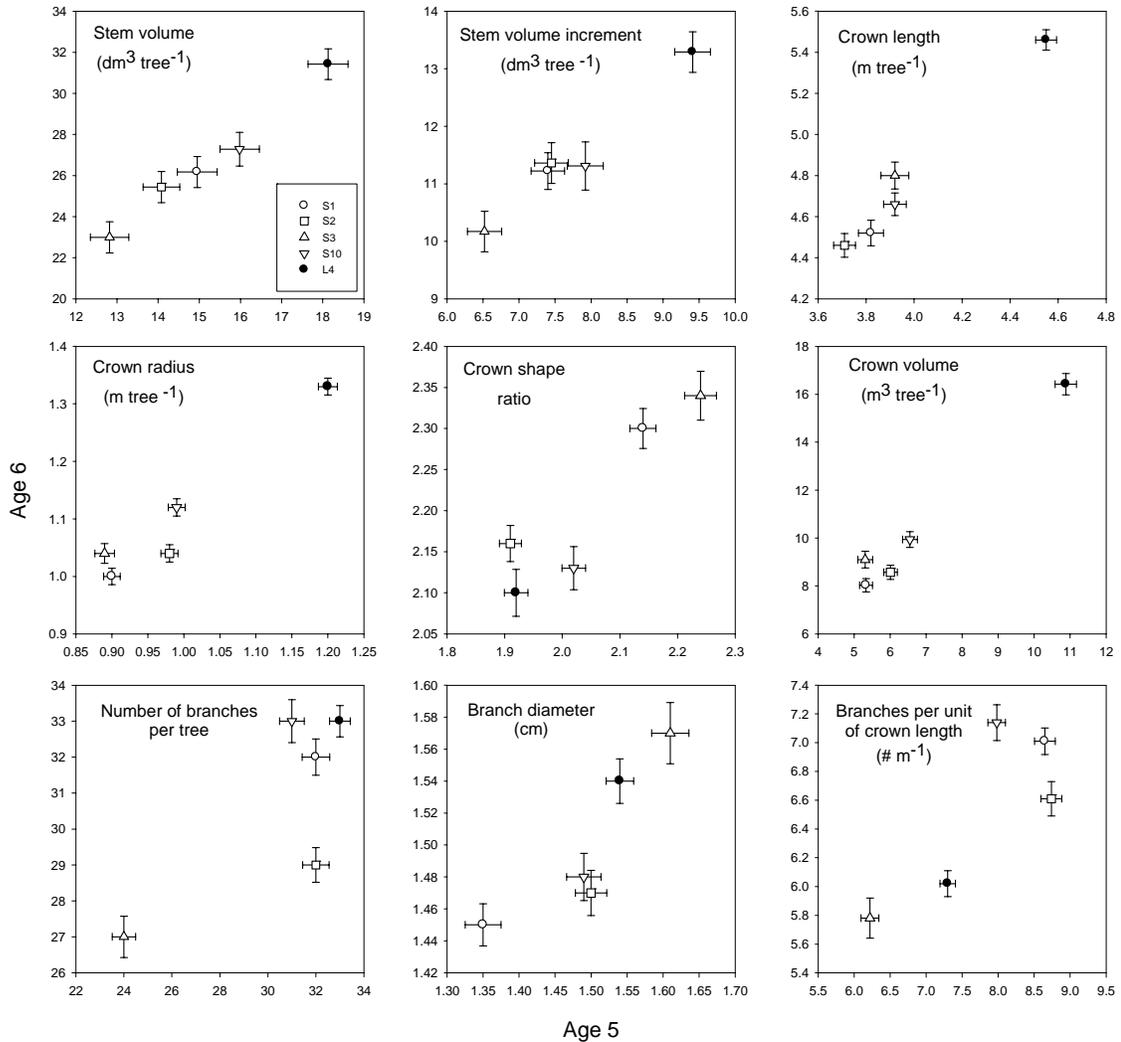


Figure 2-1. Family means and standard error bars for individual-tree growth and crown structural traits for 5 and 6 year-old loblolly and slash pine families in north central Florida. S1=family slash 1; S2= family slash 2; S3=family slash 3; S10=family slash 10; L4=family loblolly 4.

There were species-level differences in a number of crown structural traits.

Loblolly pine had longer and wider crowns at age 5 and 6 y, resulting in species differences in crown volume on the order of 85% (Table 2-1). Slash pine crowns of a given length were slightly narrower than loblolly pine crowns of a similar length, as

quantified by the crown shape ratio: 2.23 vs. 2.10 at age 6 y for slash and loblolly pine, respectively (Table 2-1). Loblolly pine branches were displayed at a more acute angle than were slash pine branches: 51.1 vs. 56.9 °, respectively (Table 2-1). Age 5-6 y radiation interception, simulated with the MAESTRA radiative transfer model, was about 20% greater in loblolly pine (11,901 MJ/tree) than the mean slash pine annual APAR (9,901 MJ/tree). Numbers of branches per crown, branch diameter, and number of branches per unit crown length were not different between species (Table 2-1).

Crown structure also varied at the family level, with crown size and shape traits (length, radius, volume, and crown shape ratio) all varying significantly among the four slash pine families ($p < 0.10$). Slash pine families also differed in numbers of branches, branch diameter (at age 5 y), numbers of branches per unit crown length, and branch angle (Table 2-1, Figure 2-1). There was no significant family-level variation in tree leaf area or annual APAR. Within families, there was significant clonal variation for all traits measured ($p < 0.0001$, Table 2-1).

Within-Family Individual-Tree Broad-Sense Heritabilities

Within-family individual-tree broad-sense heritabilities (H^2_{WF}) were low to moderate for stem volume and crown structural traits. In loblolly pine, a number of crown structural traits were moderately heritable, with crown radius at age 5 yr, crown volume at age 6 yr, leaf area at age 6 yr, number of branches at age 5 yr, and branch angle at age 5 yr having H^2_{WF} between 0.20 and 0.27. Stem volume and stem volume growth traits had lower H^2_{WF} , ranging between 0.05 and 0.18 (Table 2-1).

For volume in slash pine at different ages, H^2_{WF} was between 0.17 and 0.19, and crown structural traits showed similar ranges of variation (Table 2-1). When H^2_{WF} values were estimated separately by family due to heterogeneous variance components among

Table 2-1. Significance levels (p-values), species means and pooled within-family heritabilities (H^2_{WF}) for individual-tree growth and crown structural variables for 5 and 6 year-old loblolly and slash pine families in north central Florida.

Trait	Significance level by effect			Species mean		H^2_{WF}	
	Species	Family	Clone	Slash	Loblolly	Slash ^a	Loblolly
<i>Inventory</i>							
Volume age 4 (dm ³ tree ⁻¹)	0.2240	0.0797	<0.0001	7.13	8.72	--	0.05 (0.06)
Volume age 5 (dm ³ tree ⁻¹)	0.1007	0.0451	<0.0001	14.46	18.13	0.17 (0.04)	0.08 (0.07)
Volume age 6 (dm ³ tree ⁻¹)	0.0727	0.0802	<0.0001	25.47	31.42	0.17 (0.04)	0.18 (0.08)
Volume increment age 4-5 (dm ³ tree ⁻¹)	0.0455	0.0567	<0.0001	7.32	9.41	0.19 (0.04)	0.12 (0.07)
Volume increment age 5-6 (dm ³ tree ⁻¹)	0.0754	0.3324	<0.0001	11.02	13.29	--	0.18 (0.08)
<i>Crown structure</i>							
Live crown length age 5 (m)	0.0055	0.2358	<0.0001	3.85	4.55	0.16 (0.04)	0.09 (0.07)
Live crown length age 6 (m)	0.0028	0.0812	<0.0001	4.61	5.46	--	0.11 (0.07)
Crown radius age 5 (m)	0.0067	0.0106	<0.0001	0.94	1.20	--	0.20 (0.08)
Crown radius age 6 (m)	0.0041	0.0233	<0.0001	1.05	1.33	--	0.18 (0.08)
Crown shape ratio age 5	0.1001	0.0012	<0.0001	2.07	1.92	--	0.13 (0.07)
Crown shape ratio age 6	0.0519	0.0095	<0.0001	2.23	2.10	--	0.00 (0.00)
Crown volume age 5 (m ³)	0.0039	0.0913	<0.0001	5.80	10.88	--	0.19 (0.08)
Crown volume age 6 (m ³)	0.0020	0.0723	<0.0001	8.91	16.42	--	0.25 (0.09)
Leaf area age 5 (m ²)	0.0450	0.4940	<0.0001	33.11	44.07	0.12 (0.04)	0.08 (0.06)
Leaf area age 6 (m ²)	0.1197	0.5562	<0.0001	47.14	54.58	--	0.25 (0.09)
Number branches age 5	0.1214	0.0021	<0.0001	30	33	--	0.27 (0.09)
Number branches age 6	0.1610	0.0225	<0.0001	30	33	--	0.16 (0.07)
Branch diameter age 5 (cm)	0.5230	0.0793	<0.0001	1.49	1.54	0.14 (0.04)	0.10 (0.07)
Branch diameter age 6 (cm)	0.3292	0.1584	0.0003	1.49	1.54	--	0.19 (0.08)
Number branches/crown length age 5	0.2087	0.0018	<0.0001	7.90	7.30	--	0.11 (0.07)
Number branches/crown length age 6	0.1047	0.0136	<0.0001	6.63	6.02	--	0.14 (0.07)
Branch angle age 5 (°)	0.0099	<0.0001	<0.0001	56.9	51.1	0.18 (0.04)	0.26 (0.08)
Light interception age 5-6 (MJ tree ⁻¹)	0.0293	0.1041	<0.0001	9,901	11,901	0.17 (0.01)	0.17 (0.08)

Note: Values in parentheses are standard errors

^a-- Values of H^2_{WF} in slash pine were estimated separately by family and are in Table 2-2.

slash pine families, there was a tendency for higher heritabilities in family S2 (Table 2-2). For example, crown radius at ages 5 and 6 showed H^2_{WF} of 0.41, and crown volume at ages 5 yr and 6 yr had moderate heritability values between 0.34 and 0.36.

Within-Family Genetic and Environmental Correlations

Within slash pine families, the genetic correlations between individual-tree stem volume increment (ages 5 y and 6 y) were positive and moderate to high with: APAR between age 5 y and 6 y, crown size traits at age 5 y, and tree leaf area at age 5 y ($r_g=0.35$ to 0.74). Individual-tree stem volume increment had low positive or low negative genetic correlations with crown shape ratio and branch angle at age 5 y ($r_g=-0.33$ to 0.39, Table 2-3). For loblolly pine family L4, individual-tree volume increment between age 5 y and 6 y was moderately genetically correlated with APAR between age 5 y and 6 y ($r_g=0.64$), and crown size traits at age 5 y such as crown volume ($r_g=0.51$), crown radius ($r_g=0.47$), and crown length ($r_g=0.53$). Stem volume increment was positively, but less strongly correlated with leaf area at age 5 y ($r_g=0.31$). As in slash pine, traits such as crown shape ratio and branch angle at age 5 yr had much weaker genetic correlations with stem volume growth ($r_g=-0.20$ and 0.20, respectively Table 2-3).

Environmental correlations are measures of microsite environmental fluctuation between two traits measured on the same ramets. In slash pine families, moderately to highly positive environmental correlations were found between stem volume increment age 5 and 6 y and light interception age 5 and 6 y ($r_e=0.43$ to 0.83), implying that microsities that enhanced APAR also enhanced stem growth. At the same time, positive environmental correlations were found between stem volume increment age 5 and 6 y and crown size at age 5 (crown volume, crown radius, and crown length, $r_e=0.52$ to 0.76), and between stem volume increment age 5 and 6 y and leaf area age 5 y, number of

branches age 5 y, and branch diameter age 5 y ($r_e=0.36$ to 0.72 , Table 2-3). Finally, crown shape ratio age 5 y and branch angle age 5 had low positive or negative environmental correlations with stem volume increment age 5 and 6 y, implying that microsites that favored growth did not affect crown shape ratio age 5 y and branch angle age 5 y. For loblolly pine, environmental correlations had similar tendencies as in slash pine, with moderate positive environmental correlations between stem volume increment age 5 and 6 y and crown size at age 5 (crown volume, crown radius, and crown length, $r_e=0.49$ to 0.58), between stem volume increment age 5 and 6 y and leaf area age 5 y ($r_e=0.46$), and also between stem volume increment age 5 and 6 y and branch diameter age 5 y ($r_e=0.43$, Table 2-3). Weakly positive or negative environmental correlations were found between stem volume increment age 5 and 6 y and number of branches age 5 y, branch angle age 5 y and crown shape ratio age 5 y ($r_e=-0.12$ to 0.29).

Both APAR and crown volume at age 5 y proved to be good integrators of crown characteristics for individual trees. In general, APAR and crown volume at age 5 y had stronger genetic correlations with stem volume growth than did any other crown traits.

Discussion

At the species level, the one loblolly pine family we studied tended to grow faster than the average of our four slash pine families at ages 5 y and 6 y. At the same time, loblolly pine developed larger crowns with more acute branch angles and had more leaf area per individual-tree at age 5 y and 6 y than did the slash pine families (Table 2-1). Xiao *et al.* (2003) found similar species-level contrasts in juvenile loblolly and slash pine in north central Florida, where loblolly pine accumulated more crown volume per tree, allocated more biomass to branches, and had greater amount of leaf area than slash pine at ages 3 and 4 y. Stand-level studies have similarly confirmed the ability of loblolly pine

to develop and retain higher levels of leaf area than slash pine (Dalla-Tea and Jokela 1991; Martin and Jokela 2004).

Growth differences among slash pine families were subtle, probably because the families selected for my study were all chosen for superior growth potential. In spite of the apparent similarities in stem volume growth rate, the four slash pine families differed in a number of crown architectural traits. Contrasting families had different arrangements and sizes of branches within the crown, and varied in crown shape ratio (Table 2-1, Figure 2-1). This suggests that any of a number of crown traits may be associated with high growth rate in southern pine families (see also McGarvey *et al.* 2004). In contrast, McCrady and Jokela (1996) concluded that, among the five loblolly pine families they studied, there were significant differences in height growth but none for most branching attributes.

Within-family clonal variation was highly significant for all growth and crown structural traits, reflecting a wide spectrum of clonal performance in growth and crown development at these ages. There are few reports in the literature on clonal variation in loblolly or slash pine growth. Paul *et al.* (1997) reported that height of loblolly pine clones varied significantly at different ages, but that DBH and volume did not. To our knowledge, no published studies have quantified clonal variation in crown characteristics in loblolly or slash pine, but these traits have been studied in other forest tree species. For example, Lambeth *et al.* (1994) found large differences among *Eucalyptus grandis* clones in growth, branching, and crown density. In *Populus*, clonal differences in branch characteristics and branching patterns were found that resulted in striking differences in crown form and architecture (Ceulemans *et al.* 1990). Sylleptic branches and the

considerable leaf area that they carry have important implications for whole tree light interception, and thus, play a critical role in the superior growth and productivity of certain hybrid poplar clones. The considerable variation in branch characteristics implies a strong justification for including them in selection and breeding programs for *Populus*

Table 2-2. Age 5 y and 6 y within-family individual-tree broad-sense heritability (H^2_{WF}) for growth and crown structural traits in four slash pine families in north central Florida.

Trait	H^2_{WF}			
	Family S1	Family S2	Family S3	Family S10
Stem volume (age 4 y)	0.16 (0.08)	0.22 (0.09)	0.08 (0.08)	0.00 (0.00)
Stem volume increment (age 5-6 y)	0.21 (0.08)	0.24 (0.09)	0.02 (0.07)	0.10 (0.07)
Crown length (age 6 y)	0.21 (0.08)	0.31 (0.10)	0.13 (0.08)	0.18 (0.08)
Crown radius (age 5 y)	0.11 (0.07)	0.41 (0.11)	0.12 (0.09)	0.09 (0.07)
Crown radius (age 6 y)	0.17 (0.07)	0.41 (0.11)	0.12 (0.08)	0.09 (0.08)
Crown shape ratio (age 5 y)	0.27 (0.09)	0.33 (0.10)	0.22 (0.10)	0.25 (0.09)
Crown shape ratio (age 6 y)	0.32 (0.09)	0.05 (0.07)	0.26 (0.10)	0.17 (0.08)
Crown volume (age 5 y)	0.13 (0.07)	0.34 (0.11)	0.10 (0.08)	0.10 (0.07)
Crown volume (age 6 y)	0.17 (0.08)	0.36 (0.10)	0.12 (0.08)	0.16 (0.08)
Leaf area (age 6 y)	0.13 (0.07)	0.34 (0.10)	0.15 (0.09)	0.16 (0.08)
Number of branches (age 5 y)	0.10 (0.07)	0.26 (0.09)	0.22 (0.10)	0.14 (0.08)
Number of branches (age 6 y)	0.26 (0.09)	0.15 (0.08)	0.14 (0.09)	0.10 (0.07)
Branch diameter (age 6 y)	0.09 (0.06)	0.08 (0.07)	0.03 (0.07)	0.02 (0.06)
Number of branches per unit of crown length (age 5 y)	0.00 (0.00)	0.12 (0.07)	0.27 (0.10)	0.03 (0.06)
Number of branches per unit of crown length (age 6 y)	0.04 (0.06)	0.01 (0.07)	0.24 (0.09)	0.05 (0.06)

Note: Values in parentheses are standard errors

Table 2-3. Within-family genetic correlations among individual-tree volume increment between age 5-6 and crown structural variables at age 5, for slash (S1, S2, S3 and S10) and loblolly (L4) pine families in north central Florida.

Trait	Family S1	Family S2	Family S3	Family S10	Family L4
Stem Volume Increment (age 5-6 yr)					
<i>Genetic correlations</i>					
Light interception (age 5-6 y)	0.70 (0.08)	0.74 (0.05)	0.62 (0.47)	0.67 (1.00)	0.64 (0.10)
Crown volume (age 5 y)	0.71 (0.11)	0.61 (0.10)	0.69 (0.78)	0.41 (0.33)	0.51 (0.14)
Leaf area (age 5 y)	0.64 (0.19)	0.64 (0.10)	0.35 (0.93)	0.61 (0.45)	0.31 (0.34)
Crown shape ratio (age 5 y)	0.39 (0.23)	-0.33 (0.23)	0.02 (0.72)	0.01 (0.37)	-0.20 (0.32)
Branch diameter (age 5 y)	0.51 (0.21)	0.75 (0.10)	0.40 (0.75)	0.02 (0.71)	0.16 (0.36)
Branch angle (age 5 y)	-0.05 (0.29)	0.01 (0.26)	0.01 (0.70)	-0.22 (0.45)	0.26 (0.25)
Number of branches (age 5 y)	0.43 (0.26)	0.41 (0.18)	-0.06 (0.75)	0.03 (0.41)	0.20 (0.24)
Crown radius (age 5 y)	0.55 (0.17)	0.66 (0.09)	0.42 (0.56)	0.20 (0.43)	0.47 (0.16)
Crown length (age 5 y)	0.77 (0.09)	0.55 (0.13)	0.37 (0.76)	0.31 (0.36)	0.53 (0.23)
<i>Environmental correlations</i>					
Light interception (age 5-6 y)	0.71 (0.03)	0.78 (0.03)	0.83 (0.03)	0.43 (0.07)	0.68 (0.04)
Crown volume (age 5 y)	0.69 (0.04)	0.70 (0.04)	0.76 (0.04)	0.60 (0.05)	0.58 (0.05)
Leaf area (age 5 y)	0.44 (0.06)	0.60 (0.05)	0.72 (0.07)	0.58 (0.05)	0.46 (0.06)
Crown shape ratio (age 5 y)	-0.10 (0.08)	0.09 (0.08)	0.03 (0.09)	0.01 (0.08)	-0.12 (0.08)
Branch diameter (age 5 y)	0.36 (0.07)	0.52 (0.06)	0.70 (0.05)	0.50 (0.06)	0.43 (0.06)
Branch angle (age 5 y)	-0.16 (0.08)	-0.06 (0.08)	-0.19 (0.09)	-0.24 (0.08)	0.01 (0.08)
Number of branches (age 5 y)	0.39 (0.06)	0.41 (0.07)	0.44 (0.07)	0.39 (0.07)	0.29 (0.07)
Crown radius (age 5 y)	0.62 (0.04)	0.62 (0.05)	0.74 (0.04)	0.58 (0.05)	0.56 (0.05)
Crown length (age 5 y)	0.52 (0.05)	0.64 (0.05)	0.76 (0.04)	0.57 (0.05)	0.49 (0.06)

Note: Values in parentheses are standard errors

(Ceulemans *et al.* 1990). Wu (1994a) also reported significant clonal variation in *Populus* hybrids in crown structural traits at the leaf, branch, and whole-tree levels.

Traditionally, most complex traits, such as growth rate and crown architecture, are thought to be polygenic, determined by the expression of many genes (Falconer and Mackay 1996). This seems intuitive, given that growth rate and crown architecture are affected by many physiological parameters, phenological patterns, organ growth rates, and also by environmental factors like competition interactions, seasonal variation in water availability, nutrient status, light intensity and duration, air and soil temperature, pest and pathogen pressure.

Our results agreed with the polygenic model in that crown architectural and growth traits had low to moderate within-family broad-sense heritabilities, and are therefore likely determined by the expression of many genes. It is possible that the low genetic variation may be due to the nature of the traits we measured and their role in determining fitness. Traits connected with fitness often show low heritability, since natural selection for these traits reduces genetic variation, while traits which are less intimately tied to fitness may have higher genetic variability and so higher heritability (Falconer and Mackay 1996). Tree growth rate and crown size are potentially important components of fitness.

Broad-sense heritabilities estimated from my study were expected to be smaller than broad-sense heritabilities values usually reported in the literature, because they were estimated within full-sib families and half the additive genetic variation and one fourth of the dominance variation as well as a portion of the epistatic variance occurs among full-sib families (Falconer and Mackay 1996). Considering this, our results were comparable

with other clonal studies. With respect to stem growth traits, Paul *et al.* (1997) reported a H^2 of 0.14 for loblolly pine stem volume, while Borralho *et al.* (1992) estimated H^2 between 0.08 and 0.18 for height and sapwood area in *E. globulus*. In crown structural traits, reported H^2 values ranged from 0.27 to 0.78 in *E. grandis* and hybrid poplars (Lambeth *et al.* 1994; Wu 1994a).

Narrow-sense heritability which includes only the additive genetic variation is necessarily smaller than broad-sense heritability for the same trait. For stem growth and crown structural traits, low to moderate narrow-sense heritabilities (0.0 to 0.62) have been reported in loblolly and slash pine at young ages (Lambeth and Huber 1997; Xiao *et al.* 2003), as well as in other pine species as *Pinus brutia*, *P. radiata* and *P. sylvestris* (0.02 to 0.53; Espinel and Aragonés 1997; Haapanen *et al.* 1997; Arregui *et al.* 1999; Isik and Isik 1999).

One interesting finding was the heterogeneity of the variance components among families, which resulted in significantly different within-family broad-sense heritabilities for many traits. Slash pine family S2 showed higher H^2_{WF} values compared to the other three slash pine families (Table 2-2). Higher within-family broad-sense heritability can reflect either a larger clonal variance component (σ^2_c in the numerator of H^2_{WF}) or a smaller residual variance (σ^2_e in the denominator of H^2_{WF}), or both. In family S2, a larger proportion of clonal variance and smaller residual variance component, were found with respect to the rest of the slash pine families, and resulted in larger H^2_{WF} . Smaller residual variances in S2 corresponded also to a smaller interaction between clone and microsite for that particular family than the other slash pine families. It is possible that the two parents of family S2 had greater proportion of heterozygosity at gene loci determining

crown size, producing more segregation among their progeny than in other slash pine families. If this is true, then even for polygenic traits, it is possible to find specific pairs of parents producing more variable offspring for growth or crown traits. These families might be useful for quantitative trait loci (QTL) mapping and gene discovery (Bradshaw and Stettler 1995; Wu and Stettler 1996; Wu 1998).

An understanding of the relationship between crown architecture and tree growth might provide a basis for predicting tree growth, and could aid in the search for discovering genes involved in growth and for developing new crop ideotypes (Kuuluvainen 1988; Dickmann and Keathley 1996; Martin *et al.* 2001). Evidence of positive phenotypic association between crown architecture and tree growth is common in many species, including loblolly and slash pines, with many authors reporting the importance of the amount of light intercepted by the canopy and its correlation with growth rate (Linder 1987; Cannell 1989; Dalla-Tea and Jokela 1991; McCrady and Jokela 1998; Will *et al.* 2001).

In my study, a number of crown architectural traits were consistently genetically correlated with growth (Table 2-3), which is consistent with previous quantitative genetic analysis of crown architectural traits in other species (Wu 1994b; Espinel and Aragonés 1997; Haapanen *et al.* 1997; Arregui *et al.* 1999; Isik and Isik 1999), and production ecology work in loblolly and slash pine (*e.g.* McCrady and Jokela 1998; Martin and Jokela 2004). As we hypothesized, the more integrated measures of crown structure and function in my study, specifically APAR and crown volume, were consistently more strongly correlated with stem volume growth rate than were less integrative measures such as crown radius or length, number of branches, branch angle, or average branch

diameter. APAR was a particularly comprehensive trait, providing a time- and space-integrated index of crown dimensional traits, leaf area, tree size, and crown dimension of surrounding competitor trees. It is interesting, however, that the relatively simple trait of crown volume was as strongly or almost as strongly correlated with stem volume growth as was APAR (Table 2-3). The quantity of APAR by tree crowns is one of the major factors determining aboveground biomass accumulation throughout stand development (Wang and Jarvis 1990). The amount of light intercepted by an individual-tree crown is influenced by its leaf area quantity and display, the incident radiation, and the distribution and size of surrounding trees (Wang and Jarvis 1990).

Two crown traits consistently showed weak or non-existent genetic relationships with growth: crown shape ratio and branch angle. Similar results were obtained by Lambeth and Huber (1997), where branch angle (zero being the closest to horizontal) was weakly but negatively genetic correlated with growth rate (-0.24) (bigger trees tending to have flatter branch angle). In absolute terms, bigger trees tended to have wider crowns ($r_g=0.75$), and large branch diameter ($r_g=0.31$), but when adjustments were made for size, they tended to have smaller branches and narrower crowns for their size and fewer branches per meter of height than smaller families. Xiao *et al.* (2003) reported for loblolly and slash pines families that crown shape ratio combined two important variables (crown height, crown width) that were statistically significant among taxa, but in ratio form as crown shape ratio appeared to have little ecological significance in developing stands with respect to growth performance. Similarly, McCrady and Jokela (1996) observed significant intraspecific variation in crown shape ratio in young loblolly pine

plantations, but they did not find an advantage of narrower crowns over wider crowns in height growth increment.

In other species, such as *P. radiata*, *P. sylvestris*, *Populus* and *E. grandis*, significant positive genetic correlations were found among height, stem diameter, volume, crown diameter, and crown density and vigor. On the other hand, genetic correlations between growth and branch diameter, and growth and branch angle were species specific and variable showing favorable or unfavorable correlations (Arregui *et al.* 1999; Espinel and Aragonés 1997; Haapanen *et al.* 1997; Lambeth *et al.* 1994; Wu 1994b).

With respect to environmental correlations, microsites that favored the development of the crown, leaf area, and light interception also enhanced growth rate in all families. Branch angle and crown shape ratio showed non-significant environmental correlation with volume increment. Thus, microsites with higher levels of nutrients or water availability appear to favor tree volume growth, crown size and light interception at the same time, but do not seem to affect branch angle and crown shape ratio.

Here we reported important linkage between crown structural and functional traits with stem volume growth in loblolly and slash pine families and clones. However, what is finally translated into stem volume increment depends on complex relations with other processes and their genetic patterns. Additional studies with respect to carbon gain, water relations and hydraulic conductivity at the individual-tree level will help improve our understanding of what control stem volume growth in contrasting families and clones.

CHAPTER 3
GENETIC VARIATION IN BASAL-AREA INCREMENT PHENOLOGY AND ITS
CORRELATION WITH GROWTH RATE IN LOBLOLLY AND SLASH PINE
FAMILIES AND CLONES

Introduction

Loblolly pine (*Pinus taeda* L.) and slash pine (*Pinus elliottii* Engelm. var *elliottii*) are widely planted as commercial timber species in the southeastern United States (Smith *et al.* 2004). From the early 1950s, large-scale tree breeding programs in the southeastern United States have worked to improve forest productivity by selecting trees for superior growth rate, form, and disease resistance (McKeand *et al.* 2003), and the improved material currently being established in commercial plantations is deployed from bulked orchard seed, half-sib families, and full-sib families with growing interest in the deployment of outstanding clones.

The extensive natural range of loblolly and slash pines, spanning different environmental conditions, has resulted in accumulation of adaptative genetic variation across time and differences in growth potential among sources (Burns and Honkala 1990). To develop tree breeding programs it is necessary to understand the genetic variation of selected traits, their correlations and the effect of the environment on genotypic expression (White 1987). In Florida winter temperatures are rarely low enough to prohibit positive photosynthetic rates and considerable transpiration (McGarvey 2000; Martin 2000). These mild winter conditions, plus an abundant rainfall through the summer, may have translated into genotypes adapted to a longer growing seasons and/or faster growth.

Most pines experience a cycle of bud set and growth cessation in the latter part of the growing season, followed by deepening dormancy, cold hardening, dormancy release in the winter, and bud break in the spring (Dougherty *et al.* 1994). In the case of loblolly pine, the wide natural distribution, spanning different ecotypes and environments, contains a diverse range of chilling requirements to promote dormancy release, length of the growing period and rate of growth. For instance, while it has been established that chilling is required for loblolly pine northern ecotypes, it is unclear that there is a true dormancy and chilling requirement for southern latitude sources (Carlson 1985).

Increase in the diameter of tree stems occurs primarily from meristematic activity in the vascular cambium, a cylindrical lateral meristem located between the xylem and phloem of the stem, branches, and woody roots. The time of the year during which the cambium is active varies with climate, species, crown class, seasonal development of leaf area in trees, and different parts of stems and branches (Kozłowski and Pallardy 1997). Fluctuations in environmental stresses affect cambial growth to a large extent by altering the supply of photosynthate to the branches and stem (Kozłowski 1971; Sevanto *et al.* 2003). For example, cambial growth is sensitive to available water, with several aspects being responsive to the amount and seasonal distribution of rainfall, including number of xylem cells produced and ring width, seasonal duration of cambial growth, proportion of xylem to phloem increment, time of latewood initiation, duration of latewood production, and wood density (Kozłowski 1971; Cregg *et al.* 1988; Downes *et al.* 1999; Mäkinen *et al.* 2000, 2001; Bouriaud *et al.* 2005).

The amount of growth in a particular season is determined by the date of growth initiation, the date of growth cessation (which together determine growth duration), and

the average daily growth rate for the growth period. The cessation of shoot and cambium activity is one determining factor, and the more fully the plant can utilize the growing season, without suffering from spring and fall frost, the greater potential annual growth, final harvest, and return on the investment in planting stock. Much of the interest in forest tree phenology is related with these practical questions (Lieth 1974).

The total growth period from initiation to cessation, both for height and cambial activity, has been studied on an individual tree basis in many North American tree species, but little information on genetic variation is available. Seasonal periodicity of tree growth has been studied in evergreen and deciduous trees (Jackson 1952; Harkin 1962; Langdon 1963; Emminham 1977; Li and Adams 1994; McCrady and Jokela 1996; Zhang *et al.* 1997; Jayawickrama *et al.* 1998; Yu *et al.* 2001). Wide variation among species in duration of the period of growth was recorded by Jackson (1952). Cambial growth of some species lasted only about 80 days and others grew for up to 200 days. Several of the species which initiated growth early in the season had long periods of growth, while some of the late starting species exhibited shorter periods.

Langdon (1963) studied growth patterns of slash pine (*Pinus elliottii* Engelm. var. *densa* Little and Dorman) in south Florida (Fort Myers) for four years and found that diameter growth occurred about ten months per year (from March through December). Initiation of diameter growth was believed to be promoted by apically produced hormones (Savidge and Wareing 1984). Diameter growth has been reported to initiate before or almost simultaneously with height growth for loblolly pine (Zahner 1962) and for slash pine (Kaufmann 1977). Conifers usually continue diameter growth into the fall

after height growth has stopped, as reported for *Pseudotsuga menziesii* and loblolly pine (Emmingham 1977; Jayawickrama *et al.* 1998).

Previous research in loblolly and slash pine diameter growth phenology has provided important knowledge about the duration of cambial activity (Jackson 1952; Harkin 1962; Langdon 1963; McCrady and Jokela 1996; Zhang *et al.* 1997; Jayawickrama *et al.* 1998). However, there is a lack of information about how the duration of cambial growth might influence the differences in growth rate between species planted in the same area, and also the growth differences among families within species and clones within families.

My study examines the following hypotheses:

- There is significant genetic variation in basal-area growth phenology among slash and loblolly pine genotypes (species, families and clones);
- Where it exists, genetic variation in basal-area growth phenology is correlated with variation in annual basal-area increment.

The specific objectives are to:

- Compare two years basal-area growth phenology among species, families and clones;
- Estimate genetic parameters for basal-area growth phenology, its correlation with growth rates, and the genotype interaction with seasonal environmental changes.

Material and Methods

Study Site and Plant Material

The study area was located on lands managed by Rayonier Inc. in Bradford County, Florida. The climate is humid and subtropical, with a mean annual temperature of 21°C, mean annual rainfall of 1316 mm, and over 50% of the rainfall occurring in June through September. Periods of drought are normal in the spring and fall. Mean annual rainfall during 1999-2001 was 967 mm, in contrast to 1405 mm in year 2002 (NOAA 2002). The

soils are classified as Pomona and consist of very deep, somewhat poorly to poorly drained soils that are formed in sandy and loamy marine sediments (sandy, siliceous, hyperthermic Ultic Alaquods). Slopes are 0 to 2 %. In a typical profile, the spodic horizon occurs at 30-60 cm, with an argillic horizon at 90-120 cm. Water table is typically at a depth of 15 to 45 cm for one to three months and a depth of 25 to 100 cm for six months or more, during most years (Soil Survey Staff 1998).

The study took place in an area containing 16 full-sib and half-sib loblolly and slash pine families planted in 337 m² family plots in January 1997. The experiment was designed as a randomized complete block with four replicates (Appendix A). We used one full-sib loblolly pine family and four full-sib slash pine families. Each family plot contained 60 clones propagated as rooted cuttings from a single family, planted at 1.7 m x 3.4 m spacing (1730 trees ha⁻¹). Cuttings were taken from donor hedges in the spring, and were rooted and grown in a greenhouse for six months before planting. Each of the four plots of the same family contained the same 60 genotypes, but with the ramets planted into different, randomly-determined planting locations in the plot. In total we studied approximately 1,200 trees: 60 trees per family plot x 5 families x 4 replications. Fertilization and weed control were applied periodically to reduce interspecific competition and prevent nutrient deficiency (Appendix B).

Basal-area Increment Measurements

Phenology was evaluated as periodic basal-area growth increment as determined from repeated DBH measurements throughout growing seasons in 2002 and 2003. Families S1, S2, S3, L4 and S10 were monitored for diameter increment once a month in the summer time and every ten to fifteen days during the period of growth initiation and cessation in the spring and fall, respectively. Diameter increment was measured with a

digital caliper (model 18 ES, Mahr, Germany, resolution 0.01 mm) over 4 plexiglass plates attached to the tree stem in north-south and east-west orientations. Diameter measurements were done such that two replications were measured on day 1 and two replications on day 2 each time period.

Phenological Traits

From the periodic diameter measurements, a cumulative basal-area growth curve for two growing seasons was plotted for each tree, and dates of basal-area growth initiation and cessation were estimated by interpolation as the dates when 5% and 95% of annual growth were completed (Hanover 1963). Duration of basal-area growth (in days) was calculated as the difference between dates of cessation and initiation. Basal-area increment per year (in mm²) was calculated as the difference in individual tree basal-area between the 5% and the 95% dates of initiation and cessation. Basal-area growth rate (in mm²/day) was calculated as the ratio of annual basal-area increment and duration of basal-area growth.

Meteorological Data and Water Balance

Climatic data were collected at the Gainesville Regional Airport (about 20 km distant from the study site, NOAA 2003) and a research weather station 8 km from the study site. Meteorological variables included hourly radiation, mean air temperatures, and daily rainfall. A simple water balance model was computed to estimate soil water reserves at daily time steps, and to quantify soil water deficit. The model was given by Equation 3-1, where R_n is soil water reserve at day n , R_{n-1} is soil water reserve of the day before, P_n is precipitation and T_n is transpiration, both at day n :

$$R_n = R_{n-1} + P_n - T_n \quad (3-1)$$

The water holding capacity in 1 m depth for this site was estimated at 260 mm according to soil texture and flatwoods Spodosols moisture release curves (H.L. Gholz, personal communication). Plot-level transpiration (T_n) was estimated as follows: maximum hourly potential evapotranspiration (PET, mm) was calculated by dividing measured hourly radiation by the latent heat of vaporization of water; maximum plot-level transpiration was then calculated as 60% of PET, and was assumed to occur when all-sided leaf area index (LAI) was greater than 6.0. At LAI less than 6.0, transpiration was estimated to decline linearly with declining LAI (Martin and Jokela 2004). Plot-level leaf area index was calculated from litterfall data as in Martin and Jokela (2004). Because understory vegetation was sparse, only pine LAI was considered. The resulting model incorporated variation in environmental conditions (daily precipitation, hourly radiation), as well as plot-level leaf area index to produce a plot-level index of soil water availability.

Statistical Analyses and Genetic Parameters

Analysis of variance (ANOVA) was used for phenological and growth data separately for each year. PROC GLM in the SAS® System was used to test for significance of random effects (clone), while PROC MIXED was utilized to test the fixed effects (species and families). Equation 3-2 shows the linear model considered for the analyses, where Y_{ijkl} is the performance of the ramet of the l^{th} clone within the k^{th} family nested in the j^{th} species in the i^{th} replication; $i = 1, 2, 3,$ and 4 for replications; $j = \text{slash}, \text{loblolly}; k = 1, 2, 3, 4,$ and 10 for families; $l = 60$ identification numbers for 60 clones within each of the five families:

$$Y_{ijkl} = \mu + b_i + S_j + F_{k(j)} + c_{l(jk)} + bS_{ij} + bF_{ik(j)} + \epsilon_{ijkl} \quad (3-2)$$

μ = population mean,
 b_i = random variable of replication \sim NID (0, σ^2_b),
 S_j = fixed effect of species (slash or loblolly),
 $F_k(j)$ = fixed effect of family nested within species,
 $cl(jk)$ = random variable of clone nested within-family and species \sim NID (0, σ^2_c),
 bS_{ij} = random variable for replication x species interaction \sim NID (0, σ^2_{bS}),
 $bF_{ik}(j)$ = random variable for replication x family(species) interaction \sim NID (0, σ^2_{bF}), and
 ε_{ijkl} = error term \sim NID (0, σ^2_ε).

With so few families, estimates of genetic parameters were restricted to within-family estimates obtained from clonal variation expressed within each of the four slash families and one loblolly pine family. For each family two types of genetic parameters were estimated: within-family broad sense heritability for each trait, and within-family genetic correlations among traits. Within-family variance and covariance components were obtained using ASREML, a statistical package that fits linear mixed models using Restricted Maximum Likelihood (Gilmour 1997).

Within-family individual tree broad sense heritability was calculated using Equation 3-3, where σ^2_c is the variance among clones within-family and σ^2_ε is the residual variance as defined in Equation 3-2:

$$H^2_{WF} = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_\varepsilon} \quad (3-3)$$

Theoretically, broad sense within-family heritability contains $\frac{1}{2}$ the additive genetic variance, $\frac{3}{4}$ of the dominance genetic variance, and most of the epistatic genetic variance (Falconer and Mackay 1996). The standard error for heritability estimates was calculated from Dickerson (1962). The residual likelihood ratio test (Wolfinger 1996) was used to test heterogeneity of variances among slash pine families, and heritabilities were estimated separately ($\chi^2_{(6, 0.05)} = 12.6$), or pooled, as appropriate.

Within-family genetic correlations among basal-area phenological traits and growth rate were calculated using Equation 3-4 (Falconer and Mackay 1996), where σ_{xy} is the clonal covariance between two traits, and σ_x and σ_y are the square root of the product of the clonal variance within-family for traits x and y, respectively:

$$r_{xy} = \frac{\sigma_{xy}}{\sigma_x \sigma_y} \quad (3-4)$$

Standard error for genetic correlations was estimated using ASREML (Gilmour 1997).

The significance of the clone by year variance component was tested using the likelihood ratio test (Wolfinger 1996). The clone by year variance component was declared different from 0 when $\chi^2_{(1,0.05)}$ was equal to or greater than 3.8. For traits with a significant clone by year variance component, within-family genetic correlations between years were estimated considering the two years as two different traits using Equation 3-4.

Results and Discussion

Genetic Variation among Species and Families

In 2002, species and families within species were not significantly different at 5% for any phenological or growth trait, while in year 2003, date of growth cessation and daily basal-area growth rate were significant at the species level with loblolly pine ceasing growth earlier and growing more than slash pine (Table 3-1 and Figure 3-1). In 2002, the mean date of basal-area growth initiation was March 10 (69 days after January 1) for the single loblolly pine family and one day earlier for the slash pine families. Basal-area growth cessation for the loblolly pine family, on average occurred on November 1, resulting in a mean duration of basal-area growth of 236 days. In the case of

slash pine families, mean cessation was on October 28, and the duration of basal-area growth was 234 days (Table 3-1).

In 2003, basal-area growth started and finished one to two weeks sooner than in 2002 for both loblolly and slash pine families (Table 3-1). For loblolly pine, basal-area growth began in February 23 and finished by October 4, resulting in a mean duration of basal-area growth of 223 days (13 days fewer than in 2002). For slash pine, basal-area growth began, on average, on February 25 and ended on October 17, for duration of 235 days (only one day shorter than in 2002, Table 3-1). In 2003, more growing days for slash pine compared to loblolly pine might explain why the difference between species in total basal-area growth was less than in the previous year ($26.98-23.81=3.17 \text{ cm}^2$ in 2002 versus $24.26-21.98= 2.28 \text{ cm}^2$ in 2003). This is supported by the fact that the slash pine families with longest (S1) and shortest (S3) duration had greater differences in basal-area growth in 2003 than in 2002 ($24.94-22.12=2.82 \text{ cm}^2$ in 2002 versus $22.8-19.69=3.11 \text{ cm}^2$ in 2003). Annual basal-area increment and daily basal-area growth rate were larger for all families in 2002 than in year 2003, despite a shorter growing season for some families in 2002. Early cessation in year 2003 in comparison with year 2002, and the difference in total annual increment between years could possibly be due to the differences in amount and seasonal distribution of rainfall between 2002 and 2003 (1405 mm and wet soil conditions by the end of the year in 2002; and 1184 mm and dry conditions by the end of the year in 2003, Figure 3-2).

In general, loblolly pine tended to accumulate more stem volume through ages 6 and 7 y than slash pine. This was manifested by larger yearly and daily basis basal-area increments, but these differences among species were only significant ($p<0.05$) for daily

basal-area growth in 2003 (Table 3-1). From my study we can conclude that the differences between loblolly and slash pine accumulated slowly over time through ages 6 and 7 y.

Table 3-1. Significance levels (p-values), and species and family least square means for individual tree stem growth and phenological traits for two growing seasons for loblolly and slash pine families in north central Florida.

Trait	Significance level by effect			Species mean		Slash family means			
	Species	Family	Clone	Slash	Loblolly	Family S1	Family S2	Family S3	Family S10
<i>Year 2002</i>									
Initiation ^a	0.8160	0.1120	0.0534	68.43	69.00	70.31	63.57	70.83	68.99
Cessation ^a	0.3542	0.1870	0.2141	302.16	304.82	306.43	301.66	301.44	299.12
Duration (days)	0.5456	0.2966	0.0131	233.71	235.82	236.13	238.03	230.59	230.10
Volume 6 y (dm ³)	0.0797	0.1062	<0.0001	25.37	31.58	26.32	26.10	23.55	27.52
BA increment (cm ²)	0.0784	0.0586	<0.0001	23.81	26.98	24.94	25.04	22.12	23.14
BA growth rate (mm ² /day)	0.0945	0.2783	<0.0001	10.18	11.47	10.57	10.50	9.59	10.05
<i>Year 2003</i>									
Initiation ^a	0.0815	0.3384	0.0062	55.85	53.86	56.21	54.94	56.79	55.45
Cessation ^a	0.0459	0.0853	0.0007	291.01	276.51	297.03	291.00	280.87	295.12
Duration (days)	0.0711	0.0774	0.0050	235.18	222.64	240.82	236.12	224.09	239.69
Volume 7 y (dm ³)	0.0835	0.0687	<0.0001	38.31	44.30	38.67	38.87	34.67	41.03
BA increment (cm ²)	0.1312	0.0894	<0.0001	21.98	24.26	22.80	22.83	19.69	22.60
BA growth rate (mm ² /day)	0.0385	0.4796	<0.0001	9.35	10.81	9.45	9.63	8.87	9.45

^a Initiation and cessation are days after January 1 to complete 5 and 95% of seasonal diameter growth

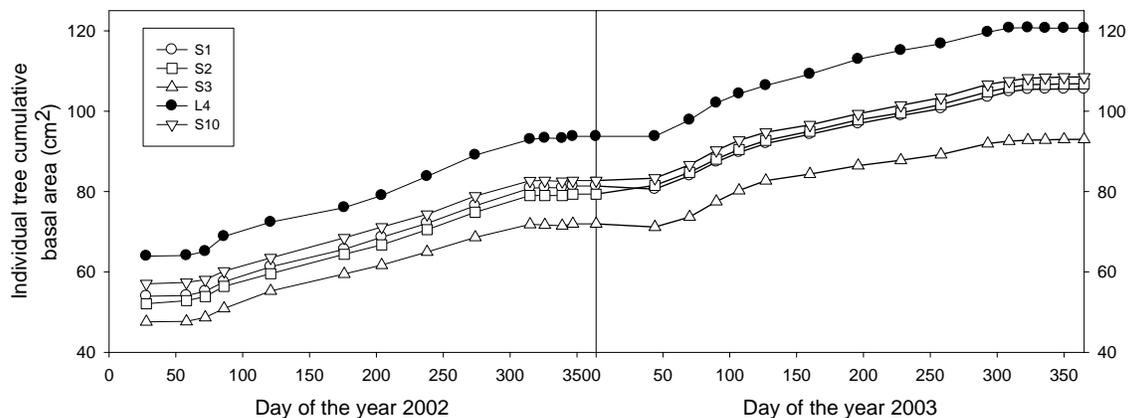


Figure 3-1. Family mean cumulative basal-area growth curves for years 2002 and 2003 in loblolly and slash pine in north central Florida.

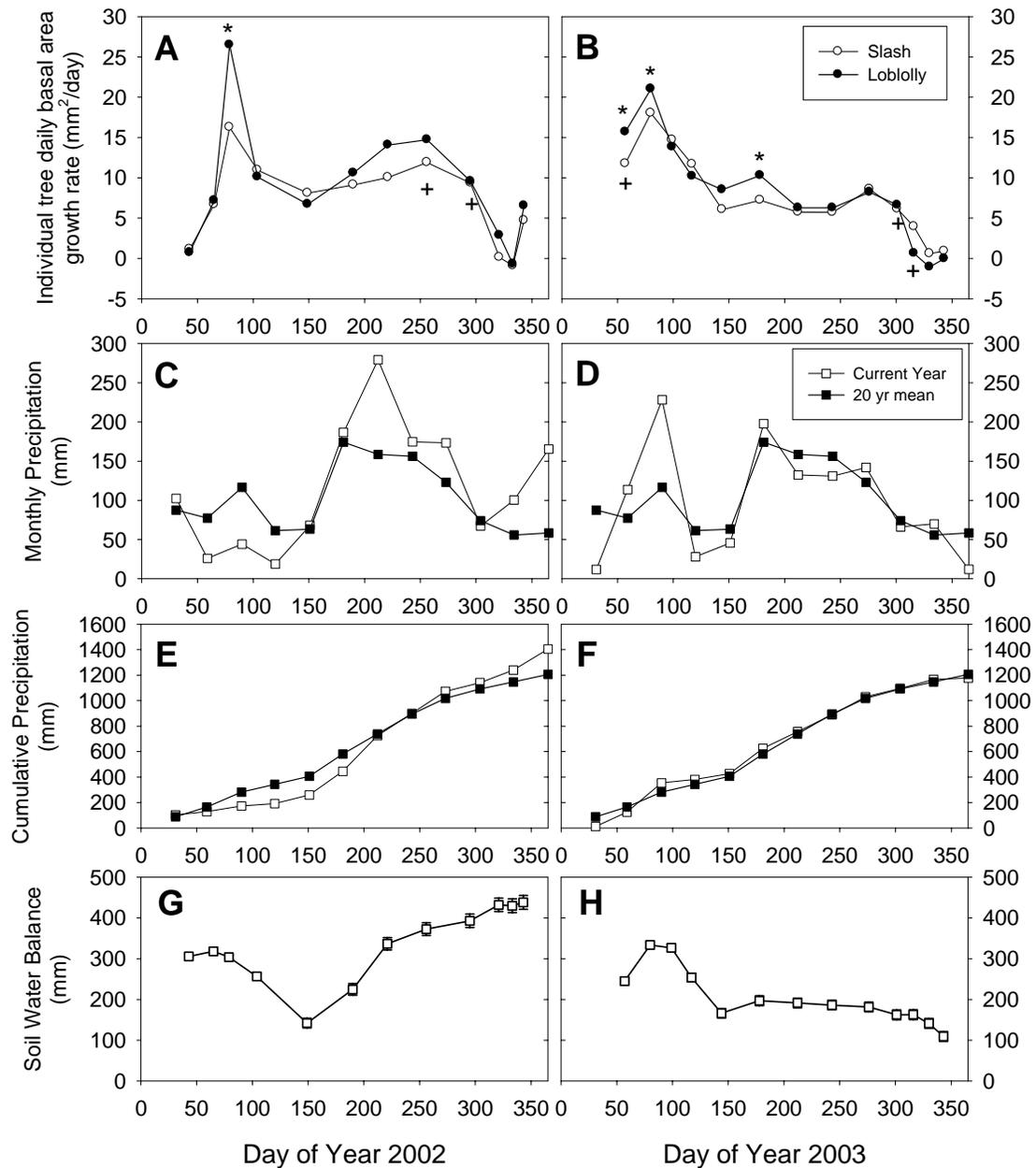


Figure 3-2. Species mean daily basal-area growth increment for loblolly and slash pine in north central Florida and environmental variables. Species mean daily basal-area growth increment in 2002 (A) and 2003 (B), where * indicates significant differences between species ($p < 0.05$) and + indicates significant differences among slash pine families ($p < 0.05$); current-year and 20-year mean monthly precipitation in 2002 (C) and 2003 (D); cumulative precipitation in 2002 (E) and 2003 (F); and mean plot-level soil water balance (error bars indicating standard errors) in 2002 (G) and 2003 (H). Precipitation data from Gainesville Regional Airport, NOAA (2003).

Because phenology is closely related to latitude (Campbell 1986; Jayawickrama *et al.* 1998; Nielsen and Jørgensen 2003), growth initiation, cessation, and duration should depend on the study location and geographic origin of the sampled seed or vegetative propagule. Our results are in agreement with what Langdon (1963) reported on growth patterns of slash pine in south Florida (Fort Myers). He found that diameter growth occurred about ten months during the year (from March through December), and the total amount of diameter growth and its seasonal distribution responded to climatic variation. With respect to diameter growth cessation date, similar results were found by Jayawickrama *et al.* (1998), for example, a loblolly pine provenance from Gulf Hammock (Florida) grew until day 299 and 313 in two different years.

Comparing studies done in northern regions with slash and loblolly pine, our results showed earlier initiation date, later cessation date and longer season length. For example, in a site close to Athens, Georgia, Jackson (1952) found that loblolly and slash pine started diameter growth between the end of March and the beginning of April, with a duration of five to six months. In South Carolina, McCrady and Jokela (1996) found that in loblolly pine diameter growth initiated by the end of March and finished by August-September, giving mean diameter growth duration of 5 months. No significant differences were found among families in initiation or cessation of diameter growth.

All families showed similar patterns of basal-area increment across the growing season in years 2002 and 2003, *i.e.* shapes of the cumulative basal-area curves were quite similar (Figure 3-1). In general, basal-area growth peaked in early spring, and then remained relatively constant throughout the remainder of the growing season (Figure 3-1, 3-2). The differences at the species level and among families within slash pine

accumulated across time. Daily average basal-area growth rate was only significantly different at the species level in 2003 (Table 3-1). Similar trends in diameter growth were found by others authors. Linear radial growth was observed over the entire growing season in slash and loblolly pine trees by Jackson (1952), except for a period of slow growth in the late summer which was probably associated with soil moisture depletion. Similar linear trends were reported by McCrady and Jokela (1996) in loblolly pine families. Cregg *et al.* (1988) reported that unlike height growth, rapid diameter growth can be maintained over the entire growing season and the rate of diameter growth of loblolly pine, observed during a year when moisture deficits did not develop was almost constant over the period from day 50 to day 290.

Despite the apparent lack of variation in basal-area growth rate indicated by the cumulative growth data (Figure 3-1), peaks in basal-area increment occurred in the early spring both years (Figure 3-2). Significant species and family differences were found for critical spring periods when growth rates were highest: in year 2002 measurement period 3 (March), and in 2003 measurement periods 1 and 2 (end of February and middle March, respectively). These results suggest that at least some of the genetic differences in cumulative growth (as shown in Figure 3-1) are manifested not through constant expression of consistent growth rate differences, but rather through elevated growth rate during very discrete periods of time (as shown in Figure 3-2). In other words, the basal-area growth rates of taxa are remarkably similar for most of the year, but in the spring some environmental variables or genetic differences in phenology trigger more rapid growth in some taxa, which essentially raises the intercept of the linear cumulative basal-area functions for the rest of the year (Figure 3-1). In other studies in loblolly and slash

pine, peaks in basal-area increment in early spring also were reported by Zhang *et al.* (1997) and Langdon (1963); accelerating growth in spring was also reported in Norway spruce (Bouriaud *et al.* 2005). But these studies in conifers did not identify genetic differences in tree growth rate at this temporal scale. In the case of hardwoods, growth and phenology studies in hybrid aspen clones (*Populus tremula* x *Populus tremuloides*) compared growth patterns in temperate climates throughout the year (Yu *et al.* 2001). Peaks in diameter growth occurred in the end of the spring and beginning of the summer. Hybrid clones had higher growth rates than the pure *P. tremula* and also accumulated larger annual diameter increment.

In 2002, daily basal-area growth was weakly negatively correlated with soil water balance (Daily BA growth = $13.7929 - 0.0187 \times$ soil water balance, $R^2=0.11$, $p<0.0001$, Figure 3-3). In contrast, in 2003, daily basal-area growth was positively associated with calculated soil water balance (Daily BA growth = $-1.9424 + 0.0478 \times$ soil water balance, $R^2=0.49$, $p<0.0001$, Figure 3-3). The total amount of rainfall in 2002 was 1405 mm, 177 mm above average. Wet conditions were present especially between June and December and presumably had a negative effect on growth (Figure 3-2). On the other hand, in a year with average rainfall, like in 2003, where rainfall totaled 1184 mm (44 mm less than a normal year), a strong correlation was observed with more growth associated with higher levels of soil water availability. In year 2003, we found that daily basal-area growth followed same pattern as that of soil water balance (Figure 3-2B). For both years and both species, the highest growth rates in basal-area were reached in conditions where the soil water balance was around 300 mm. This analysis implies that basal-area growth rate increased as water soil availability increased, when water was limiting, but excess water

available in the soil had a negative effect on growth, perhaps caused by plant stress due to prolonged root inundation.

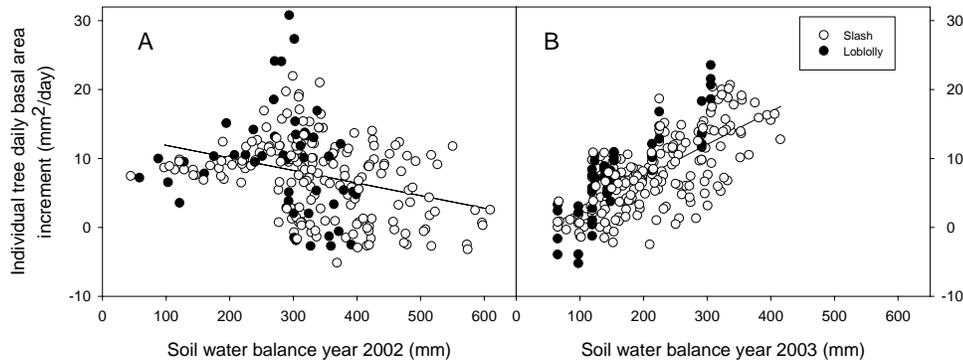


Figure 3-3. Relationship between individual tree daily basal-area increment and simulated daily plot-level soil water balance in loblolly and slash pine in 2002 (A) and 2003 (B). The line shows a linear regression through data. A: Daily BA growth = $13.7929 - 0.0187 \times \text{soil water balance}$, $R^2=0.11$, $p<0.0001$. B: Daily BA growth = $-1.9424 + 0.0478 \times \text{soil water balance}$, $R^2=0.49$, $p<0.0001$.

Studies in flatwoods soils in north-central Florida have shown reduced radiation use efficiency when soil water balance was high/wet, and this effect can have a direct impact on tree growth rates (Martin and Jokela 2004). Langdon (1963) also reported that excess soil water appeared to depress growth. From the four years of their study with slash pine, for the year with high rainfall (1929 mm) and high ground-water levels during summer and early fall, both diameter and height growth were considerably below the other 3 years. Water table depth was found to be associated with growth in the flatwoods in Florida (White and Pritchett 1970). Larger height and diameter growth was reported in slash and loblolly pine with controlled water table depth conditions at 46 and 92 cm from the surface, in comparison with natural fluctuating water table conditions. Bouriaud *et al.* (2005) studied the influence of climatic variables on annual radial growth and wood density on *Picea abies*. They found numerous decreases in radial growth rate closely related to the calculated soil water deficit. Also, wood density increased with decreasing

radial growth rate in the second half of the growing season affected by drought. Similar results were reported by Cregg *et al.* (1988), where early season diameter growth rate for loblolly pine was a function of available soil moisture and temperature.

Clonal Variation and Within-Family Inheritance of Phenological Traits and Stem Growth

At the clone within-family level (pooled across families), differences in initiation, cessation and duration of basal-area increment in the growing season were more apparent than at the family and species level differences in both 2002 and 2003 (Table 3-1). Traits related to individual tree stem growth, such as volume, and yearly and daily basal-area increment were also different among clones within families in both years (Table 3-1). Analyses of the data separately by family showed that phenological traits differed among clones for some families and not for others in both years. Family S2 was the only one that showed significant clonal variation in all phenology traits in 2003. Volume, yearly basal-area increment, and daily basal-area growth had significant clonal variation within-family in 2002 and 2003 for all families (Table 3-2).

For phenology traits, individual tree broad sense heritabilities were low to moderate, ranging from 0.01 to 0.24 (Table 3-3). In contrast, within-family heritabilities for stem growth traits were moderate to high in both years ranging from 0.10 to 0.37 (Table 3-3). Family S2 tended to have higher within-family broad sense heritabilities than the other slash pine families, in most cases due to higher clonal variation within that family as opposed to lower residual environmental variance. These heritabilities are expected to be smaller than broad sense heritabilities values usually reported in the literature, because they are estimated within full-sib families and half the additive genetic variation and one fourth of the dominance variation as well as a portion of the epistatic

variance occurs among full-sib families (Falconer and Mackay 1996). Still, phenotypic expressions of phenological traits associated with basal-area growth were under weak genetic control.

Table 3-2. Significance levels (p-values) for clone within-family for tree stem growth and phenological traits for two growing seasons in loblolly and slash pine families in north central Florida.

Trait	Significant level within-family (clonal variation)				
	Family L4	Family S1	Family S2	Family S3	Family S10
<i>Year 2002</i>					
Initiation	0.0003	0.0311	0.1964	0.1577	0.1880
Cessation	0.3200	0.3489	0.1145	0.1541	0.3753
Duration	0.0695	0.1608	0.0498	0.2409	0.1593
Volume 6 y	0.0015	<0.0001	<0.0001	0.0720	0.0390
BA increment	<0.0001	0.0042	<0.0001	0.0191	0.0277
BA growth rate	<0.0001	0.0028	<0.0001	0.0279	0.0286
<i>Year 2003</i>					
Initiation	0.0253	0.4211	0.0011	0.0706	0.4932
Cessation	0.2165	0.3028	0.0028	0.1302	0.1550
Duration	0.3215	0.3770	0.0068	0.1146	0.3893
Volume 7 y	0.0001	0.0003	<0.0001	0.0206	0.0318
BA increment	<0.0001	0.0043	<0.0001	0.0524	0.0004
BA growth rate	<0.0001	0.0016	<0.0001	0.0536	<0.0001

Heritability estimates for phenological traits are available for a few species and are usually presented for leaf phenology rather than basal-area or shoot phenology. For example, narrow-sense h^2 estimates ranged between 0.67 to 0.96 in *Juglans nigra*, and between 0.28 and 0.71 in *Picea glauca* for initiation and cessation of leaf development, depending on experimental conditions, age, and method of computations (Leith 1974). In pole-size *P. menziesii*, individual tree heritabilities were higher for bud burst and bud set ($h^2=0.73$ and 0.81, respectively) than for duration of shoot growth ($h^2=0.17$, Li and Adams 1993). With respect to diameter growth, Li and Adams (1994) estimated individual heritabilities for diameter growth initiation ($h^2=0.23$), and cessation ($h^2=0.11$) in 15 y-old *P. menziesii*, values that are comparable with my study. At the same time, Li and Adams (1994) did not detect significant family differences in duration of diameter

increment, suggesting that the small variation in date of diameter growth cessation among families may have been related to summer dry conditions. Other studies have shown that summer drought has little effect on variation in cambial growth initiation, but reduces variation in cambial growth cessation among coastal *P. menziesii* provenances (Emmingham 1977). In my study, we did not detect an association between clonal variation within-family for initiation or cessation and soil water balance and the presence of relatively dry spring or late summer.

Table 3-3. Within-family individual-tree broad-sense heritabilities for growth phenology traits and basal-area growth increment by year in loblolly and slash pine families growing in north central Florida.

Trait	H^2_{WF}				
	Family L4	Family S1	Family S2	Family S3	Family S10
<i>Year 2002</i>					
Initiation	0.20 (0.07)	0.10 (0.06)	0.07 (0.07)	0.09 (0.08)	0.00 (0.00)
Cessation	0.00 (0.00)	0.00 (0.00)	0.08 (0.07)	0.07 (0.08)	0.00 (0.00)
Duration	0.08 (0.06)	0.07 (0.06)	0.11 (0.07)	0.05 (0.08)	0.00 (0.00)
Volume 6 y	0.18 (0.07)	0.25 (0.07)	0.26 (0.08)	0.10 (0.08)	0.10 (0.07)
BA increment	0.24 (0.07)	0.15 (0.07)	0.37 (0.08)	0.15 (0.09)	0.13 (0.07)
BA growth rate	0.23 (0.07)		0.19 (0.04) ^a		
<i>Year 2003</i>					
Initiation	0.12 (0.07)	0.03 (0.06)	0.24 (0.08)	0.10 (0.09)	0.03 (0.06)
Cessation	0.05 (0.06)	0.05 (0.06)	0.19 (0.08)	0.08 (0.08)	0.09 (0.07)
Duration	0.04 (0.06)	0.05 (0.06)	0.16 (0.08)	0.09 (0.08)	0.03 (0.06)
Volume 7 y	0.20 (0.07)	0.20 (0.07)	0.29 (0.08)	0.14 (0.09)	0.11 (0.07)
BA increment	0.34 (0.07)		0.21 (0.05) ^a		
BA growth rate	0.32 (0.07)		0.23 (0.05) ^a		

Note: Values in parentheses are standard errors

^a Values of H^2_{WF} in slash pine were pooled by family since variance components were homogeneous.

Genetic Correlations among Phenological Traits and Stem Growth

When phenological traits did not differ significantly among clones within-family, genetic correlations were not estimated. Among the estimates, many genetic correlations relating phenology traits to growth were not significantly different from zero (Table 3-4). In 2002, genetic correlations between initiation and duration were strong and negative in family L4, S1 and S2, which indicated that clones with early growth initiation also had a

tendency to grow longer, and that clones that initiated later also tended to have a shorter growing season. On the other hand, genetic correlations between cessation and duration were positive and strong in family S2 and S3, meaning that clones that had a tendency to cease growth late in the year also grew for a longer period of time.

In 2003, genetic correlations between initiation and cessation were significant and moderately positive only for family S2. The genetic correlations were positive and strong between cessation and duration for all families, meaning that clones that stopped growth later also grew for a longer period of time. In general, these results suggest that variation in duration of the growing season among individuals in these families was more a function of cessation date than initiation date, but all of these traits were weakly inherited (Table 3-3).

With respect to genetic correlations between stem growth variables and phenological variables, significant correlations were found primarily in family S2, varying from moderate to strongly positive ($r = 0.31$ to 0.85 , Table 3-5). In 2002, duration had a positive strong genetic correlation with basal-area increment in S2. In 2003, initiation, cessation, and duration had moderate positive genetic correlations with basal-area increment in S2. At the same time, initiation in 2003 for L4 showed a strong positive genetic correlation with basal-area increment ($r_g=0.86$). Among the variables we investigated, daily basal-area growth rate in both years showed the strongest genetic correlation with yearly basal-area increment across all families. Correlations of phenology variables with total volume after the 2002 and 2003 growing seasons were similar to the patterns of correlation with yearly basal-area increment, reflecting consistency between phenology and increment during the year and phenology and

cumulative stem growth. These results suggest that clones that grew faster between initiation and cessation were also the ones with more yearly basal-area increment and total volume. The high genetic correlation between daily basal-area growth rate and yearly basal-area increment in a year was explained in part because of the autocorrelation between these two variables.

Genetic correlations among clonal values for basal-area growth and phenological traits are scarce in the literature; most of the reported results are phenotypic correlations at the family level. One of the few studies on genetic control of cambial phenology found that *P. menziesii* genotypes with early growth initiation also tended to cease growth early ($r_g=0.60$, Li and Adams 1994). They also suggested that variation in growth duration among individuals is primarily a function of variation in date of growth cessation ($r_g=0.77$). Height phenology studies in *P. abies* in northern Europe showed that early start of shoot growth was genetically correlated with early shoot growth cessation. Also, there was a consistently low or no correlation between the shoot elongation period and either total height or leader length (Ekberg *et al.* 1994).

Reported phenotypic correlations in southern pines for both diameter and height growth are more closely related to growth rate, than with phenological traits such as cessation (McCrary and Jokela 1996; Jayawickrama *et al.* 1998). Jackson (1952) found that there was no consistent relationship between the starting date and faster growth in slash and loblolly pine trees. For *P. menziesii* saplings, most of the differences among populations in one season's growth were related to growth rate rather than growth duration (Emmingham 1977). In *Picea mariana*, cambial growth cessation and total

Table 3-4. Within-family genetic correlations between growth phenology traits in 2002 (above the diagonal) and 2003 (below the diagonal) in loblolly and slash pine families growing in north central Florida. I: initiation; C: cessation; and D: duration of basal-area growth.

Trait	Family L4			Family S1			Family S2			Family S3			Family S10		
	I	C	D	I	C	D	I	C	D	I	C	D	I	C	D
I	-	^a	-0.98 (0.33)	-	^a	-0.85 (0.41)	-	-0.47 (0.91)	-0.82 (0.35)	-	0.46 (0.72)	0.02 (0.86)	-	^a	^a
C	0.96 (0.61)	-	^a	0.60 (2.06)	-	^a	0.67 (0.28)	-	0.91 (0.21)	-0.25 (0.72)	-	0.89 (0.17)	1.00 (2.6)	-	^a
D	0.93 (1.04)	0.99 (0.06)	-	0.49 (2.59)	1.00 (0.09)	-	0.56 (0.34)	0.99 (0.01)	-	-0.42 (0.67)	0.98 (0.02)	-	1.00 (2.6)	1.00 (0.31)	-

Note: Values in parentheses are standard errors

^a Was not estimated because within-family clonal variance was 0

Table 3-5. Within-family genetic correlations between growth and phenology traits by year in loblolly and slash pine families growing in north central Florida

Traits	Basal-area increment 2002					Basal-area increment 2003				
	L4	S1	S2	S3	S10	L4	S1	S2	S3	S10
Initiation	-0.05 (0.26)	-0.12 (0.45)	^a	^a	^a	0.86 (0.27)	^a	0.59 (0.22)	0.22 (0.84)	^a
Cessation	^a	^a	^a	^a	^a	^a	^a	0.59 (0.23)	^a	^a
Duration	0.32 (0.35)	^a	0.85 (0.28)	^a	^a	^a	^a	0.53 (0.25)	^a	^a
BA growth rate	0.99 (0.01)	1.00 (0.01)	0.99 (0.00)	0.99 (0.01)	0.99 (0.01)	1.00 (0.01)	0.99 (0.01)	0.99 (0.02)	0.96 (0.09)	0.99 (0.02)
	Volume age 6					Volume age 7				
Initiation	0.03 (0.29)	0.06 (0.37)	^a	^a	^a	0.94 (0.32)	^a	0.31 (0.25)	0.76 (0.50)	^a
Cessation	^a	^a	^a	^a	^a	^a	^a	0.66 (0.23)	^a	^a
Duration	0.29 (0.40)	^a	0.83 (0.36)	^a	^a	^a	^a	0.65 (0.25)	^a	^a
BA growth rate	0.99 (0.04)	1.00 (0.00)	0.99 (0.02)	0.91 (0.11)	1.00 (0.11)	1.00 (0.03)	0.89 (0.06)	0.93 (0.04)	0.89 (0.13)	1.00 (0.13)

Note: Values in parentheses are standard errors

^a Was not estimated because within-family clonal variance was 0

height had a positive phenotypic correlation. Although in continental *P. abies* populations, this correlation was zero and sometimes negative (Dietrichson 1967, 1969).

On the other hand, studies in aspen hybrids in temperate regions suggested that the fast overall growth is largely explained by longer vegetative period (r_p between growth period and diameter was 0.67-0.91 and highly significant, Yu *et al.* 2001).

Because cambial phenology traits appear to be weakly inherited and have small and inconsistent genetic correlations with growth, indirect responses in cambial phenology from selection of bole basal-area or volume are expected to be small. The practical implications of these findings are that selection programs aimed at increasing growth rate are very unlikely to impact dates of initiation or cessation; thus there are few concerns about increasing the likelihood of frost damage. Also, another important point to consider in indirect responses, as suggested by Langdon (1963), is the effect of length of growing season on wood properties. Trees that are capable of growing longer into the season may produce a higher proportion of summerwood to spring wood and have higher wood density than genotypes that cease growth early. Future research could help to understand whether families or clones which cease growth earlier do, in fact, have lower wood density. If so, this could then be incorporated into selection programs.

Analysis across Years 2002-2003

There were no significant clone by year interactions for any basal-area phenology traits, and only basal-area increment for L4 and basal-area growth rate for S2 showed significant clone by year interactions (data not shown). Still, genetic correlations between years were high for these two traits with significant clone x year interactions (0.91 for L4 basal-area increment, and 0.93 for S2 basal-area growth rate), indicating that the interactions were not biologically important. From this analysis we can conclude that

each of the basal-area growth phenology traits and each of the basal-area growth rate traits were genetically controlled by a similar set of genes in years 2002 and 2003. So, the clones were consistent across years in both phenology and growth traits that we measured. Nevertheless, stability needs to be tested for a longer period of time, since environment played an important role in the control of the inheritance of basal-area phenology traits (*i.e.* severe environmental conditions may change the results).

Clonal studies in *Betula pendula* revealed significant clone-by-year interactions for bud burst and also large interannual variation among clones in the date of bud burst and, especially, in the termination of growth (Rousi and Pusenius 2005). These interactions indicated that in addition to genetic effects, environmental factors have a strong influence on both bud burst and growth termination. In *P. menziesii*, provenance-by-year interaction existed for bud burst date in a three-year study (White *et al.* 1979). In a two-year study with loblolly pine, Jayawickrama *et al.* (1998) found no significant provenance-by-year interaction; and significant year-by-family within provenance interactions were found for height growth and height growth cessation.

We conclude that the significant genetic variation among clones within-family in basal-area growth and the stability of ranks across years found in my study, contribute to understanding the potential impact that clonal selection can have on future forest plantation productivity. Poor consistency in direction and strength of genetic correlations between basal-area increment and phenological traits indicated that in these slash and loblolly pine families, initiation, cessation or duration of growth were traits that did not have biological importance in determining how much a genotype will grow during the season. Basal-area growth in loblolly and slash pine families and clones was sensitive to

soil water availability, with stem growth declining both above and below an “optimum” soil water balance level. Finally, while there were significant size differences among taxa (species and families) at age 6 y and 7 y, genetic differences in basal-area growth rate were only expressed during short, discrete time periods in the spring and fall. This finding may have important implications for the timing of investigations attempting to determine the mechanisms underlying genetic growth differences, since growth rate, and possibly the physiological or gene expression traits controlling growth rate, may be similar throughout most of the growing season among taxa with contrasting long-term cumulative growth.

CHAPTER 4
CARBON ISOTOPE DISCRIMINATION, CROWN CONDUCTANCE, GROWTH
AND THEIR GENETIC PARAMETERS IN LOBLOLLY AND SLASH PINE
FAMILIES AND CLONES

Introduction

Plants fractionate carbon isotopes during photosynthesis. The magnitude of the fractionation varies with photosynthetic type, environment, genotype, and other factors, and this variation in magnitude can be used to study a variety of issues in plant physiology (O'Leary 1981, 1988, 1993; Farquhar *et al.* 1989). During photosynthesis, the stable isotope ratio ($^{13}\text{C}/^{12}\text{C}$) of carbon dioxide assimilated differs from that of the source CO_2 and is about 2 ‰ lower in plants than air (Farquhar *et al.* 1989). There are two primary processes that cause carbon isotope ratios to change during photosynthesis: diffusional fractionation and enzymatic fractionation. Carbon dioxide molecules containing ^{12}C are lighter, and therefore, diffuse into the leaf at a faster rate (by a factor of 1.0044, or 4.4 ‰) than molecules containing ^{13}C (Craig 1954; Farquhar and Lloyd 1993). The primary carboxylating enzyme in C_3 plants, ribulose-1,5-biphosphate carboxylase, preferentially uses $^{12}\text{CO}_2$ (by a factor of 1.029 or 29‰) and so discriminates against $^{13}\text{CO}_2$ (Roeske and O'Leary 1984; Guy *et al.* 1993). The carbon isotope ratio of leaf organic material depends on the relative influence of diffusional and enzymatic fractionation, which in turn is determined by the ratio of intercellular CO_2 (p_i) and atmospheric CO_2 (p_a) partial pressures (Farquhar *et al.* 1982, 1989). Changes in the ratio p_i/p_a and the leaf carbon isotope ratio are a function of changes in either, or both, photosynthetic rate and stomatal conductance (Farquhar *et al.* 1989).

Since the carbon isotope ratio in the leaf provides information about processes integrated over the whole life of a leaf, it is particularly useful for examining subtle genetic differences in photosynthetic and water use characteristics. There has been considerable interest in using carbon isotope discrimination ($\Delta^{13}\text{C}$, determined from carbon isotope ratio of the sample with respect to a standard; lower discrimination against ^{13}C , means $\Delta^{13}\text{C}$ value closer to 0, than high discrimination against ^{13}C) to estimate integrated water use efficiency (WUE) in both agronomic plants and trees. WUE measures the ratio between photosynthetic rate (A) and transpiration rate (E), or in other words the ratio between carbon fixation and water losses.

Genetic variation in $\Delta^{13}\text{C}$ has been reported for several tree species. Family differences in $\Delta^{13}\text{C}$ were reported for *Pseudotsuga menziesii* (Zhang *et al.* 1993), *Larix occidentalis* (Zhang *et al.* 1994), *Picea mariana* (Flanagan and Johnsen 1995; Johnsen *et al.* 1999), *Picea glauca* (Sun *et al.* 1996), *Araucaria cunninghamii* (Prasolova 2000), *Pinus pinaster* (Brendel *et al.* 2002), *Castanea sativa* (Lauteri *et al.* 2004). Significant clonal variation has also been demonstrated in foliar carbon isotope composition in F1 hybrids clones between slash pine (*Pinus elliottii*) and Caribbean pine (*Pinus caribaea*) (Xu *et al.* 2000; Prasolova *et al.* 2003, 2005), in *Eucalyptus globulus* (Osorio and Pereira 1994, Osorio *et al.* 1998), loblolly pine (*Pinus taeda*) (Gebremedhin 2003) and poplar hybrid clones (Marron *et al.* 2005). Therefore, understanding of the genetic basis of variation in $\Delta^{13}\text{C}$ could be very useful for ranking genotypes and may serve as a guide for tree breeding programs.

Most studies reported in the literature on $\Delta^{13}\text{C}$ and WUE in tree species have been associated with a small number of species or genetic entries. Only a few recent

publications (Johnsen *et al.* 1999; Prasolova *et al.* 2003) have reported the results of $\Delta^{13}\text{C}$ with relatively large sets of genetic materials in tree breeding trials, and these have been used to obtain reasonable estimates of genetic parameters such as heritability for foliar $\Delta^{13}\text{C}$ and genetic correlations between physiological traits. From an operational point of view, such information is crucial when introducing new genetic entries, such as untested clones, into plantation schemes.

The growth of individual plants may be either positively or negatively correlated with leaf carbon isotope discrimination values depending on whether variation in discrimination is associated with changes in photosynthetic capacity or stomatal conductance (Farquhar *et al.* 1989). During photosynthetic gas exchange, discrimination will be reduced in a plant when photosynthetic rate is increased, if stomatal conductance remains constant. The higher photosynthetic rate may also translate into faster growth, if other factors remain constant. Therefore, carbon isotope discrimination values should be negatively correlated with plant growth when variation in discrimination results from changes in photosynthetic rates (Farquhar *et al.* 1989). In contrast, if variation in discrimination is caused by changes in stomatal conductance, then carbon isotope discrimination values should be positively associated with growth. This results because an increase in stomatal conductance will result in higher assimilation of carbon, thereby increasing growth, and will also enhance discrimination against ^{13}C during gas exchange (Farquhar *et al.* 1989).

Previous results have suggested that carbon isotope ratio in the leaf can be used in early selection in tree improvement programs (Farquhar *et al.* 1989; Bond and Stock 1990; Zhang *et al.* 1993; Sun *et al.* 1996; Johnsen *et al.* 1999; Xu *et al.* 2000; Pita *et al.*

2001; Prasolova *et al.* 2003). Due to the advantages of early selection in tree improvement programs of loblolly and slash pine, the use of the carbon isotope ratio technique might help to increase forest productivity in future plantations by selecting families or clones that show greater water use efficiency or photosynthetic rate or a combination of both.

Stomata respond to environmental variation and regulate water loss and carbon dioxide gain, and thus biosphere–atmosphere exchange of mass and energy. Ideally, stomatal conductance should remain in balance with variations in soil-leaf hydraulic conductance. This coordination would contribute to maintenance of leaf water potential above minimum values associated with leaf desiccation, nonstomatal inhibition of photosynthetic carbon acquisition, and xylem cavitation (Wullschleger *et al.* 1998). In the last decade, development and calibration of techniques that allow measurement of water movement through the sapwood and crown as sap flow, make it possible to calculate crown conductance parameters such as stomatal sensitivity to changes in environmental conditions, like radiation and vapor pressure deficit in longer spatial and temporal scales (Granier 1987; Martin *et al.* 1997; Ewers *et al.* 1999; Martin *et al.* 2001; Lu *et al.* 2004; Martin *et al.* 2005). Stomatal sensitivity to environmental changes will affect gas exchange levels, photosynthesis, carbon fixation and growth (Sperry 2000; Tyree 2003). Because one of the processes that define carbon isotope discrimination in the leaf is stomatal conductance, it is important to know the correlation between discrimination and conductance in loblolly and slash pine clones.

In this paper we examine how changes in carbon isotope discrimination are related to both differences in stem growth increment and differences in tree-level crown

conductance observed within full-sib families and clones of loblolly and slash pine. The following hypotheses were considered:

- Carbon isotope discrimination varied among genotypes;
- Fast-growing genotypes tend to have lower carbon isotope discrimination and higher water use efficiency, so stem growth will be negatively correlated with leaf stable carbon isotope discrimination; and
- Genotypes that tend to have higher stomatal sensitivity to changes in water pressure deficits tend to have lower values of discrimination against ^{13}C .

Material and Methods

Study Site and Plant Material

The study area was located on lands managed by Rayonier Inc. in Bradford County, Florida. The climate is humid and subtropical, with a mean annual temperature of 21°C, mean annual rainfall of 1316 mm, and over 50% of the rainfall occurring in June through September. Periods of drought are normal in the spring and fall. Mean annual rainfall during 1999-2001 was 967 mm, in contrast to 1405 mm in year 2002 (NOAA 2002). The soils are classified as Pomona and consist of very deep, somewhat poorly to poorly drained soils that are formed in sandy and loamy marine sediments (sandy, siliceous, hyperthermic Ultic Alaquods). Slopes are 0 to 2 %. In a typical profile, the spodic horizon occurs at 30-60 cm, with an argillic horizon at 90-120 cm. Water table is typically at a depth of 15 to 45 cm for one to three months and a depth of 25 to 100 cm for six months or more, during most years (Soil Survey Staff 1998).

The study took place in an area containing 16 full-sib and half-sib loblolly and slash pine families planted in 337 m² family plots in January 1997. The experiment was designed as a randomized complete block with four replicates (Appendix A). We used one full-sib loblolly pine family and four full-sib slash pine families. Each family plot

contained 60 clones propagated as rooted cuttings from a single family, planted at 1.7 m x 3.4 m spacing (1730 trees ha⁻¹). Cuttings were taken from donor hedges in the spring, and were rooted and grown in a greenhouse for six months before planting. Each of the four plots of the same family contained the same 60 genotypes, but with the ramets planted into different, randomly-determined planting locations in the plot. For growth and carbon isotope discrimination we studied approximately 1,200 trees: 60 trees per family plot x 5 families x 4 ramets per clone distributed as one ramet in each of the 4 complete blocks. For sap flow and crown conductance analysis we measured approximately 300 trees: 30 trees per family x 5 families x 2 ramets per clone distributed across the 4 complete blocks as described later. Fertilization and weed control were applied periodically to reduce interspecific competition and prevent nutrient deficiency (Appendix B).

Tree Growth and Carbon Isotope Discrimination

Stem volume growth in the 2001, 2002, and 2003 growing seasons (ages 4-5 yr, 5-6 yr, and 6-7 yr, respectively) was determined from dormant season measurements of tree diameter at 1.37 m height (DBH) and total tree height (HT). Outside-bark individual tree stem volume was calculated using Equation 4-1 (Hodge *et al.* 1996), where DBH and HT were entered in m:

$$\text{VOL (dm}^3\text{)} = (0.25 * 3.14 * (\text{DBH})^2 * (1.37 + 0.33 (\text{HT} - 1.37))) * 1000 \quad (4-1)$$

Also, periodic diameter increment was measured in March-April 2002 to calculate sapwood cross-sectional area for tree-level transpiration analysis as explained later.

Foliar samples for stable carbon isotope discrimination analysis were collected from the five families from the first flush formed in the spring. Samples were taken in middle summer 2001 and 2003 from a branch on the south side of the upper canopy

(exposed to full sun, to help avoid extraneous differences in isotope values). The tissue was dried at 65 °C for several days, and finely ground. The relative abundance of ^{13}C and ^{12}C was determined in 3 mg subsamples with a Delta Plus isotope ratio mass spectrometer (Cornell University Stable Isotope Laboratory). Stable carbon isotope ratio ($\delta^{13}\text{C}$) was expressed as $^{13}\text{C}/^{12}\text{C}$ ratio relative to international PDB (Pee Dee Belemnite, Craig 1954). Carbon isotope discrimination values ($\Delta^{13}\text{C}$) were calculated from $\delta^{13}\text{C}$ values using Equation 4-2 (Farquhar *et al.* 1989), where δ_p is the isotope composition of the plant material and δ_a is that of the air (assumed to be -8‰):

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \quad (4-2)$$

The accuracy and precision of this analysis for foliar $\delta^{13}\text{C}$ were ascertained by making repeated measurements of $\delta^{13}\text{C}$ in each batch of the samples and using an internal foliar standard in each of the sample batches. We concluded that carbon isotope measurements are repeatable and accurate with a standard error of 0.14‰.

Use of $\Delta^{13}\text{C}$ to compare A/E among genotypes requires several assumptions: first, that leaf temperatures, and therefore leaf-to-air vapor pressure differences, are similar among the plants being compared; second, that $\delta^{13}\text{C}$ of source of CO_2 is identical among genotypes being compared; and third, that biosynthetic fractionation is similar among the genotypes. These assumptions are well met by the needle shape of conifer leaves, because they are narrow and their boundary layer conductances are therefore high (Marshall and Zhang 1993, Ewers and Oren 2000).

Meteorological Data

Air temperature, photosynthetically active radiation, and relative humidity were measured in year 2002 by a weather station installed in the study area. Variables were

read every minute and averages recorded every 15 minutes by a data logger. Vapor pressure deficit (D) was calculated from relative humidity (R_H) and air temperature (T_A) measurements based on equations adapted from Goff and Gratch (1946).

Individual Tree Transpiration

A subsample of 300 trees from 5 families (30 clones per family and 2 ramets per clone) was used to monitor sap flow on a daily basis. Selection of clones within families was based on three criteria: genotypes were selected across the range of growth performance (good, medium and poor growers); genotype performance for growth was consistent across replications in the test; and finally genotypes were free of disease. Selection of ramets within clone was done by selecting the two ramets more representative of the growth performance category and free of disease.

Water flux in the xylem was estimated using the constant heat method of Granier (1987). Heat dissipation gauges were installed in each of the 300 trees, a constant 0.2 W of power was applied to the probe, and the degree to which heat is dissipated from the probe was measured. A heat probe of 20 mm long was inserted into the tree stem, at 40 cm from the base, and was paired with an unheated probe located 10 cm below the heated probe. To avoid thermal gradients from direct radiation, all sensors were installed in the north side of the stem and covered with aluminum shelters.

Measurements of sap flux were taken every minute and stored as a 15-min average for two months (March-April in 2002) with dataloggers (Campbell Scientific, Logan, UT). Equation 4-3 shows how sap flux (J_S , kg H₂O m⁻² s⁻¹) was calculated using an empirical relationship (Granier 1987):

$$J_S = 0.119 ((\Delta T_m - \Delta T) / \Delta T)^{1.231} \quad (4-3)$$

which measured the daily maximum temperature difference between heated and unheated probes during times of zero flux (ΔT_m) as a baseline. Temperature difference (ΔT) was also measured during the day as water carried heat away from the probe. Deviation from the baseline was used to estimate water flux.

Whole-tree transpiration per unit of projected crown area (E_L , $\text{kg H}_2\text{O m}^{-2} \text{A}_C \text{s}^{-1}$) was calculated by multiplying tree sapwood cross-sectional area (A_S , m^2) by the sap flux density measurement and standardized by projected crown area (A_C , m^2 , calculated as projected crown area on the ground):

$$E_L = J_S (A_S/A_C) \quad (4-4)$$

To estimate E_L we assumed that water uptake, as estimated from sap-flow measurements, does not significantly lag actual canopy transpiration.

Calculations of individual tree sapwood cross-sectional area contained two assumptions: 1) For young southern pine, the entire cross-sectional area of the tree was composed of active sapwood; and 2) Sap flux density for sapwood further than 20 mm from the cambium (where sap flow probes are located) was the same as outer cambium. The first assumption was probably met in these young pine trees, while the second was almost certainly not. Several studies have shown that sap flux density tends to be higher near the cambium, and declines with radial depth into the sapwood (Phillips *et al.* 1996; Wullschleger and King 2000; James *et al.* 2002). However, for the purposes of my study, the estimates of whole-tree water use were acknowledged to be biased upward, but should still be useful for relative comparison of the genotypes.

Crown Conductance and Stomatal Sensitivity Calculations

We calculated whole-tree crown conductance of water vapor (G_S , mm s^{-1}) by substituting the transpiration data and meteorological measurements into the inverted

Penman-Monteith equation using the formula suggested by Monteith and Unsworth (1990), where λ is the latent heat of evaporation of water (J kg^{-1}), γ is the psychrometer constant (kPa K^{-1}), ρ_a is the density of dry air (kg m^{-3}), C_p represents the specific heat capacity of the air ($\text{J kg}^{-1} \text{K}^{-1}$), and D is the vapor pressure deficit (kPa):

$$G_S = 1000 (E_L \lambda \gamma) / (\rho_a C_p D) \quad (4-5)$$

G_S values were converted from mm s^{-1} to $\text{mmol m}^{-2} \text{s}^{-1}$ using Pearcy *et al.* (1989).

Equation 4-5 requires the following conditions (Ewers and Oren 2000): (1) D is close to the leaf-to-air vapor pressure deficit, namely boundary layer conductance is high; (2) There is no vertical gradient in D through the canopy; and (3) There is negligible water stored above the J_S measurement position. We assumed that these conditions were met at my study.

As the vapor pressure deficit between leaf and air increases, stomata generally respond by partial closure (Lange *et al.* 1971). Responses of stomatal conductance to increasing D generally follow an exponential decrease described by the empirical Equation 4-6 (Oren *et al.* 1999), where $-m$ is the sensitivity of G_S response to $\ln D$ or the slope of G_S vs $\ln D$ ($-dG_S/d\ln D$) and $G_{S\text{ref}} = G_S$ at $D = 1 \text{ kPa}$:

$$G_S = -m \ln D + G_{S\text{ref}} \quad (4-6)$$

Given the uncertainties under low levels of incoming radiation (or limited light throughout the canopy) and low D situations, we filtered the data to conditions where $D \geq 0.6 \text{ kPa}$ and incoming radiation $> 500 \text{ Wm}^{-2}$. This screening allowed us to keep errors in the estimation of G_S below 10% (Ewers and Oren 2000).

Genetic Parameters and Statistical Analyses

Analysis of variance (ANOVA) was used to analyze $\Delta^{13}\text{C}$, crown conductance parameters in response to vapor pressure deficit, and growth data by year. SAS® PROC GLM was used to test for significance of random effects (clone), while PROC MIXED were utilized to test the fixed effects (species and families). Equation 4-7 shows the linear model considered for the analyses, where Y_{ijkl} is the performance of the ramet of the l^{th} clone within the k^{th} family nested in the j^{th} species in the i^{th} replication; $i = 1, 2, 3,$ and 4 for replications; $j = \text{slash, loblolly}; k = 1, 2, 3, 4,$ and 10 for families; $l = 60$ identification numbers for 60 clones within each of the five families:

$$Y_{ijkl} = \mu + b_i + S_j + F_{k(j)} + c_{l(jk)} + bS_{ij} + bF_{ik(j)} + \varepsilon_{ijkl} \quad (4-7)$$

μ = population mean,

b_i = random variable of replication $\sim \text{NID}(0, \sigma^2_b)$,

S_j = fixed effect of species (slash or loblolly),

$F_{k(j)}$ = fixed effect of family nested within species,

$c_{l(jk)}$ = random variable of clone nested within-family and species $\sim \text{NID}(0, \sigma^2_c)$,

bS_{ij} = random variable for replication x species interaction $\sim \text{NID}(0, \sigma^2_{bS})$,

$bF_{ik(j)}$ = random variable for replication x family(species) interaction $\sim \text{NID}(0, \sigma^2_{bF})$, and

ε_{ijkl} = error term $\sim \text{NID}(0, \sigma^2_\varepsilon)$.

With so few families, estimates of genetic parameters were restricted to within-family estimates obtained from clonal variation expressed within each of the four slash families and one loblolly pine family. For each family two types of genetic parameters were estimated: within-family heritability for each trait, and within-family genetic and environmental correlations among traits. Within family variance and covariance components were obtained using ASREML, a statistical package that fits linear mixed models using Restricted Maximum Likelihood (Gilmour 1997).

Within-family individual-tree broad-sense heritability was calculated as in Equation 4-8, where σ_c^2 is the variance among clones within family and σ_ε^2 is the residual variance as defined in Equation 4-7:

$$H^2_{WF} = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_\varepsilon^2} \quad (4-8)$$

The standard error for heritability estimates was calculated from Dickerson (1962). The residual likelihood ratio test (Wolfinger 1996) was used to test heterogeneity of variances among slash pine families, and heritabilities were estimated separately ($\chi^2_{(0.05,6)} = 12.6$), or pooled, as appropriate.

Within-family genetic and environmental correlations between $\Delta^{13}\text{C}$ and growth rate and between $\Delta^{13}\text{C}$ and crown conductance parameters were calculated with the Equation 4-9 (Falconer and Mackay 1996), where σ_{xy} is the clonal or residual covariance between two traits, while σ_x and σ_y are the square root of the product of the clonal or residual variance within family for traits x and y, respectively:

$$r_{xy} = \frac{\sigma_{xy}}{\sigma_x \sigma_y} \quad (4-9)$$

Standard error for genetic and environmental correlations was estimated using ASREML (Gilmour 1997, asymptotic properties and the Taylor series approximation of the variance of a ratio).

The significance of the clone-by-year variance component was tested using a likelihood ratio test (Wolfinger 1996). The clone-by-year variance component was declared different from 0 when $\chi^2_{(1,0.05)}$ was equal to or greater than 3.8. For traits with a significant clone-by-year variance component, within-family genetic correlations

between years were estimated considering the two years as two different traits, using Equation 4-9.

Results

Carbon Isotope Discrimination

Family within-species and clone within-family were significant sources of variation for foliar carbon isotope discrimination in 2001 and 2003 (Table 4-1), but at the species level no significant differences were detected ($p < 0.05$). Mean family values for $\Delta^{13}\text{C}$ ranged between 21.39‰ to 22.98‰ (Table 4-1), and at the clonal level mean values for $\Delta^{13}\text{C}$ ranged from 19‰ to 25.41‰ (data not shown). Among slash pine families, family S2 tended to have the lowest values for $\Delta^{13}\text{C}$ in both years 2001 and 2003 (Figure 4-1). In contrast, family S3 showed the highest value for both years. Lower values of $\Delta^{13}\text{C}$ in year 2001 than in year 2003 samples for all families might be associated with lower rainfall between February and June 2001 in comparison with same period in 2003 (near 50 % less rainfall in year 2001 than in year 2003, Figure 4-2). Clonal within-family genetic variation was significant in all 5 families for $\Delta^{13}\text{C}$ in 2001, and for all families except S3 in 2003 (Table 4-2).

Within-family heritabilities ranged from 0.01 to 0.32 for discrimination in years 2001 and 2003 (Table 4-3). There was no evidence for genotype-by-year interaction for any family. A strong within-family genetic correlation between $\Delta^{13}\text{C}$ in year 2001 and year 2003 occurred for all families (Table 4-4), indicating that the ranking of clones remained constant across years for carbon isotope discrimination.

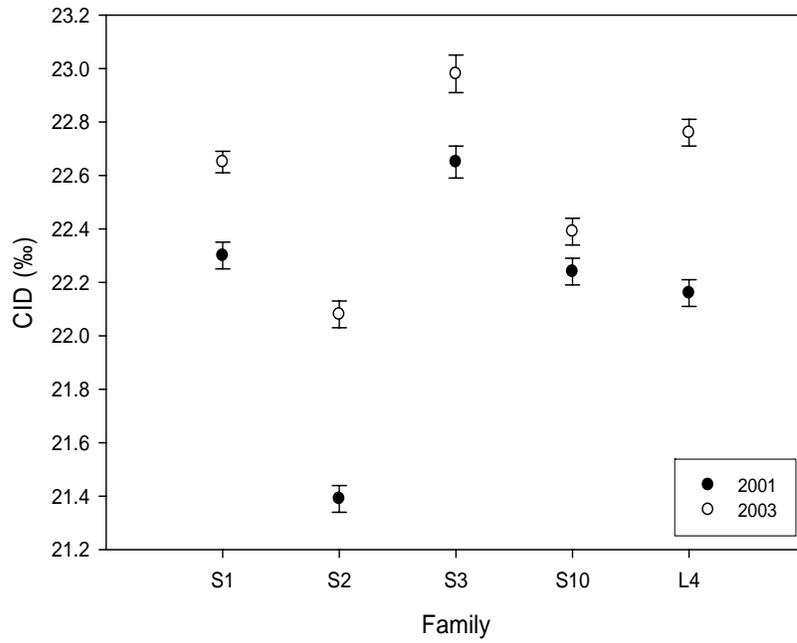


Figure 4-1. Family means and standard errors in carbon isotope discrimination in year 2001 and 2003.

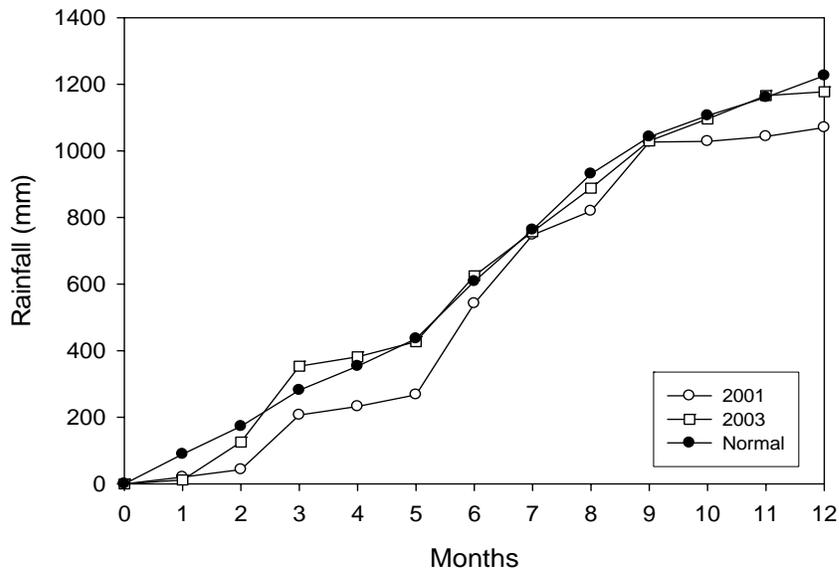


Figure 4-2. Accumulated monthly precipitation in years 2001, 2003 and mean normal year from Gainesville Regional Airport, Gainesville, Florida (NOAA 2003).

Table 4-1. Significance levels (p-values), species and within family least square means for volume, carbon isotope discrimination ($\Delta^{13}\text{C}$) for three growing periods, and crown conductance variables (G_{Sref} and $G_{\text{Sensitivity}}$) in slash and loblolly pine families in north central Florida.

Variable	Code	Significance level by effect			Species mean		Slash family means			
		Species	Family	Clone	Slash	Loblolly	S1	S2	S3	S10
$\Delta^{13}\text{C}$ Year 2001 (‰)	CID2001	0.9005	<0.0001	<0.0001	22.14	22.16	22.30	21.39	22.65	22.24
$\Delta^{13}\text{C}$ Year 2003 (‰)	CID2003	0.2852	0.0175	<0.0001	22.53	22.76	22.65	22.08	22.98	22.39
G_{Sref} ($\text{mmol m}^{-2} \text{s}^{-1}$)	G_{Sref}	0.0840	0.4340	0.2195	754.36	553.86	798.98	702.17	735.94	780.33
$G_{\text{Sensitivity}}$ ($\text{mmol m}^{-2} \text{s}^{-1} \ln(\text{kPa})^{-1}$)	$G_{\text{Sensitivity}}$	0.0910	0.1640	0.0801	465.91	330.55	511.17	410.11	457.54	484.84
Volume increment age 4-5 ($\text{dm}^3 \text{tree}^{-1}$)	VI45	0.0472	0.0767	<0.0001	7.32	9.41	7.40	7.42	6.55	7.90
Volume increment age 6-7 ($\text{dm}^3 \text{tree}^{-1}$)	VI67	0.8318	0.0786	<0.0001	12.29	12.51	12.35	12.35	10.97	13.47

Table 4-2. Significance levels (p-values) for clone within family in carbon isotope discrimination for two growing periods and crown conductance variables for loblolly and slash pine families in north central Florida.

Trait	Significance level within family (clonal variation)				
	L4	S1	S2	S3	S10
$\Delta^{13}\text{C}$ 2001	<0.0001	0.0006	0.0009	0.0208	<0.0001
$\Delta^{13}\text{C}$ 2003	0.0025	<0.0001	0.0070	0.5361	<0.0001
G_{Sref}	0.0384	0.2386	0.4424	0.3361	0.6081
$G_{\text{Sensitivity}}$	0.0195	0.1801	0.3011	0.2645	0.6019
VI45	0.0112	0.0003	<0.0001	0.2153	0.0043
VI67	0.0032	0.0289	0.0001	0.0401	0.0234

Table 4-3. Within-family individual-tree broad-sense heritabilities for stable carbon isotope discrimination ($\Delta^{13}\text{C}$) by year, and crown conductance variables in loblolly and slash pine families growing in north-central Florida

Trait	Family L4	Family S1	Family S2	Family S3	Family S10
$\Delta^{13}\text{C}$ 2001	0.23 (0.08)		0.20 (0.04) ^a		
$\Delta^{13}\text{C}$ 2003	0.17 (0.07)	0.32 (0.09)	0.14 (0.07)	0.01 (0.00)	0.25 (0.08)
G _{Sref}	0.30 (0.23)		0.08 (0.09) ^a		
G _{Ssensitivity}	0.38 (0.23)	0.22 (0.19)	0.11 (0.19)	0.21 (0.19)	0.00 (0.00)
VI45	0.12 (0.07)		0.19 (0.04) ^a		
VI67	0.16 (0.07)		0.15 (0.04) ^a		

Note: Values in parentheses are standard errors

^a Variance components were pooled across slash pine families

Table 4-4. Genetic correlations between years 2001 and 2003 by family for carbon isotope discrimination ($\Delta^{13}\text{C}$) and between 4-5 yr and 6-7 yr stem volume increment (VI) for loblolly and slash pine families in north central Florida.

Trait	Family L4	Family S1	Family S2	Family S3	Family S10
$\Delta^{13}\text{C}$	0.70 (0.23)	0.82 (0.17)	1.00 (0.25)	-- ^a	1.00 (0.14)
VI	0.90 (0.15)	0.78 (0.13)	0.96 (0.05)	0.62 (0.42)	0.81 (0.17)

Note: Values in parentheses are standard errors

^a -- Genetic variation was not significant in carbon isotope discrimination year 2003

Stem Growth

Clonal differences in stem growth increments (age 4-5 y and age 6-7 y) were more apparent than differences at the family and species levels (Table 4-1). Loblolly pine tended to growth faster than slash pine. Within slash pine, family S10 was the fastest grower and S3 was the slowest in both years (Table 4-1). We found greater rates of stem volume increment in year 2003 than in 2001 for all families most likely to higher rainfall during the growing season in 2003 (Figure 4-2). Clone within-family variation changed from one family to another in terms of significance, with family S2 having the highest variation among clones and family S3 the smallest clonal variation in stem volume increment (Table 4-2). This difference in clonal variation was directly related with levels of inheritance. However, when testing for significance of clonal variance components among slash pine families, differences were not detectable at $p=0.05$, so families were pooled to increase precision of heritability estimates for both years of measurements. In

general, within-family heritabilities for stem growth increment were low to moderate in both loblolly and slash pine (0.12 to 0.19, Table 4-3). On the other hand, the clone-by-year interaction component was not significant and year-to-year genetic correlations were high for all families, except for family S3 in which the correlation was moderate with a wide confidence interval (Table 4-4). The lack of year-by-clone interaction indicates that clones that had a high value for stem volume increment at age 4-5 y also had a high value for the same trait at age 6-7 y.

Whole-Tree Crown Conductance and Stomatal Sensitivity

Whole-tree level crown conductance was calculated at 15 minute intervals for approximately 300 trees (half of the ramets per clone, and half of the clones per family in 5 families). Tree-level stomatal conductance was negatively associated with D, with an exponential decrease in G_S as D increased, as shown for a representative slash pine ramet (Figure 4-3). From this relationship we estimated G_{Sref} , defined as the value of G_S when $D=1$ kPa, and $G_{Ssensitivity}$ which quantified the sensitivity of G_S to changes in D, solving the parameters in Equation 4-6 for each ramet. Across all 5 families, there was a significant linear relationship between G_{Sref} and $G_{Ssensitivity}$ with no intercept (averaged across family $R^2=0.77$, data not shown).

Genetic variation in G_{Sref} and $G_{Ssensitivity}$ was difficult to detect at the species, family and clonal levels ($p<0.05$). This may be caused by microsite variation and sample size (Table 4-1). G_{Sref} and $G_{Ssensitivity}$ were 36 and 40% higher in slash pine than in loblolly pine ($p=0.08$ and 0.09 , respectively). Conductance in slash pine genotypes tended to be more sensitive to changes in D than loblolly pine genotypes. At the same time, slash pine families had on average higher G_{Sref} than loblolly pine, meaning that slash pine conductance to CO_2 was higher at a reference D of 1 kPa.

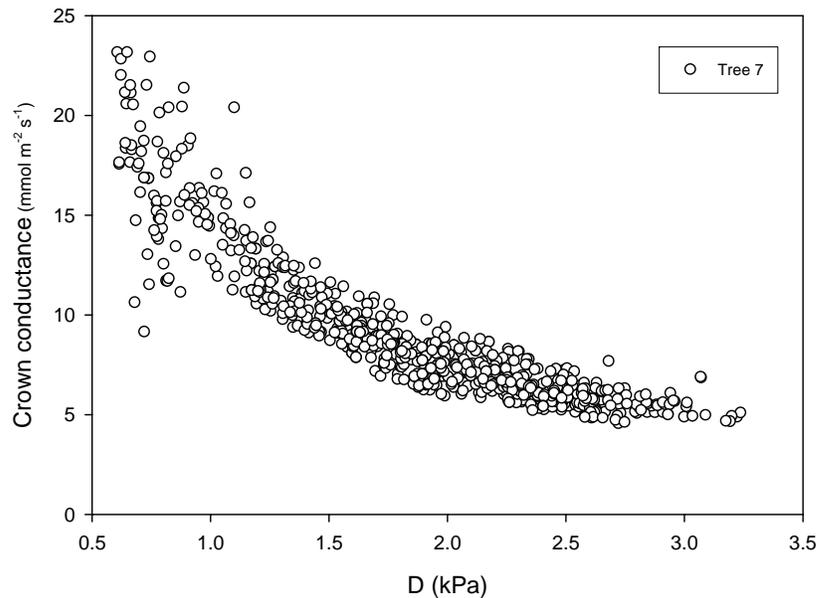


Figure 4-3. Representative relationship between canopy average stomatal conductance (G_s) and vapor pressure deficit (D) on a half hourly basis for a typical slash pine ramet.

When we analyzed clonal variation family by family, only family L4 showed significant differences for G_{Sref} and $G_{Ssensitivity}$ (Table 4-2), and variable levels of genetic control were found among slash pine families. Within-family individual-tree broad-sense heritabilities were high for $G_{Ssensitivity}$ in family L4 ($H^2_{WF}=0.38$), lower in slash pine families S1, and S3, and very low in families S2 and S10 (Table 4-3). For G_{Sref} a moderate level of heritability was found in family L4, but low heritability was found for the pooled slash pine families. This result was likely influenced by lack of genetic variation in family S10. Interestingly, within-family broad-sense heritabilities for crown conductance parameters and $\Delta^{13}C$ were generally higher than for stem growth increment, meaning that these physiological traits were under stronger genetic control than growth traits. The same time, however, heritability estimates for crown conductance parameters were associated with large standard errors (Table 4-3).

Genetic Correlations between Carbon Isotope Discrimination and Growth and Whole-Tree Crown Conductance

Genetic correlations among families were not significantly different from zero between $\Delta^{13}\text{C}$ and stem volume growth (Table 4-5). Only the genetic correlation between $\Delta^{13}\text{C}$ year 2001 and stem volume increment age 4-5 yr in family S10 was significantly negative (-0.54), meaning that faster growing clones showed less discrimination against ^{13}C during gas exchange. In 2003, family S3 had no variation for isotope composition so its correlation with growth increment could not be estimated. Of the nine estimable correlations for the 5 families across 2 years, the average genetic correlation was -0.26 which may indicate a slightly negative general relationship between carbon isotope discrimination and growth in these slash and loblolly pine clones.

Table 4-5. Within-family correlations between volume increment of the growing season and stable carbon isotope discrimination ($\Delta^{13}\text{C}$) by year in loblolly and slash pine families growing in north-central Florida

Trait	Volume increment				
	Family L4	Family S1	Family S2	Family S3	Family S10
<i>Genetic</i>					
$\Delta^{13}\text{C}$ 2001	0.09 (0.31)	-0.29 (0.27)	0.05 (0.26)	-1.00 (0.80)	-0.54 (0.24)
$\Delta^{13}\text{C}$ 2003	0.01 (0.32)	-0.38 (0.26)	-0.33 (0.29)	-- ^a	0.01 (0.31)
<i>Environmental</i>					
$\Delta^{13}\text{C}$ 2001	-0.05 (0.08)	-0.08 (0.08)	-0.21 (0.08)	-0.10 (0.09)	-0.08 (0.08)
$\Delta^{13}\text{C}$ 2003	-0.05 (0.08)	-0.40 (0.06)	-0.32 (0.08)	-0.33 (0.08)	-0.28 (0.07)

Note: Values in parentheses are standard errors

^a -- Not estimated because genetic variation was not significant in carbon isotope discrimination year 2003.

We found non-significant environmental correlations between $\Delta^{13}\text{C}$ and stem volume increment in year 2001, and low negative environmental correlations for all families in year 2003 but family L4.

We estimated genetic and environmental correlations between mean $\Delta^{13}\text{C}$ and crown conductance parameters using averages since not all measurements were made in the same year. For $\Delta^{13}\text{C}$ we took the average between samples collected in years 2001

and 2003 per individual tree (leaves formed in the springs of both years), and for crown conductance we estimated the parameters from data collected from March through April 2002. We assumed that the environmental conditions in the springs of all years were similar. Despite the considerable effort of measuring sap flow in 300 trees, and estimating integrated crown conductance parameters, we could not accept or reject our hypothesis that related low $\Delta^{13}\text{C}$ with high stomatal conductance sensitivity to changes in D. We found that genetic and environmental correlations were unstable across families and had wide confidence intervals, so they were not significantly different from zero (Table 4-6). This lack of precision in the estimation can be related to two sources: (1) small sample size; and (2) sensitivity of the physiological measurements to subtle changes in microsite. The lack of correlations between $\Delta^{13}\text{C}$ and crown conductance parameters might be due to the separation in years of sap flow measurement and leaf collection, so the assumption of similar environment was not valid.

Table 4-6. Genetic and environmental correlations between mean carbon isotope discrimination (mean $\Delta^{13}\text{C}$) and G_{Sref} and $G_{\text{Sensitivity}}$ for loblolly and slash pine families in north central Florida

Trait	Mean $\Delta^{13}\text{C}$					
	Family L4	Family S1	Family S2	Family S3	Family S10	All slash
<i>Genetic</i>						
Gs ref	-0.22 (0.40)	-0.20 (0.43)	0.97 (1.07)	-0.39 (0.79)	-- ^a	0.24 (0.29)
Gs sensitivity	-0.16 (0.35)	0.06 (0.40)	0.84 (0.51)	-0.41 (0.70)	-- ^a	0.28 (0.24)
<i>Environmental</i>						
Gs ref	0.12 (0.17)	-0.04 (0.17)	0.01 (0.17)	0.12 (0.19)	-0.17 (0.15)	0.00 (0.08)
Gs sensitivity	0.10 (0.18)	-0.03 (0.17)	-0.05 (0.17)	0.17 (0.19)	-0.13 (0.16)	0.00 (0.09)

Note: Values in parentheses are standard errors

^a -- Not estimated because genetic variation was not significant in carbon isotope discrimination year 2003

Discussion

Information on clonal variation in southern pines has become more common in the last two decades (Foster 1988; Paul *et al.* 1997; Isik *et al.* 2003; Schmidting *et al.* 2004; Baltunis *et al.* 2005). At the same time, clonal forestry appears to offer an excellent

opportunity for the early capture of the benefits generated by tree improvement and biotechnology programs (Ahuja and Libby 1993; Libby and Ahuja 1993; Schmidting *et al.* 2004). The novelty of my study was its analysis of clonal genetic variation among families, the number of clones involved per family, and the possibility of analyzing growth and physiological traits under field conditions.

We found significant within-family clonal genetic variation in $\Delta^{13}\text{C}$ and stem volume increment in both years of measurements, reflecting a wide spectrum of clonal performance for growth and gas exchange. At the same time, greater rates of stem volume increment were detected in year 2003 compared to 2001 for all families. These differences may have been caused by variation in seasonal rainfall pattern and total amount of annual precipitation, or simply due to tree size. There are few reports in the literature of clonal variation in loblolly or slash pine growth. Paul *et al.* (1997) reported that height of loblolly pine clones varied significantly at different ages, but that DBH and volume did not. To our knowledge, no other published studies have quantified clonal variation in $\Delta^{13}\text{C}$ in loblolly or slash pine under field conditions.

Clonal variation in foliar $\Delta^{13}\text{C}$ fluctuated from family to family, with family S3 having the lowest range of clonal values. In general, the range of phenotypic clonal mean values we found in our selected families (from 19 to 25.41‰) had a wider distribution in comparison to what had been reported in similar studies; for example, in F1 hybrid pine clones between slash pine and Caribbean pine (19.6 to 20.7‰ and 18 to 21.84‰, Xu *et al.* 2000, Prasolova *et al.* 2003, respectively), loblolly pine clones (23.3 to 22.3‰, Gebremedhin 2003). In *P. menziesii*, family means $\Delta^{13}\text{C}$ ranged between 19.7 to 22.43‰ (Zhang *et al.* 1993), and in *E. globulus* family means for $\Delta^{13}\text{C}$ ranged between 16.7 to

18.1‰ (Pita *et al.* 2001). Genetic variation in $\Delta^{13}\text{C}$ should reflect differences in C_i/C_a , a consequence of the balance between stomatal supply and mesophyll demand of CO_2 (Farquhar *et al.* 1989).

In my study, low to moderate levels of heritabilities for growth, $\Delta^{13}\text{C}$, and crown conductance parameters suggested that these are complex traits determined by the expression of many genes, each one having a small effect on the phenotypic expression of the individual (Falconer and Mackay 1996). However, the heritabilities we estimated are expected to be smaller than broad sense heritabilities values usually reported, because they are estimated within full-sib families and half the additive genetic variation and one fourth of the non-additive variation occurs among full-sib families (Falconer and Mackay 1996). Levels of genetic control for $\Delta^{13}\text{C}$ in a clonal study reported low heritabilities of 0.08 in loblolly pine under different watering regimes (Gebremedhin 2003), and 0.09 to 0.15 in F1 hybrid slash x Caribbean pine (Prasolova *et al.* 2003). In other conifers, studies were carried out in full-sib families and $\Delta^{13}\text{C}$ narrow-sense heritability range from low to moderate, 0.54 for *P. mariana* (Johnsen *et al.* 1999), 0.17 in *P. pinaster* (Brendel *et al.* 2002), between 0.4 to 1.0 in *A. cunninghamii* (Prasolova 2000). In the hardwood *C. sativa*, narrow sense heritability was moderate ($h^2=0.31$, Lauteri *et al.* 2004). Genetic analysis of variation in crown conductance parameters has not been reported in the literature, so it was difficult to make comparisons with slash, loblolly or other pine species or hardwoods. Nevertheless, the within-family, individual-tree broad-sense heritabilities values we reported for whole-tree crown conductance and stomatal sensitivity were large in several cases (Table 4-3). However, the precision of our estimates were low. Increased precision likely requires a much larger number of

replications per clone than was possible in my study. Heterogeneous soil conditions in the study site might have lowered heritabilities due to lack of optimal silvicultural treatments (weed control and fertilization in several years).

Consistency of genotypic ranking across years is essential for breeding to be effective in modifying a particular quantitative trait. We found significant positive correlations across years for both stem volume increment and $\Delta^{13}\text{C}$. Similar results in consistency in across years in $\Delta^{13}\text{C}$ have been reported in *P. mariana* (Johnsen *et al.* 1999).

In my study we tested the hypothesis of correlations between $\Delta^{13}\text{C}$ and stomatal sensitivity to changes in D. We found negative results in the sense that wide confidence intervals around the estimation in genetic correlations gave small confidence in making conclusions or possible explanations on association between these two variables. However, future research in this avenue is needed to understand the underlying mechanism behind the intrinsic photosynthesis-stomatal conductance relationship. Here, we analyzed the relationship between $\Delta^{13}\text{C}$ and crown conductance, but the knowledge of the relationship of $\Delta^{13}\text{C}$ with photosynthetic capacity is also needed. Then, we can understand if genetic variation in $\Delta^{13}\text{C}$ is due to changes in stomatal conductance, or in photosynthetic capacity, or both.

We hypothesized that traits which integrated information over space and/or time would be more highly correlated with growth (see Chapter 2). In this case, $\Delta^{13}\text{C}$ corresponds to an integrated measurement of photosynthesis and stomatal conductance during the time of formation of the leaf. Our results did not support that hypothesis, and genetic correlations between $\Delta^{13}\text{C}$ and stem volume increment were not stable across

families, across years, and not significantly different from zero. We can conclude that $\Delta^{13}\text{C}$ and stem growth were controlled by largely independent sets of genes. Similarly, environmental correlations between $\Delta^{13}\text{C}$ and stem volume increment were low, meaning that microsites which increased discrimination, also increased or decreased stem growth independently.

The observed independence of $\Delta^{13}\text{C}$ from stem growth and the absence of year-by-clone interaction in both growth and $\Delta^{13}\text{C}$ still provide opportunities for selecting loblolly and slash pine clones combining high productivity and high water-use efficiency (low $\Delta^{13}\text{C}$). The changes from year to year in genetic and environmental correlations between these two traits might be associated with changes in seasonal weather patterns, for example the amount of rainfall and soil moisture conditions during the time of leaf formation in the case of $\Delta^{13}\text{C}$ and the total growing season in the case of stem volume increment. As in Figure 4-2, rainfall between February and June was lower in year 2001 than in 2003, and by the end of the 2001 growing season, the decrease in rainfall may have affected growth increment too. Unfortunately, we did not measure soil moisture content to confirm this hypothesis.

On the other hand, the presence of mild weather years in my study, where stem growth was not limited by water supply and water use efficiency may affect the degree of correlations between $\Delta^{13}\text{C}$ and stem growth increment. Condon and Richards (1993) showed that in wheat genotypes, the relationship between crop biomass production and leaf carbon isotope discrimination values changed when crops were grown on different-quality sites. It was only on the driest site that the negative relationship between growth and leaf carbon isotope discrimination predicted from gas exchange characteristics was

supported for wheat genotypes (Condon and Richards 1993). The same situation was described in *P. mariana* by Flanagan and Johnsen (1995), where the strongest correlation between height and $\Delta^{13}\text{C}$ was found in the driest site. In *C. sativa*, the genetic correlations between $\Delta^{13}\text{C}$ and growth traits were generally strong and negative (-0.5 to -1.0), especially in two high temperature treatments (Lauteri *et al.* 2004).

In the literature, negative, positive, or no correlations between $\Delta^{13}\text{C}$ and growth have been reported. Low, moderate and strong negative genetic correlations were reported in some conifers, as for example *P. mariana* (-0.96, Johnsen *et al.* 1999), F1 hybrid between slash pine and Caribbean pine (-0.19 to -0.36 depending on site and sampling season, based on clonal means, Prasolova *et al.* 2003; and -0.83 to -0.96 based on clonal means, Xu *et al.* 2000), *P. menziesii* (-0.65 to -0.67, Zhang *et al.* 1993). Positive phenotypic correlations have been demonstrated in eucalyptus species, like *E. globulus* (Pita *et al.* 2001), and some eucalyptus hybrids (Le Roux *et al.* 1996), and also in loblolly pine clones (0.86, but might be associated to wide standard errors because of low heritability, Gebremedhin 2003). No genetic correlations at all between $\Delta^{13}\text{C}$ and ring width was found in *P. pinaster* (0.02), and in agreement there was the lack of co-location of QTLs between both traits (Brendel *et al.* 2002). Similarly, Marron *et al.* (2005) did not find a significant correlation between discrimination and total biomass in hybrid poplar clones.

Some authors explain the lack of consistent correlation between $\Delta^{13}\text{C}$ and growth in pines as follows: (1) if $\Delta^{13}\text{C}$ is mainly determined by assimilation rate, and if growth is not primarily determined by assimilation rate, then there might be no correlation between $\Delta^{13}\text{C}$ and growth (Brendel *et al.* 2002); (2) if genetic control is moderate for both traits,

this might lower the significance of a genetic correlation (Brendel *et al.* 2002); and (3) if water supply is not limiting growth, then water use efficiency might not be defining growth (Prasolova *et al.* 2003).

On the other hand, differences in allocation of carbon between photosynthetic tissue and root can alter the relationship between $\Delta^{13}\text{C}$ values and growth when water is not limiting. For example slash or loblolly pine clones that have high discrimination, and a low ratio of photosynthesis to stomatal conductance, may also have a high ratio of photosynthetic tissue to root tissue. A higher allocation to photosynthetic tissue on a site that is not limited by water availability, however, may overcome any restriction on growth imposed by low assimilation rates (Flanagan and Johnsen 1995).

Future research in analyzing associations between $\Delta^{13}\text{C}$ and photosynthetic capacity will be needed, and also the corresponding measurements of amount of leaf area. Some authors supported the thesis that differences in photosynthetic capacity have been observed to be the primary cause of genetic variation in $\Delta^{13}\text{C}$ in several coniferous species (Flanagan and Johnsen 1995; Guehl *et al.* 1995; Johnsen and Major 1995; Sun *et al.* 1996; Johnsen *et al.* 1999; Xu *et al.* 2000; Prasolova *et al.* 2003, 2005). Also, the possibility to repeat the study in the same field with severe weather conditions is recommended to make comparisons with our results. Replicated studies in different site conditions are also a good source of information to capture genetic variation across different environments.

CHAPTER 5 SUMMARY AND CONCLUSIONS

Loblolly and slash pines are widely planted as commercial timber species in the southeastern United States. Knowledge about the biology of physiological processes and their genetic parameters give us insight into what are the key functional and structural traits that determine genotype performance differences in southern pines.

The overall goal of this dissertation was to investigate biological traits and the genetic structure of these traits in 300 clones from five different full-sib loblolly and slash pine families. One peculiarity of this study was the number of clones represented in each full-sib family and also the advantage of having them in field conditions at an early stage of stand development.

The study was divided in three main areas of research:

- Detailed quantification of crown structure and estimation of the total amount of radiation absorbed by each tree over a year using the process model MAESTRA;
- Seasonal dynamics and phenology of basal area growth and its association with soil water balance;
- Leaf carbon isotope discrimination and whole-tree sap flow

The common objectives in each main research area of the study were to:

- Determine species, family within-species and clone within-family genetic variation for all variables measured or estimated;
- Where genetic variation exists, estimate genetic control and environmental influence on structural and functional variables

Based on the results of the previous chapters the main conclusions and implications of this study were summarized into three themes:

- Genetic variation among species and families;
- Clonal variation and within-family inheritance;
- Correlations

Genetic Variation among Species and Families

Differences in stem growth and crown structural traits between species and among slash pine families were subtle. In general, the one loblolly pine family we studied tended to grow faster than the average of our four slash pine families at ages 5 yr and 6 yr. At the same time, loblolly pine developed larger crowns with more acute branch angles and had more leaf area per individual-tree at age 5 yr and 6 yr than did the slash pine families. In spite of the apparent similarities in stem volume growth rate, the four slash pine families differed in a number of crown architectural traits. Contrasting families had different arrangements and sizes of branches within the crown, and varied in crown shape ratio. This suggests that any of a number of crown traits may be associated with high growth rate in southern pine families.

When we analyzed the repeated basal area growth measurements we found that loblolly pine tended to have larger yearly and daily basis basal area increments than slash pine at ages 6 and 7 yr. From this study, we concluded that the differences between loblolly and slash pine accumulated slowly over time through ages 6 and 7 yr. Loblolly and slash pine families considered in this study tended to grow about eight months per year, from March through October. We did not find significant differences at species and family level in initiation, cessation or duration of basal area growth both years 2002 and 2003. In both years, peaks in basal area increment occurred in short (2-3 week) periods in the early spring for all families, followed by relatively constant rates of basal area growth

until cessation. While there were significant size differences among taxa (species and families) at age 6 yr and 7 yr, genetic differences in basal area growth rate were only expressed during short, discrete time periods in the spring and fall.

When we studied environmental effects on seasonal basal area growth, we found that basal area growth rate increased during periods when water soil availability increased (up to 300 mm), but an excess in water availability in the soil had a negative impact on growth. Integration of climatic data with physiological variables and soil conditions in a water balance model allowed us to better understand the interactions between basal area growth in loblolly and slash pine families and soil water availability.

The study of leaf and crown integrated physiological processes, such as $\Delta^{13}\text{C}$ and whole-tree sap flow gave us the opportunity to explore genetic variation at the species, family and clonal level in slash and loblolly pines at larger temporal and spatial scales and in much more detail than is typical in field ecophysiological investigations. Family within-species was a significant source of variation for foliage carbon isotope discrimination in 2001 and 2003, but at the species level no significant differences were detected. Genetic variation in G_{Sref} and $G_{\text{Ssensitivity}}$ was difficult to detect at the species level and among the four slash pine families. Microsite variation and the small sample size may have been responsible.

Conductance in slash pine genotypes tended to be more sensitive to changes in D than loblolly pine genotypes. At the same time, slash pine families on average had higher crown conductance per unit of projected crown area than loblolly pine.

Clonal Variation and Within-Family Inheritance

Within-family clonal variation was highly significant for all growth and crown structural traits, reflecting a wide spectrum of clonal performance in growth and crown

development at these ages. Within-family individual-tree broad-sense heritabilities (H^2_{WF}) were low to moderate for stem volume and crown structural traits for all five families (0.05 to 0.41). One interesting result we found was the heterogeneity in variance components among slash pine families; there was a tendency for higher heritabilities in family S2 than the rest of the slash pine families in many traits, meaning that even for polygenic traits, it is possible to find specific pairs of parents producing more variable offspring for growth or crown structural traits.

H^2_{WF} for basal area growth phenological traits ranged from low to moderate for all traits (0.00 to 0.24). In general, heritabilities were higher for growth traits than for phenological traits for all families.

Clone within-family was a significant source of variation for foliage $\Delta^{13}C$ in 2001 and 2003, but not for crown conductance parameters. H^2_{WF} for $\Delta^{13}C$ and crown conductance parameters were in general higher than that for stem growth increment (0.01 to 0.38), meaning that these physiological traits were under stronger genetic control than growth traits. But, at the same time heritability estimates for crown conductance parameters were associated with large standard errors.

Correlations

As we hypothesized, the more integrated measures of crown structure and function in this study, specifically APAR and crown volume, were consistently more strongly correlated with stem volume growth rate than were less integrative measures such as crown radius or length, number of branches, branch angle, or average branch diameter. At the same time, microsites that favored the development of the crown, leaf area, and light interception also enhanced growth rate in all families. Branch angle and crown shape ratio showed non-significant environmental correlation with volume increment. An

understanding of the relationship between crown architecture and tree growth might provide a basis for predicting tree growth, and could aid in the search for discovering genes involved in growth and for developing new crop ideotypes.

Both the strength and direction of correlation between basal area phenological traits and basal area growth rate varied across families and years, and many times was not significantly different from zero. There were no significant clone-by-year interactions for any basal area phenology traits. We can conclude that each of the basal area growth phenology traits and each of the basal area growth rate traits were genetically controlled by a similar set of genes in years 2002 and 2003.

Genetic correlations between $\Delta^{13}\text{C}$ and stem volume increment were not stable across families, across years, and not significantly different from zero. It might be that in the years 2001 and 2003, weather and field conditions were mild enough throughout the growing season that stem growth was not limited by water supply and water use efficiency, and the genetic correlation between $\Delta^{13}\text{C}$ and stem volume increment did not have any biological importance. There was no evidence of genotype-by-year interaction in any family for $\Delta^{13}\text{C}$ and stem volume increment, indicating that the ranking of clones remained constant between years. We found non-significant environmental correlations between $\Delta^{13}\text{C}$ and stem volume increment in year 2001, and low negative environmental correlations for all families in year 2003.

Genetic and environmental correlations between $\Delta^{13}\text{C}$ and stomatal sensitivity to changes in vapor pressure deficit were difficult to conclude due to wide confidence intervals for all families.

In conclusion, for the particular loblolly and slash pine families studied here, there was a wide spectrum of clonal within-family performance in stem growth, crown development, and measured physiological traits, making interesting the possibility of clone within-family selection for traits that increase productivity. We found low to moderate levels of within-family heritability in many key structural and functional traits (crown structure, basal area growth phenology, $\Delta^{13}\text{C}$, and crown conductance parameters), but just crown structural traits had stable and higher genetic correlation with stem growth increment.

Here we reported important linkage between crown structural and functional traits with stem volume growth in loblolly and slash pine families and clones. However, what is finally translated into stem volume increment depends on complex relations with other processes and their genetic patterns. Additional studies with respect to carbon gain, water relations and hydraulic conductivity at the individual-tree level will help improve our understanding of what controls stem volume growth in contrasting families and clones.

The results from this study should positively impact future tree growth modeling and will help in decisions that involve genotype deployment and silvicultural treatments.

APPENDIX A
DESIGN AND LAYOUT STUDY SITE

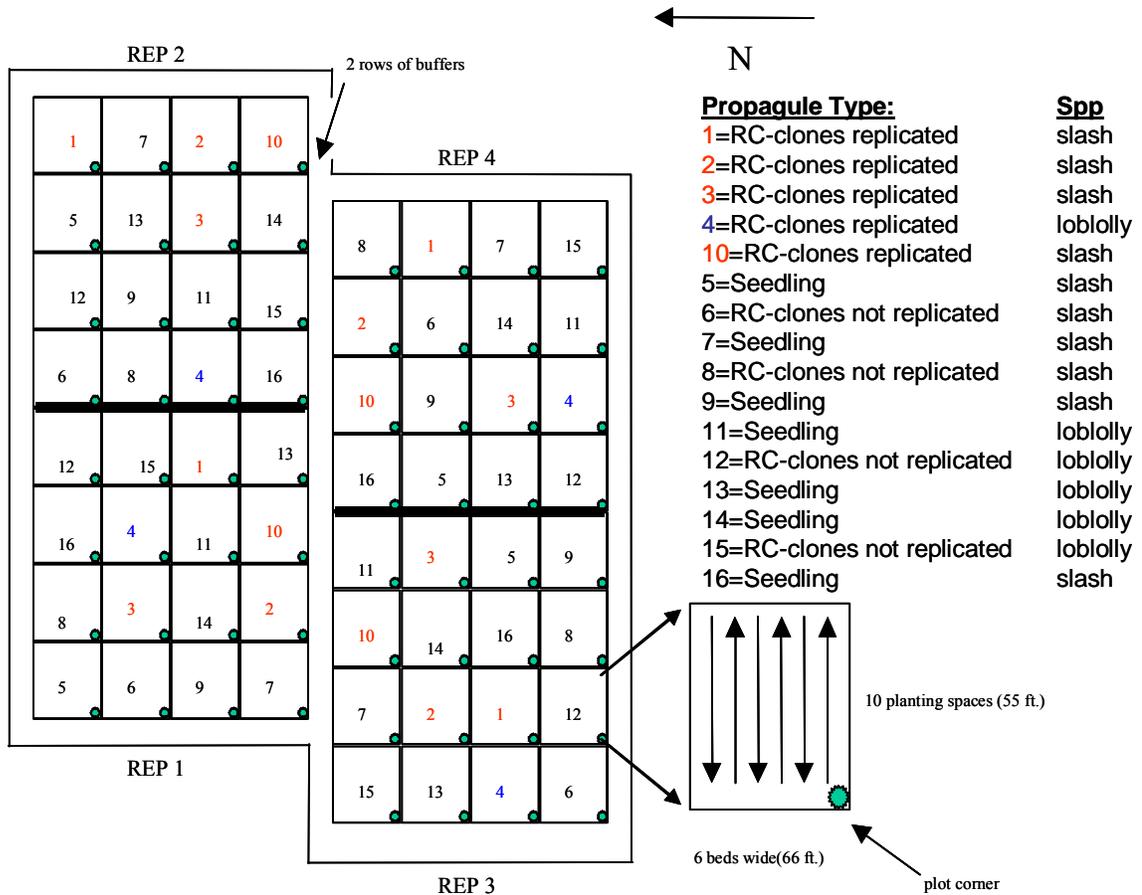


Figure A-1. Design and layout of full-sib family block plot study at Rayonier, Inc.

APPENDIX B
SILVICULTURAL TREATMENTS AT STUDY SITE

Table B-1. Treatment regimes applied in the research location at Rayonier, Inc.

Year	Treatment
1997-January	Double bedding and planting
1997-March	Chemical weed control (arsenal imazapyr) - banded
1997-June	Chemical weed control (arsenal imazapyr and sulfometuron methyl) - broadcast
1997-October	Fertilization 220 kg/ha diammonium phosphate
1999-August	Fertilization 220 kg/ha diammonium phosphate
2000-July	Mechanical weed control
2000-November	Fire line plowed
2001-May	Chemical weed control (glyphosate) - broadcast
2002-June	Fertilization 500 kg/ha ammonium nitrate

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BIOGRAPHICAL SKETCH

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