GENETIC EFFECTS OF ROOTING ABILITY AND EARLY GROWTH TRAITS IN LOBLOLLY PINE CLONES

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Loblolly pine is the most important commercial tree species in the southern United States with over 1.1 billion seedlings planted annually. With elite genotypes becoming available, several forest industry companies in the southeastern United States are developing rooted cutting and somatic embryogenesis programs aiming towards deployment of tested clones or families. However, before clones can be deployed, sufficient data need to be collected on the population in order to have reliable information about the clones for deployment decisions.

This dissertation reports on the genetic effects of rooting ability and early growth traits in nearly 2,200 clones of loblolly pine from 70-full-sib families. More than 239,000 stem cuttings were set in five rooting trials over two years. Overall rooting success across the five trials was 43%, and significant seasonal effects were observed. Heritability of rooting ability was estimated both on the observed binary scale and on the transformed underlying normal scale.

Rooted cuttings from these trials along with seedlings from the same full-sib families were established at several sites, and early growth traits through age two were compared between propagule types. All growth traits demonstrated genetic variation, and parental and full-sib family rankings were similar for both propagule types. However, estimates of dominance genetic variance in the seedling population appear to be inflated at the expense of additive effects due to a lack of randomization of seedlings prior to field establishment. Little genotype x environment interaction was observed across sites for all traits.

A successful clonal forestry program for loblolly pine based on rooted cutting technology needs to consider selection for both rooting ability and subsequent growth. There was a positive genetic correlation between rooting ability and 2nd year height at the parental, full-sib family, and clonal levels indicating that selection for one trait will also lead to improvement of the other. The genetic gains in rooting ability and 2nd year height associated with several selection and deployment strategies are discussed. Moderate to high family and clonal mean heritabilities, moderate to high type B correlations, and substantial among-family and among-clone genetic variation indicate the potential for increasing rooting efficiency and improving growth.

CHAPTER 1
INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is the most important commercial tree species in the United States with over one billion seedlings planted annually (McKeand *et al.* 2003). Most commercially important tree species remain relatively undomesticated, and loblolly pine is no exception. Genetic improvement of loblolly pine has been occurring since the 1950's in several tree improvement programs. There are three cooperative tree improvement programs in the southern United States that focus on improvement of southern pine species including loblolly pine: the Cooperative Forest Genetics Research Program (CFGRP), North Carolina State University-Industry Cooperative Tree Improvement Program (NCSUITIP), and the Western Gulf Forest Tree Improvement Program (WGFTIP). Loblolly pine tree improvement programs in the South are beginning their 3rd generation of breeding with gains in volume per unit area up to 30% over unimproved loblolly pine (McKeand *et al.* 2003).

Long-term tree improvement programs aim to increase the population mean breeding value of a few key traits such as stem volume, disease resistance, and wood properties through breeding and selection of superior genotypes. Loblolly pine tree improvement programs are based on recurrent selection for general combining ability, which captures only the additive portion of the genetic variance. However, the nonadditive portion of genetic variation, dominance and epistasis, may be important components of variation for traits.

Both additive and nonadditive genetic variation can be captured by deploying full-sib families or clones. However, deployment decisions should be based on reliable information. Field trials established with full-sib seedlings allow the genetic variation to be partitioned into additive and nonadditive components. These trials not only provide ranks of parents or individuals for selection, but also of full-sib families in order to provide information for making deployment decisions.

In any given generation of breeding, maximum genetic gains can be achieved in the deployment population by capturing all of the genetic variation through operational propagation and deployment of selected clones. However, clonal forestry is not a breeding method to develop better genotypes. Clonal forestry is a method to mass-produce well-tested genotypes. Short-term genetic gains may be maximized through deployment of well-tested clones, but long-term gains need to involve both clonal selection and recurrent selection for additive genetic variation through repeated selection and breeding.

Clonal tests derived from full-sib families do provide an opportunity to estimate additive and nonadditive components of variance associated with a particular trait or set of traits. However, tests should be designed with a sufficient genetic structure in order to precisely quantify the genetic variation. For example, Frampton and Huber (1995) reported that they had low power in partitioning the genetic variation because of the lack of a mating design among the parents of the full-sib crosses in a loblolly pine clonal study. In another study comparing clones from 30 full-sib families derived from two disconnected 4x4 factorials, Paul *et al.* (1997) concluded that future clonal studies should include more parents in the mating design. Although Isik *et al.* (2003) estimated the

genetic variances from both clones and seedlings from the same nine full-sib families of loblolly pine, they identified a weakness of their study in that there were a limited number of parent trees used in the mating design. Finally, Frampton and Foster (1993) warned that interpretation of the results may be difficult for studies that only include seedlings and cuttings from a common checklot to be compared to the clonal propagules from select parents and families. In this case, any differences in the field performance because of propagule type may be confounded with the differences in genetic improvement (Frampton and Foster 1993).

Clonally replicated progeny trials have been suggested as part of a tree improvement strategy for radiata pine (Jayawickrama and Carson 2000) and for loblolly pine (Foster and Shaw 1987; Isik *et al.* 2004; Byram *et al.* 2004) for a number of reasons. First, field trials established with clonally replicated progeny allow for further partitioning of the genetic variation into the additive, dominance, and epistatic genetic variation (Foster and Shaw 1988). Second, clonally propagated seedlings can provide genetic information more efficiently and with greater precision than zygotic seedling progeny (Burdon and Shelbourne 1974; Isik *et al.* 2004). Finally, clonal testing and selection strategies can provide greater gain than seedling options (Shaw and Hood 1985; Mullin and Park 1994; Isik *et al.* 2004).

Based on current technologies, several forest industries in the southeastern United States are pursuing clonal forestry programs with loblolly pine (Weber and Stelzer 2002). In the initial stages of these clonal forestry programs, forest managers needed assurance that the clonal propagules' growth corresponded to that of seedlings. Therefore, most of the earlier studies were designed to test whether cuttings grew similarly to seedlings.

Based on those results, it is generally accepted that cuttings rooted from juvenile stock plants grow and perform comparably to seedlings. For example, Foster *et al.* (1987) reported that loblolly pine rooted cuttings should perform comparably to seedlings when the cuttings come from vigorous juvenile stock plants. In addition, McRae *et al.* (1993) concluded that for loblolly pine there were no significant differences between seedlings and rooted cutting propagules from common checklots through five years of growth. Similar results were obtained by Frampton *et al.* (2000) where they reported no significant differences between the means of rooted cuttings and seedlings for height, diameter at breast height, and volume through six years in the field.

Trials established with clones and seedlings from the same families provide an opportunity for comparing both half-sib and full-sib family performances for both propagules. Genetic correlations between propagule types can provide further assurance that selections made through traditional tree improvement activities for recurrent selection for general combining ability can also be used successfully in breeding families to test in a clonal forestry program. Although a number of studies have been reported comparing rooted cutting and seedlings, very few have been designed to estimate the genetic correlation for a trait between propagule types.

Two main criteria need to be met prior to operational deployment of loblolly pine clones. First, loblolly pine clones must perform well, *e.g.*, meet the selection criteria for the desired traits. This involves the accumulation of reliable data for the clones from greenhouse screening, field trials, etc. Second, the selected clones have to be propagated in large enough numbers for deployment. For a rooted cutting based clonal program, this involves bulking up the number of hedges (ramets) of a particular clone or group of

selected clones through serial propagation and then producing reforestation stock efficiently from the bulked-up clones. Only those clones that can be propagated easily and in sufficient numbers will be economically feasible for deployment. A clone that grows well in the field but roots poorly may not be economically feasible to include in a clonal program based on rooted cutting technology.

This dissertation is unique in that a complex genetic structure was utilized in order to increase the power in quantifying the genetic variation associated with several traits in loblolly pine clones and seedlings. In Chapter 2 rooting ability was assessed for nearly 2,200 clones of loblolly pine from 70 full-sib families derived from a partial diallel mating design in order to estimate genetic parameters associated with rooting. More than 1,200 of these clones along with zygotic seedlings from the same full-sib families were established together on multiple sites across the southeastern United States. Genetic parameter estimates are compared between propagule types for early growth traits in Chapter 3. Finally, selection for both rooting ability and field growth is addressed in Chapter 4.

CHAPTER 2
GENETIC EFFECTS OF ROOTING LOBLOLLY PINE STEM CUTTINGS FROM A PARTIAL DIALLEL MATING DESIGN

Introduction

Loblolly pine is the most important commercial tree species in the southern United States with over 1.1 billion seedlings planted annually (McKeand *et al.* 2003). There are three cooperative tree improvement programs in the southern United States that focus on improvement of southern pine species including loblolly pine: the Cooperative Forest Genetics Research Program (CFGRP), North Carolina State University-Industry Cooperative Tree Improvement Program (NCSUITIP), and the Western Gulf Forest Tree Improvement Program (WGFTIP). Loblolly pine tree improvement programs in the South are beginning their 3rd generation of breeding with gains in volume per unit area up to 30% over unimproved loblolly pine (McKeand *et al.* 2003).

Long-term tree improvement programs aim to increase the population mean breeding value of a few key traits through breeding and selection of superior genotypes. These programs are based on recurrent selection for general combining ability which captures only the additive portion of the genetic variance. In any given generation of breeding, maximum gains can be achieved in the deployment population by capturing all of the genetic variation (additive and nonadditive components) through operational propagation of selected clones. With elite genotypes becoming available, several forest industries in the southeastern United States are developing rooted cutting programs for

loblolly pine aiming towards deployment of tested clones or families (Weber and Stelzer 2002).

Clonal tests derived from full-sib families provide an opportunity to estimate additive and nonadditive genetic components of variance associated with a particular trait or set of traits (Isik *et al.* 2003; Isik *et al.* 2004). In clonal field trials traits of interest may include height, volume, wood quality, and disease resistance. However, in order to establish clonal field trials the clones must first be propagated. Therefore, clonal rooting trials are important for estimating genetic variance components associated with rooting.

Previous rooting studies of loblolly pine have been relatively small in size ranging from several hundred (Goldfarb *et al.* 1998; Foster *et al.* 2000) to several thousand cuttings (Foster 1990; Anderson *et al.* 1999). Many studies contained a small number of families from factorial mating designs (Goldfarb *et al.* 1998; Anderson *et al.* 1999; Cooney and Goldfarb 1999; Frampton *et al.* 1999) and few have been designed to estimate genetic parameters associated with rooting in loblolly pine (Foster 1978; Foster 1990; Anderson *et al.* 1999). The current study is unique in that a large number of cuttings were set in each trial (> 34,000), and rooting was assessed on nearly 2,200 clones of loblolly pine from 70 full-sib families derived from a partial diallel mating design in order to estimate genetic parameters associated with rooting. The objectives of the study were to (*i*) evaluate the rooting ability of stem cuttings from nearly 2,200 loblolly pine clones, (*ii*) determine the causal components of variance in rooting of stem cuttings, (*iii*) assess heritability estimates for rooting from five trials both on the observed binary scale and the underlying normal scale, and (*iv*) determine the Type B genetic correlations for

both additive and dominance genetic effects to measure the correspondence in rooting performance across five setting dates.

Materials and Methods

Population

The parental population for this study was selected from the Loblolly Pine Lower Gulf Elite Population (LPLGEP) which consists of selections from all three southern pine tree improvement cooperatives: CFGRP, NCSUITIP, and WGFTIP. Twenty 1st generation and ten 2nd generation selections representing the Atlantic Coastal Plain, Florida, and Lower Gulf provenances were selected from this population. Two additional slow-growing parents were included to provide linkage with another study. These parents were crossed in a circular diallel mating design (Appendix A) with some additional off-diagonal crosses, resulting in a total of 70 full-sib families. On average each parent was involved in approximately four crosses.

Seeds from the 70 families were sown in March 2000 into Ray Leach SuperCells (Stuewe and Sons, Corvallis, OR). The seedlings were grown in a greenhouse at International Paper Company's (IPC) facility in Jay, FL, and after three months of growth the seedlings were pruned back to a height of about 10-12 cm. Approximately 32 seedling hedges (ortets) per full-sib family were transplanted into 3-gallon containers and given unique clonal identifications in September 2000. The hedges were repeatedly sheared in order to minimize the effects of maturation and increase the number of shoots available for the rooting trials. The ortets were randomized in a containerized hedge orchard in order to reduce spurious C effects at the family level. However, C effects at the clonal level could not be accounted for, because all cuttings originated from a single seedling ortet.

Table 2-1. Experimental design for 5 rooting trials of loblolly pine stem cuttings. All trials were established in randomized complete block designs with 4 to 6 blocks and 4 to 9 ramets per clone in a row plot within each block.

Trial	Date set	No. of families	No. of clones	No. of plots per clone	No. of cuttings per plot	Total cuttings
Spring01	May 7-11, 2001	70	2194	4	4	34,707
Summer01	July 2-6, 2001	70	2157	5	4	43,048
Winter02	Jan 14-18, 2002	61	1648	6	5	49,315
Spring02	Apr 29-May 3, 2002	61	1254	6	9	67,059
Summer02	June 24-28, 2002	61	947	6	9	45,108

Experimental Design

Two rooting trials were conducted in 2001 and three were conducted in 2002. Stem cuttings between 3 and 8 cm in length were harvested from the seedling ortets in May 2001, July 2001, January 2002, April/May 2002, and June 2002, for trials Spring01, Summer01, Winter02, Spring02, and Summer02, respectively. Cutting size was relatively consistent within any trial, and the cuttings set in Winter02 were the smallest. The experimental design differed among the trials due to number of families, clones and available cuttings from each ortet (Table 2-1). The reduction in the number of families and clones between the first and last rooting trials was a result of a number of factors. First, random hedge mortality was a major factor: disease, repeated severe pruning, and uneven watering (inadequate) all contributed to the random loss of hedges. Since cuttings of a clone originated from a single ortet, by default mortality resulted in a truncated population for future rooting trials. Second, not all hedges produced an adequate number of cuttings at every harvest. The primary objective of the three settings

in 2002 was to produce propagules for field trials. Therefore, clones that were not producing enough shoots to ultimately be planted across six field sites were culled regardless of rooting frequency. This resulted in the reduction in the number of families in the last three rooting trials because of too few surviving clones in some of the families to meet the goal for field designs. We were striving for a balanced field design with 15 clones from each of 61 full-sib families.

Cuttings were randomly set in 4-, 5-, or 9-cutting clonal row plots (Table 2-1) into pre-formed plugs consisting of peat moss, perlite, and a binding resin. Each plug was approximately 13 mL in volume and was held by the V-13 HIKO tray (135 cells; Stuewe and Sons, Corvallis, OR). Cuttings were either treated prior to setting with a 1.0% indole-3-butyric acid and 0.5% napthalene-1-acetic acid (NAA) basal dip or after setting using a foliar NAA application according to IPC protocols. Trays contained 15 to 30 clones depending on the trial and were randomly placed in an environmentally controlled greenhouse. There were 4 to 6 complete replications depending on trial (Table 2-1).

Rooting assessments were made 9-weeks after setting for both the Spring01 and Summer01 trials. Cuttings were measured for presence (1) or absence (0) of roots. Cuttings with a root $\geq$ 1 mm were considered rooted (Goldfarb *et al.* 1998, Foster *et al.* 2000). For trials Winter02, Spring02, and Summer02 assessments were at 11-weeks following setting. Cuttings in these trials were also scored for presence or absence of roots. However, only cuttings that had at least one visible root on the exterior of the plug regardless of length were considered rooted.

Statistical Analyses

For binomial traits, such as rooting, the unit of analysis can be the individual observations (Huber *et al.* 1994; Dieters *et al.* 1996) or plot means combined with a

transformation such as arcsin or logistic (Sohn and Goddard 1979; De Souza *et al.* 1991). We chose to analyze the observed 0,1 data for several reasons. First, REML estimation of variance components has been shown to be robust to violations of the underlying normality assumptions (Banks *et al.* 1985; Westfall 1987) suggesting that analyses using individual observations of binary data yields satisfactory results. Second, simulation studies have shown that the use of individual observations is superior to the use of plot means in REML, and that these variance component estimates perform well across mating designs and imbalanced data (Huber *et al.* 1994). Huber *et al.* (1994) showed that a lower variance among estimates was obtained using individual observations as compared to plot means and that this advantage increased with increasing imbalance. Third, when heritability is low and the incidence close to 50%, there is little difference between heritability estimates on the binary and transformed scale (Dempster and Lerner 1950). In fact these two estimates are equivalent when the incidence is exactly 50% for low heritability traits. Lopes *et al.* (2000) demonstrated that the Dempster and Lerner (1950) threshold model closely estimates the true underlying heritability at incidences between 25% and 75% for traits with low heritability ($h^2 \leq 0.3$). Finally, Lopes *et al.* (2000) also demonstrated (for traits with low heritability) that heritability estimates from the observed binary data without transformation of data result in predicted gain close to the realized gain, while transformations can suffer from issues of back transformation when one wishes to predict gains on the original scale.

All variance components for rooting ability of loblolly pine stem cuttings were estimated using the individual binary observations using REML estimation for each of the 5-rooting trials using GAREML (Huber 1993). However, upwardly biased estimates

of genetic variances result when variance components are estimated from single-site (trial) analyses since the estimated genetic variance also contains the genotype x environment interaction (Comstock and Moll 1963). Therefore, across-trial analyses were performed to separate the genotype x environment interactions in order to remove this bias.

[2-1] $$y_{ijklmno} = \mu + T_i + R_{j(i)} + tray_{k(j(i))} + gca_l + gca_m + sca_{lm} + c(fam)_{n(lm)} + t*gca_{il} + t*gca_{im} + t*sca_{ilm} + t*c(fam)_{in(lm)} + r*fam_{j(i)lm} + error_{ijklmno},$$

where $y_{ijklmno}$ is the rooting response (0 or 1) of the o^{th} ramet of the n^{th} clone within the lm^{th} full-sib family in the k^{th} tray within the j^{th} rep of the i^{th} trial

μ is the population mean

T_i is the fixed effect of trial

$R_{j(i)}$ is the fixed effect of rep

$tray_{k(j(i))}$ is the random variable tray (incomplete block) ~ IID$(0, \hat{\sigma}^2_{TRAY})$

$gca_{l \text{ and } m}$ is the random variable female (l) and male (m) general combining ability (gca) ~ IID$(0, \hat{\sigma}^2_{GCA})$

sca_{lm} is the random variable specific combining ability (sca) ~ IID$(0, \hat{\sigma}^2_{SCA})$

$c(fam)_{n(lm)}$ is the random variable clone within family ~ IID$(0, \hat{\sigma}^2_{CLONE})$

$t*gca_{il \text{ and } im}$ is the random variable test by female gca and test by male gca interaction ~ IID$(0, \hat{\sigma}^2_{TESTxGCA})$

$t*sca_{ilm}$ is the random variable test by full-sib family interaction ~ IID$(0, \hat{\sigma}^2_{TESTxFAM})$

$t*c(fam)_{in(lm)}$ is the random variable test by clone interaction ~ IID$(0, \hat{\sigma}^2_{TESTxCLONE})$

$r*fam_{j(i)lm}$ is the random variable rep by family interaction ~ IID$(0, \hat{\sigma}^2_{REPxFAM})$

$error_{ijklmno}$ is the random error which includes among plot and within plot ~ IID(0, $\hat{\sigma}^2_{ERROR}$).

The single trial model is identical except all model factors with subscript 'i' are removed (sources involving test).

Genetic parameters were estimated and standard errors were calculated according to Foster and Shaw (1988) using the appropriate variance components from the individual or across trial model. Estimates of additive and dominance genetic variance are upwardly biased because they are confounded with fractions of epistasis (Cockerham 1954). Epistastic genetic variance is also only approximated because it contains only a fraction of the total epistasis plus any C effects, if they exist.

[2-2] $\hat{V}_A = 4\hat{\sigma}^2_{GCA} = V_A + \frac{1}{4}V_{AA} + \frac{1}{16}V_{AAA} + ...$ = estimate of additive genetic variance

[2-3] $\hat{V}_D = 4\hat{\sigma}^2_{SCA} = V_D + \frac{1}{2}V_{AA} + \frac{1}{2}V_{AD} + \frac{1}{4}V_{DD} + ...$ = estimate of dominance genetic variance

[2-4] $\hat{V}_I = \hat{\sigma}^2_{CLONE} - 2\hat{\sigma}^2_{GCA} - 3\hat{\sigma}^2_{SCA} = \frac{1}{4}V_{AA} + \frac{1}{2}V_{AD} + \frac{3}{4}V_{DD} + ...$ = estimate of epistatic genetic variance

[2-5] $\hat{V}_G = 2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}$ = estimate of total genetic variance

[2-6]

$\hat{V}_P = 2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + \hat{\sigma}^2_{REPxFAM} + \hat{\sigma}^2_{ERROR}$ = phenotypic variance for across trial model (the phenotypic variance from the individual trial model is the same but drop $2\hat{\sigma}^2_{TESTxGCA}$, $\hat{\sigma}^2_{TESTxFAM}$, and $\hat{\sigma}^2_{TESTxCLONE}$).

Biased and unbiased heritability estimates for rooting based on observed 0,1 data were derived using the estimated variance components from the single and across trial

models, respectively. In addition the proportion of dominance ($\hat{d}^2$) and epistasis ($\hat{i}^2$) were estimated. Standard errors of these estimates were calculated using a Taylor series expansion (Kendall and Stuart 1963; Namkoong 1979; Huber *et al.* 1992; Dieters 1994).

[2-7]

$$\hat{h}^2_{0,1} = \frac{4\hat{\sigma}^2_{GCA}}{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + \hat{\sigma}^2_{REPxFAM} + \hat{\sigma}^2_{ERROR}} =$$

across-trial narrow-sense hertibility based on observed binary data

[2-8]

$$\hat{H}^2_{0,1} = \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}}{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + \hat{\sigma}^2_{REPxFAM} + \hat{\sigma}^2_{ERROR}} =$$

across-trial broad sense heritability based on observed binary data

[2-9]

$$\hat{d}^2_{0,1} = \frac{4\hat{\sigma}^2_{SCA}}{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + \hat{\sigma}^2_{REPxFAM} + \hat{\sigma}^2_{ERROR}} =$$

across-trial dominance proportion

[2-10]

$$\hat{i}^2_{0,1} = \frac{\hat{\sigma}^2_{CLONE} - 2\hat{\sigma}^2_{GCA} - 3\hat{\sigma}^2_{SCA}}{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + \hat{\sigma}^2_{REPxFAM} + \hat{\sigma}^2_{ERROR}} =$$

across-trial epistatic proportion.

The main problem with calculating heritability on the observed 0,1 data is that the relationship between h^2 on the observed scale and h^2 on the underlying normal scale depends on the mean incidence (*e.g.*, % survival, % infected individuals, rooting percentage), and therefore the conversion results in a biased estimate (Van Vleck 1972). However, this is not a problem for low heritability traits with intermediate incidences

(Lopes *et al.* 2000). In order to make valid comparisons to heritability estimates in other rooting trials that have different mean rooting percentages $\hat{h}^2_{0,1}$ needs to be transformed to h^2 on the underlying normal scale. Therefore, narrow- and broad-sense heritability estimates on the observed 0,1-scale were transformed using a threshold model to an underlying normal scale (Dempster and Lerner 1950).

[2-11] $\hat{h}^2_N = \hat{h}^2_{0,1}\frac{(p)(1-p)}{z^2}$, where

$\hat{h}^2_N$ is the heritability on the underlying normal scale

p is the rooting percent

z is the ordinate of the normal density function which corresponds to probability p.

Full-sib family mean heritability and clonal mean heritability for rooting were estimated for both the single- and across-trial analyses. Standard errors for these estimates were calculated using Dickerson's Method which assumes the phenotypic variance (denominator) is a known constant (Dickerson 1969).

[2-12]

$$\hat{H}^2_{FS} = \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA}}{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \frac{\hat{\sigma}^2_{CLONE}}{c} + \frac{2\hat{\sigma}^2_{TESTxGCA}}{t} + \frac{\hat{\sigma}^2_{TESTxFAM}}{t} + \frac{\hat{\sigma}^2_{TESTxCLONE}}{tc} + \frac{\hat{\sigma}^2_{REPxFAM}}{tr} + \frac{\hat{\sigma}^2_{ERROR}}{ctrn}}$$

= across-trial family mean heritability, where, c = harmonic mean number of clones per family, t = number of trials, r = harmonic mean number of reps per test, and n = harmonic mean number of ramets per clone per plot.

[2-13]

$$\hat{H}^2_{CL} = \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}}{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + \frac{2\hat{\sigma}^2_{TESTxGCA}}{t} + \frac{\hat{\sigma}^2_{TESTxFAM}}{t} + \frac{\hat{\sigma}^2_{TESTxCLONE}}{t} + \frac{\hat{\sigma}^2_{REPxFAM}}{tr} + \frac{\hat{\sigma}^2_{ERROR}}{trn}}$$

= across-trial clonal mean heritability.

Type B genetic correlations for rooting across all 5 trials were estimated for both additive and nonadditive components (Yamada 1962; Burdon 1977). Standard errors of Type B correlations were calculated using the Taylor Series Expansion method.

[2-14] $\hat{r}_B = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \hat{\sigma}^2_{ge}}$,

where $\hat{r}_B$ is the estimate of the Type B genetic correlation, $\hat{\sigma}^2_g$ is the genetic variance component (either additive or dominance), and $\hat{\sigma}^2_{ge}$ is the G x E interaction (additive and dominance).

Results and Discussion

Average Rooting

A total of over 239,000 stem cuttings from nearly 2,200 clones of loblolly pine were set in five rooting trials. Overall rooting across the five trials was 43% and is comparable to other rooting studies involving loblolly pine. Goldfarb *et al.* (1998) reported 44% rooting of loblolly pine stem cuttings from 400 seedling hedges from one open-pollinated family. Over four rooting trials, Anderson *et al.* (1999) reported 33% rooting from 90 clones of loblolly pine from 9 full-sib families. Foster (1990) reported overall rooting from three settings of 42% for 546 clones of loblolly pine derived from 54 full-sib families. However, studies with fewer families of loblolly pine have yielded

substantially higher rooting percentages (Cooney and Goldfarb 1999; Murthy and Goldfarb 2001; LeBude *et al.* 2004).

Rooting of loblolly pine cuttings was variable over the five trials (Table 2-2). Spring cuttings rooted at greater than 50%, while summer cuttings in the two summer trials averaged 38% and 24% respectively. Winter cuttings were intermediate at 45% rooting. Broad inference linear contrasts were constructed using the estimates of the fixed effects in order to test seasonal rooting responses. Cuttings set in the two spring trials rooted at a significantly greater frequency then cuttings set in the summer trials ($p < 0.0001$). This implies that we would always expect a greater rooting percent in spring settings than in summer settings under this propagation system.

Table 2-2. Summary of rooting from 5 loblolly pine trials set over two years and three seasons.

Trial	Rooting %	Half-sib family range	Full-sib family range	Clone range
Spring01	54%	36-70%	28-77%	0-100%
Summer01	38%	24-54%	18-69%	0-100%
Winter02	45%	17-60%	17-67%	0-100%
Spring02	51%	36-67%	28-75%	0-98%
Summer02	24%	9-43%	8-53%	0-89%

Seasonal rooting responses for loblolly pine stem cuttings have been observed in other studies. Early rooting trials of loblolly pine cuttings reported best rooting from cuttings set from September through January (Cech 1958; Reines and Bamping 1960; Grigsby 1962; Marino 1982). These early experiments concluded that increased temperatures in the greenhouse in spring and summer trials decreased rooting. In fact,

Cech (1958) reported a 3-fold increase in rooting under cool conditions rather than under warm conditions. However, Foster *et al.* (2000) observed an overall rooting of 50% for a rooting trial established in March, while only 20% rooting for a trial established in September. They hypothesized that the reduction in September rooting was due to a decrease in metabolic activity due to the decrease in photoperiod. Rowe *et al.* (2002a; 2002b) reported trends in rooting similar to those of the current study. They observed 59% rooting for spring cuttings *versus* 40% rooting for winter cuttings and 35% rooting for summer cuttings. Cooney and Goldfarb (1999) also reported high rooting percentages for spring cuttings (62% and 83% in two successive years). In contrast, Murthy and Goldfarb (2001) reported higher rooting percentages for winter cuttings (85%) than for spring cuttings (60%). Winter cuttings often take longer to root but overall rooting may not be different than spring cuttings. Perhaps the slight reduction in rooting frequency for the Winter02 setting *versus* the two spring settings was a function of rate of rooting.

The reduction in rooting seen here for summer settings may be a result of increased temperatures during the collection and propagation phases of the experiment. The higher temperatures and humidity experienced during summer months may have resulted in an increased abundance or activity of pathogens, and hence a higher rate of decay was observed in the two summer trials. The time delay between collection and setting of cuttings may also have contributed to this reduction in rooting. Murthy and Goldfarb (2001) reported a decline in rooting percentage with increasing drying time of cuttings.

Observed Variance Components

Variance components were estimated for all single- and across-trial analyses (Appendix B). Even with the reduction in the number of clones and families throughout the study, there was no apparent reduction in the variance component estimates over time

as evidenced by parameter estimates, *e.g.*, additive genetic variance estimates were relatively constant over time (Figure 2-1). The variance associated with general combining ability was 2% of the total phenotypic variation associated with rooting. Half-sib family rooting percentages ranged from a high of 36-70% for Spring01 to a low of 9-43% for Summer02 (Table 2-2). The proportion of the total variation in rooting that was accounted for by specific combining ability was only 0.3-1.1%. Full-sib family means for rooting for the two spring settings ranged from 28-76%. Although overall rooting was greater for these two spring trials, the net difference in the range of family means was approximately the same (~45-51%). Similar ranges in family mean rooting percentages have been reported (Foster 1990; Anderson *et al.* 1999).

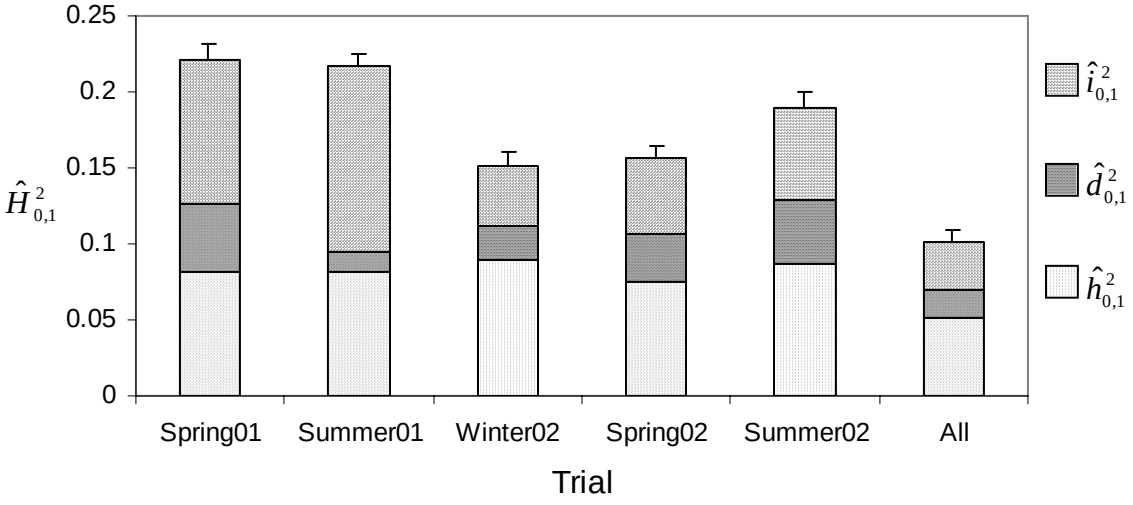


Figure 2-1. The proportion of the additive ($\hat{h}^2_{0,1}$), dominance ($\hat{d}^2_{0,1}$), and epistasis ($\hat{i}^2_{0,1}$) genetic variances on the observed binary scale for rooting of loblolly pine stem cuttings from each of the five separate trials (biased heritabilities) and from the combined analysis of all five trials. Standard error bars for broad-sense heritability estimates are included.

Clones within families accounted for approximately 10-17% of the total variation in rooting in the five trials (Appendix B). Foster (1990) reported that the clone within family source of variation in rooting of 546 clones of loblolly pine was 3.7 % of the total variation. However, in another study involving cuttings of loblolly pine, the among clone source of variation accounted for nearly 22% of the total variation (Foster 1978). In the current study, the variance associated with clone within family was 4.5-8.4 times greater than the *gca* variance and 13-56 times greater than the *sca* variance based on single-trial analyses.

Just as there was a large range in rooting among families, there was also a large range in rooting among clones within families (Table 2-2). Rooting for clones within families ranged from 0-100% for the first three trials, and ranged from 0-98% and 0-89% in the last two trials, respectively. Anderson *et al.* (1999) observed ranges in rooting frequency for clones within family similar to those observed in this study. Foster (1990) reported significant variation for clones within family with rooting percentages ranging from 6.7-85.0%.

A large variation in the rooting environment has been reported in many rooting experiments of loblolly pine and other species, with an error variance ranging from 46-71% (Foster 1978; Foster 1990; Sorensen and Campbell 1980; Cunningham 1986). In the current study, the majority of the observed variance in rooting was attributable to the error variance. The error variance accounted for 76.3-83.7% of the total variance observed in the five trials. Each rooting trial was spread over an entire greenhouse due to the large size of the experiments. Apparently, the rooting environment was not uniform throughout the greenhouse. There are many factors that can contribute to a variable

rooting environment. Differential temperature gradients, unequal airflow, unequal misting of cuttings, edge effects, and disease incidence can all contribute to a variable rooting environment.

Causal Variance Components

The observed variance components were used to estimate additive, dominance and epistatic variances using equations 2-2, 2-3, and 2-4, respectively. Additive genetic variance was approximately 2 to 6 times larger than the dominance genetic variation for rooting in the five trials (Figure 2-1). In Foster (1990), the additive genetic variance was also about 6 times larger than the dominance genetic variance. However, Anderson *et al.* (1999) found three times and Foster (1978) found 2.2 times greater dominance genetic variance in rooting compared to the additive variance in loblolly pine stem cuttings. The epistatic genetic variance was also estimated in the current study and was approximately 0.44 to 1.49 times as much as the additive genetic variance. The lowest amount of epistasis was observed for the winter setting and was the only setting that had a nonadditive to additive ratio less than one.

C effects can lead to upwardly biased estimates of total genetic and nonadditive genetic components of variance when analyzing clonal data (Libby and Jund 1962). If C effects are present, then total genetic variation associated with clones will be overestimated (Libby and Jund 1962). Significant C effects are likely to occur in traits that are measured soon after propagation (such as rooting traits, early shoot growth, etc.), but apparently lessen for traits measured at later times (Libby and Jund 1962). Foster *et al.* (1984) used a secondary cloning approach to separate C effects from the genetic variance in rooting of western hemlock cuttings. They found significant C effects associated with rooting and that these non-genetic effects biased the genotypic values of

clones. However, low or non-significant C effects for rooting of tamarack and balsam poplar cuttings have been reported (Farmer *et al.* 1989; Farmer *et al.* 1992). In the current study, estimates of epistatic genetic variance components are confounded with C effects, because ramets of a clone came from the original seedling ortet. However, estimates of additive and dominance genetic effects are not confounded with C effects, because the clones within families were randomized in the hedge orchard.

Heritability Estimates

Rooting of loblolly pine stem cuttings was weakly controlled by additive effects. Individual tree narrow-sense heritability using the observed variance components ($\hat{h}^2_{0,1}$) ranged from 0.075 to 0.089 in the five separate trials (Figure 2-1). These estimates are in agreement with Foster (1978) who reported $\hat{h}^2$ as 0.07 for rooting percentage of loblolly pine. A slightly higher $\hat{h}^2$ was reported by Foster (1990). However, Anderson *et al.* (1999) reported $\hat{h}^2$ of 0.26 for rooting percentage in loblolly pine stem cuttings. However, all of these previous studies of loblolly pine rooted cuttings analyzed data based on plot rooting percentage, as opposed to using 0,1 data as in this study, and this leads to an increased estimate of heritability due to the reduction of the impact of the within plot portion of the error variance in any plot means or plot percentage analysis for rooting. When the data in the current study were analyzed based on rooting percentage, $\hat{h}^2$ was 0.18 (from Spring01, data not shown), which falls in the middle of the range previously reported.

The unbiased estimates of individual tree narrow-sense heritability ($\hat{h}^2_{0,1}$) using the 0,1 data from the pairwise test analyses ranged from 0.045 to 0.074. When all of the data

from the 5 trials were analyzed together, $\hat{h}^2_{0,1}$ was 0.051 (Table 2-3; Figure 2-1). The proportion of dominance ($\hat{d}^2_{0,1}$) was estimated for each of the trials. The upwardly-biased estimates for this parameter ranged from 0.014 to 0.044 (Figure 2-1). When all of the data were analyzed together from the 5 trials, $\hat{d}^2$ was 0.018 (Table 2-3; Figure 2-1). The epistatic proportion ($i^2_{0,1}$) was also estimated and ranged from 0.032 to 0.095 (Table 2-3; Figure 2-1).

Table 2-3. Genetic parameter estimates (standard error) for rooting of loblolly pine stem cuttings across 5 trials.

Parameter	Estimate
Narrow-sense heritability on observed binary scale ($\hat{h}^2_{0,1}$)	0.051 (0.017)
Broad-sense heritability on observed binary scale ($\hat{H}^2_{0,1}$)	0.101 (0.008)
Narrow-sense heritability on the underlying normal scale ($\hat{h}^2_N$)	0.08 (0.027)
Broad-sense heritability on the underlying normal scale ($\hat{H}^2_N$)	0.16 (0.013)
Narrow-sense family mean heritability ($\hat{H}^2_{FS}$)	0.833 (0.24)
Broad-sense clonal mean heritability ($\hat{H}^2_{CL}$)	0.815 (0.074)
Additive genetic variance ($\hat{V}_A$)	0.0117 (0.004)
Dominance genetic variance ($\hat{V}_D$)	0.0042 (0.002)
Epistatic genetic variance ($\hat{V}_I$)	0.0074 (0.002)
Phenotypic variance ($\hat{V}_P$)	0.2297 (0.002)
Type B additive genetic variance correlation	0.68 (0.23)
Type B dominance genetic variance correlation	0.61 (0.27)
Type B total genetic variance correlation	0.53 (0.048)

Broad-sense heritability ($\hat{H}^2_{0,1}$) ranged from approximately 0.15 to 0.22 (Figure 2-1). Broad-sense heritability was 0.101 when all of the data from the five trials were combined (Table 2-3; Figure 2-1). Foster (1990) observed very little nonadditive genetic

variance and reported $\hat{H}^2$ of 0.13 for rooting percentage. In another study $\hat{H}^2$ was reported as 0.23 for rooting percentage (Foster 1978). However, Anderson *et al.* (1999) reported a much higher $\hat{H}^2$ (0.63) for rooting percentage of loblolly pine. When the data in this study were analyzed based on rooting percentage, then $\hat{H}^2$ was 0.47.

Narrow- and broad-sense heritability estimates on the observed 0,1-scale were transformed to an underlying normal scale assuming a threshold model (Equation 2-11). Narrow-sense heritability based on the underlying normal scale ($\hat{h}_N^2$) ranged from 0.12 to 0.16 (Figure 2-2). Broad-sense heritability based on the underlying normal scale ($\hat{H}_N^2$) ranged from 0.24 to 0.36 (Figure 2-2). When all of the data from the five settings were analyzed together, then $\hat{h}_N^2$ was 0.08 and $\hat{H}_N^2$ was 0.16 (Table 2-3; Figure 2-2). The transformation of $\hat{h}_{0,1}^2$ and $\hat{H}_{0,1}^2$ to the underlying normal scale allows direct comparisons of heritability estimates among rooting studies when the rooting percentages are different.

Family mean heritability ranged from 0.84 to 0.9, while clonal mean heritability ranged from 0.82 to 0.92 (Figure 2-3). When all of the data from the five trials were combined then $\hat{H}_{\overline{FS}}^2$ was 0.83 and $\hat{H}_{\overline{CL}}^2$ was 0.82 (Table 2-3; Figure 2-3). Foster (1990) reported lower estimates of both family-mean heritability and clonal-mean heritability for a rooting study consisting of 540 clones of loblolly pine from 54 full-sib families, 0.46 and 0.40, respectively. In another loblolly pine rooting study with 27 full-sib families consisting of 10 clones each, family-mean heritability was reported as 0.31, while the clonal-mean heritability was 0.87 (Anderson *et al.* 1999).

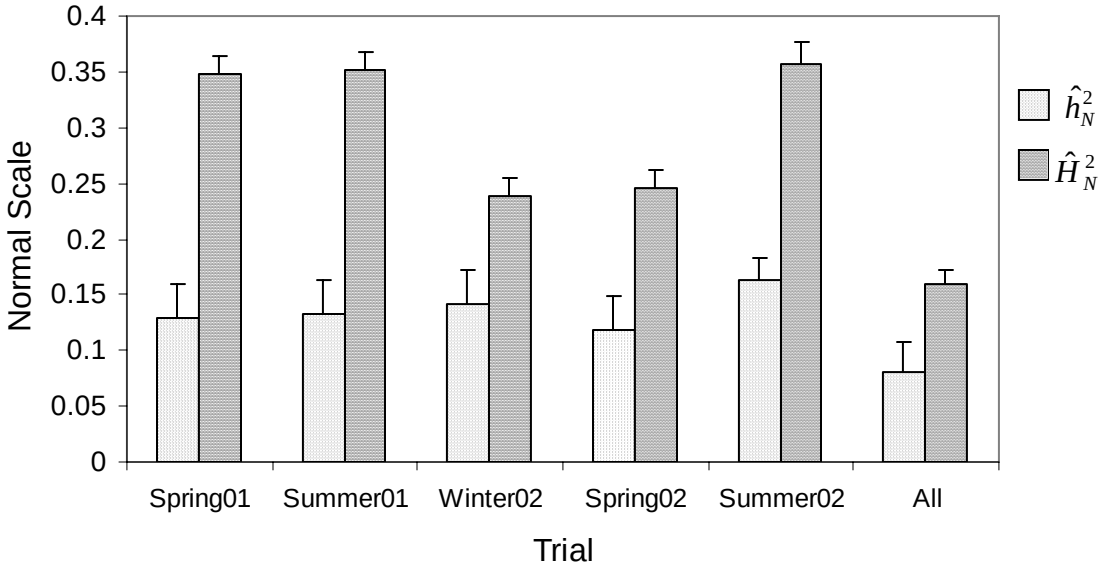


Figure 2-2. Narrow-sense ($\hat{h}_N^2$) and broad-sense ($\hat{H}_N^2$) heritability estimates for rooting of loblolly pine stem cuttings transformed to the underlying normal scale using the threshold model of Equation 2-11. Standard error bars are included.

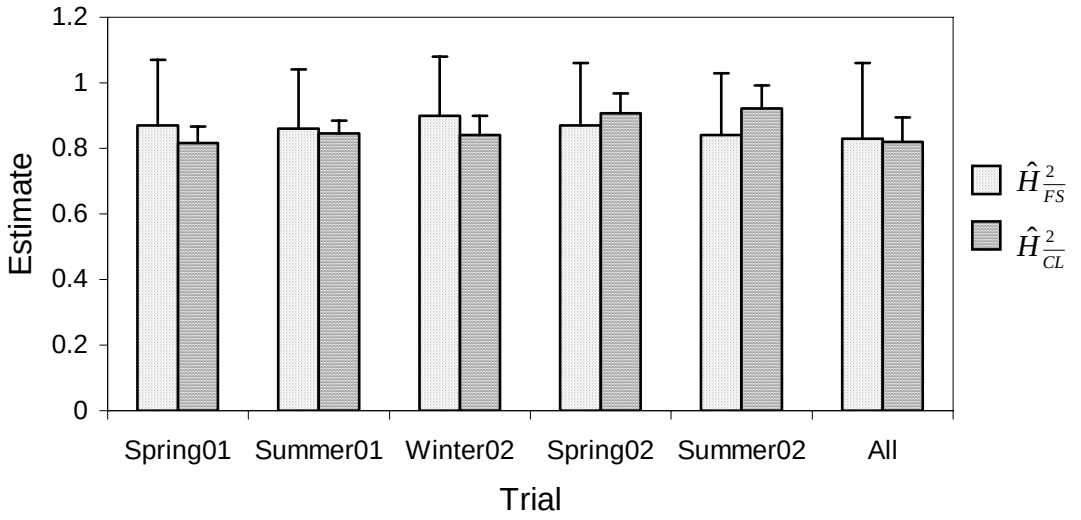


Figure 2-3. Full-sib family mean ($\hat{H}_{FS}^2$) and clonal mean ($\hat{H}_{CL}^2$) heritability estimates for rooting success of 2,200 clones from 70 full-sib families of loblolly pine. Standard error bars are included.

Type B Genetic Correlations

The type B correlations for additive effects were moderately high and ranged from 0.53 to 0.91 (Table 2-4) indicating that parental rankings of the 32 parents were moderately to strongly correlated among pairs of trials. When all of the data were analyzed together from the five trials, the type B additive correlation was 0.68. The highest type B correlation was observed between the two spring settings which were one year apart, while the lowest correlations were observed between the winter setting (Winter02) and other trials. Spring and summer cuttings were, in general, actively growing, succulent material, while winter cuttings were generally smaller and more lignified. Perhaps, some of the genes controlling rooting of dormant winter cuttings are different than those controlling spring and summer cuttings.

Table 2-4. Type B additive and dominance variance correlations among pairs of rooting trials for loblolly pine stem cuttings (above and below diagonal, respectively). Standard errors are given in parentheses.

	Spring01	Summer01	Winter02	Spring02	Summer02
Spring01		0.701 (0.11)	0.59 (0.15)	0.911 (0.07)	0.851 (0.08)
Summer01	0.823 (0.19)		0.634 (0.12)	0.673 (0.12)	0.634 (0.13)
Winter02	0.192 (0.30)	0.629 (0.34)		0.656 (0.12)	0.525 (0.16)
Spring02	0.493 (0.24)	0.533 (0.35)	0.724 (0.24)		0.907 (0.06)
Summer02	0.777 (0.22)	0.973 (0.37)	0	0.703 (0.24)	

Estimated type B correlations for dominance effects measure the correspondence of dominance across pairs of trials and were estimated to be moderate to high (Table 2-4)

with two exceptions. First, the type B dominance correlation between the Spring01 and Winter02 trials was 0.192. Also, no dominance genetic variance was detected in the pairwise analysis of theWinter02 and Summer02 trials. However, when all of the data were analyzed together, the type B dominance correlation was 0.61.

Selection for Rooting

Before clones of loblolly pine can be deployed operationally, two things must occur. First, the clones have to be field-tested and selected for desirable traits, *e.g.*, growth, disease resistance, and wood properties. Second, the selected clones have to be be propagated in large enough numbers for deployment. For a rooted cutting-based program, this involves bulking up the number of hedges of a particular clone or group of clones through serial propagation and then producing reforestation stock efficiently from the bulked-up clones. Only those clones that can be propagated easily will be economically feasible for deployment. Therefore, selection of clones for rootability as well as field performance should be considered as part of a clonal forestry program based on rooted cutting technology (Foster *et al*. 1985; Foster *et al*. 2000). In the current study, selection of the top 10% of clones for rooting (~220 clones) would result in a gain of about 24% in rooting, where

$$Gain = i\hat{H}^2_{\overline{CL}}\sqrt{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + \frac{2\hat{\sigma}^2_{TESTxGCA}}{t} + \frac{\hat{\sigma}^2_{TESTxSCA}}{t} + \frac{\hat{\sigma}^2_{TESTxCLONE}}{t} + \frac{\hat{\sigma}^2_{REPxFAM}}{tr} + \frac{\hat{\sigma}^2_{ERROR}}{trn}}$$

(Foster 1990).

Selecting the top 1% of clones for rooting would result in a gain of nearly 37% in rooting of loblolly pine stem cuttings in the current generation. Alternatively, genetic

gain in rooting success in the next generation can be achieved through selection and breeding.

$$Gain = i_F \hat{H}^2_{\overline{FS}} \sqrt{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \frac{\hat{\sigma}^2_{CLONE}}{c} + \frac{2\hat{\sigma}^2_{TESTxGCA}}{t} + \frac{\hat{\sigma}^2_{TESTxSCA}}{t} + \frac{\hat{\sigma}^2_{TESTxCLONE}}{tc} + \frac{\hat{\sigma}^2_{REPxFAM}}{tr} + \frac{\hat{\sigma}^2_{ERROR}}{ctrn}}$$

$$+ \frac{i_I 2\hat{\sigma}^2_{GCA}}{\sqrt{\hat{\sigma}^2_{CLONE} + \frac{\hat{\sigma}^2_{TESTxCLONE}}{t} + \frac{\hat{\sigma}^2_{REPxFAM}}{tr} + \frac{\hat{\sigma}^2_{ERROR}}{trn}}}$$

By selecting the best rooting clone from the top 25 out of 70 families, gain in rooting success of 16.8% can be expected in the next generation by breeding these 25 selections.

Conclusion

Loblolly pine is the most important commercial tree species in the southeastern United States. Several forest industry companies are developing rooted cutting programs for loblolly pine in order to maximize genetic gains through deployment of tested clones. With rooting data from 2,200 clones from 70 full-sib families, the current study gives better estimates of genetic components of variance for rooting than several previous studies. These results show a great deal of genetic variation for rooting among families and clones of loblolly pine. Only those clones that can be propagated easily will be economically feasible for deployment. Therefore, selection of clones for rooting ability, as well as field performance should be considered as part of a clonal forestry program based on rooted cutting technology. Combined with moderate to high estimates of family- and clonal-mean heritabilities and type B correlations, these results indicate the

potential for increasing rooting efficiency by selecting good rooting families and clones or culling poor rooters.

CHAPTER 3
GENETIC ANALYSIS OF EARLY FIELD GROWTH OF LOBLOLLY PINE CLONES AND SEEDLINGS FROM THE SAME FULL-SIB FAMILIES

Introduction

Loblolly pine (*Pinus taeda* L.) is the most important commercial tree species in the United States with over one billion seedlings planted annually (McKeand *et al.* 2003). Most commercially important tree species remain relatively undomesticated, and loblolly pine tree improvement programs are only now beginning their 3rd generation of breeding and testing (McKeand and Bridgewater 1998). Tree improvement programs for loblolly pine have relied on recurrent selection for general combining ability for improvement of a few key traits. These programs have historically utilized seedling progeny trials in order to predict breeding values for these traits. Traditional tree improvement programs using open-pollinated seed orchard seedlings for deployment only capture additive genetic variation. However, nonadditive genetic variation may be an important component of variation for some traits, and additive and nonadditive genetic variation can be captured by deploying full-sib families or clones.

More efficient field-testing has been implemented in order to gain information on full-sib families. For example, the Cooperative Forest Genetics Research Program at the University of Florida is using a partial diallel mating design to cross slash pine selections to generate full-sib seedlings for progeny trials (Gezan *et al.* 2004). Tests established with full-sib seedlings allow the genetic variance to be partitioned into additive and dominance components (Falconer and Mackay 1996). These trials not only provide ranks

of parents or individuals for selection, but also of full-sib families in order to provide information for making deployment decisions.

For a number of reasons, clonally replicated progeny trials have been suggested as part of a tree improvement strategy for radiata pine (Jayawickrama and Carson 2000) and for loblolly pine (Foster and Shaw 1987; Isik *et al.* 2004; Byram *et al.* 2004). First, field trials established with clonally replicated progeny allow for further partitioning of the genetic variation into the additive, dominance, and epistatic genetic components (Foster and Shaw 1988). Second, clonally propagated seedlings can provide genetic information more efficiently and with greater precision than zygotic seedling progeny (Burdon and Shelbourne 1974; Isik *et al.* 2004). Finally, clonal testing and selection strategies can provide greater gain for operational deployment than seedling options (Shaw and Hood 1985; Mullin and Park 1994; Isik *et al.* 2004).

Based on current technologies, several forest industries in the southeastern United States are pursuing clonal forestry programs with loblolly pine (Weber and Stelzer 2002). In the initial stages of these clonal forestry programs, forest managers needed assurance that the clonal propagules' growth corresponded to that of seedlings. Therefore, earlier studies were designed to test whether cuttings grew similarly to seedlings. Based on those results, it is generally accepted that cuttings rooted from juvenile stock plants grow and perform comparably to seedlings. For example, Foster *et al.* (1987) reported that loblolly pine rooted cuttings should perform comparably to seedlings when the cuttings come from vigorous juvenile stock plants. In addition, McRae *et al.* (1993) concluded that for loblolly pine there were no significant differences between seedlings and rooted cutting propagules from common checklots through five years of growth. Similar results

were obtained by Frampton *et al.* (2000) where they reported no significant differences between the means of rooted cuttings and seedlings for height, diameter at breast height, and volume through six years in the field.

Trials established with clones and seedlings from the same families provide an opportunity for comparing both half-sib and full-sib family performances across propagule types. Genetic correlations between propagule types can provide further assurance that selections made through traditional tree improvement activities for recurrent selection for general combining ability in seedling tests can also be used successfully in breeding families to test in a clonal forestry program. Although a number of studies have compared rooted cutting and seedlings, very few have been designed to estimate the genetic correlation between propagule types for a trait.

While clonal tests derived from full-sib families provide an opportunity to estimate additive and nonadditive components of variance, tests should be designed with sufficient genetic structure in order to precisely quantify the genetic variation. Several clonal studies have reported deficiencies in mating designs, number of parents and families (Frampton and Huber 1995; Paul *et al.* 1997; Isik *et al.* 2003). For example, Frampton and Huber (1995) reported that they had low power in partitioning the genetic variation because of the lack of a mating design among the parents of the full-sib crosses in a loblolly pine clonal study. In addition, Frampton and Foster (1993) warned that interpretation of the results may be difficult for studies that only include seedlings and cuttings from a common checklot to be compared to the clonal propagules from select parents and families. In this case, any differences in the field performance because of

propagule type may be confounded with the differences in genetic improvement (Frampton and Foster 1993).

The current study employs a complex genetic structure to increase the power in quantifying the genetic variation associated with several growth traits in loblolly pine. More than 1,200 clones together with zygotic seedlings from the same 61 full-sib families were tested on multiple sites across the southeastern United States. Because of the test and mating designs, genetic correlations can be directly calculated between propagule types and within propagule types across sites. The specific objectives of this study are to 1) determine heritability estimates for various growth traits for loblolly pine clones and seedlings, 2) compare the performance between parents and full-sib families when grown as rooted cuttings and seedlings, and 3) determine the extent of genotype x environment interaction by looking at the genetic correlations for parents, families, and clones across multiple sites.

Materials and Methods

Population

The parental population consisted of twenty first-generation and ten second-generation selections, subset from the Loblolly Pine Lower Gulf Elite Population. Two additional first-generation, slow-growing parents were included. The parental selections represent the Atlantic Coastal Plain, Florida, and Lower Gulf provenances of loblolly pine (see FBRC 2000 for details). Briefly, these thirty-two loblolly pine parents were mated in a partial diallel design and created 70 full-sib families from which more than 2,000 seedling hedges were generated and given unique clonal identifications (Appendix A). On average, each parent was involved in approximately four crosses.

Propagation

The propagation of the rooted cuttings for the field trials has been previously described (Chapter 2; Baltunis *et al.* 2005). But briefly, the seedling hedges were repeatedly sheared to slow down the effects of maturation and increase the number of shoots available for collection. Cuttings were collected from seedling hedges from 61 full-sib families, placed randomly in clonal-row plots, and replicated six times in a greenhouse in January 2002, April 2002, and June 2002. At the time of collection, the hedges were 22, 25, and 27 months old from seed, respectively. Cuttings were assessed for rooting at 11-weeks after setting (Baltunis *et al.* 2005). Rooted cuttings were then transplanted into Ray Leach Supercells (Steuwe and Sons, Corvalis, Oregon) and randomized into their designated field planting order and grown to size. The clonal propagules for a field trial came from a single rooting trial (Table 3-1).

Table 3-1. Location of six field trials, establishment date and total number of test trees for each test.

Test	Sticking Date	Location	Latitude Longitude	Date Planted	Total No. Test Trees
A	January 2002	Worth County, Georgia	31°44′20″ N 83°55′50″ W	October 2002	9,216
B	April 2002	Morgan County, Georgia	33°24′55″ N 83°29′45″ W	November 2002	8,960
C	April 2002	Putnam County, Florida	29°38′ N 81°46′ W	November 2002	8,960
D	April 2002	Nassau County, Florida	30°45′23″ N 81°54′27″ W	February 2003	8,960
E	April 2002	Randolph County, Georgia	31°48′03″ N 84°41′30″ W	December 2002	4,400
F	June 2002	Santa Rosa County, Florida	30°50′05″ N 87°11′57″ W	April 2003	6,912

A single crop of seedlings, on the other hand, was used to produce all the seedlings for the field trials. Loblolly pine seed from the same full-sib families that the clones were derived from were stratified for about 30 days and then sown in May 2002. The seedlings were grown in family blocks in a different greenhouse than the cuttings. The seedlings were then moved outdoors under shade cloth and kept in their family blocks in a separate area from where the rooted cuttings were growing. The seedlings were not randomized into their designated field order until just prior to planting.

Field Design

In total 47,408 measurement trees were established at six field sites across the southeastern United States (Table 3-1). Three trials each were established in Florida and Georgia (Table 3-1). An additional field trial was established in Virginia with a subset of the clones but was not included in any of these analyses. There were four replications in each of two cultural treatments (high and low intensity) in each test, except for Test E where there was only one cultural treatment and four replications. The goal for the high intensity treatment was to push the trees to their utmost potential by reducing competition and providing a non-limiting supply of nutrients, while the low intensive culture provides insights into family and clonal performance under a less optimal cultural regime (FBRC 2000). Both cultural intensities were treated similarly during the first year with cultural differences implemented at the beginning of the second growing season.

Each trial contained 756-974 clones with approximately 15 clones from each of 61 full-sib families (Table 3-2). In total, more than 1,200 clones were planted in field trials (Table 3-2). The trials were designed to have four zygotic seedlings from each of the same 61 full-sib families within each replication. However, because of poor germination for some of the families or mortality in the nursery, each full-sib family is represented, on

average, by approximately 27 zygotic seedlings per test (Table 3-2). Both the rooted cuttings and seedlings were planted in single-tree plots utilizing a resolvable alpha incomplete block design (Williams *et al.* 2002) in which incomplete block size ranged from 10-14 trees. The variables measured were 1st year height, 2nd year height, height increment, and crown width.

Table 3-2. Total number of clones, full-sib families, half-sib families, average number of clones per full-sib and half-sib family, and average number of seedlings per full-sib and half-sib family established at the six field trials.

	Test A	Test B	Test C	Test D	Test E	Test F	Total
Total # clones	974	941	942	956	868	756	1,212
Total # FS families	61	61	61	61	61	61[a]	61
Total # HS families	32	32	32	32	32	32[b]	32
Ave. # clones/FS family	16	15.4	15.4	15.7	14.2	12.4	19.9
Ave. # clones/HS family	60.9	58.8	58.9	59.5	54.2	47.2	75.7
Ave. # seedlings/ FS family	27.9	27	27	27	15.2	35.1	151.2
Ave. # seedlings/ HS family	106.5	103	103	103	58	106.3	576.5

[a] Only 47 full-sib families and [b] 31 half-sib families are represented in the seedling population of Test F.

Statistical Analyses

All growth variables, 1st year height, 2nd year height, height increment, and crown width, were analyzed in ASREML (Gilmour *et al.* 2002) using a parental model in order to estimate the genetic variance components associated with those traits. Analyses were conducted for each trial separately and across all six trials for each propagule type. The

across-trial analyses assumed a different error variance for each trial. For the clonal population, the following across-trial model was used.

[3-1]

$$y_{ijklmnop} = \mu + T_i + T*C_{ij} + R_{k(ij)} + incbk_{l(ijk)} + gca_m + gca_n + sca_{mn} + clone_{o(mn)} + t*gca_{im} + t*gca_{in} + t*fam_{imn} + t*clone_{io(mn)} + t*c*gca_{ijm} + t*c*gca_{ijn} + t*c*fam_{ijmn} + t*c*clone_{ijo(mn)} + r*gca_{k(ij)m} + r*gca_{k(ij)n} + r*fam_{k(ij)mn} + e_{ijklmnop}$$

where,

$y_{ijklmnop}$ is the measured growth trait of the *p*th ramet of the *o*th clone within the *mn*th full-sib family in the *l*th incomplete block within the *k*th replication of the *j*th cultural treatment in the *i*th test

μ is the clonal population mean

T_i is the fixed effect of trial, $i = 1,\ldots,6$

$T*C_{ij}$ is the fixed effect of the interaction between trial and culture, $j = 1,2$

$R_{k(ij)}$ is the fixed effect of replication, $k = 1,2,3,4$

$incbk_{l(ijk)}$ is the random variable incomplete block associated with each test ~ NIID(0, $\hat{\sigma}^2_{INC_i}$)

gca_m and gca_n are the random variables female (*m*) and male (*n*) general combining ability, respectively ~ NIID(0, $\hat{\sigma}^2_{GCA}$)

sca_{mn} is the random variable specific combining ability ~ NIID(0, $\hat{\sigma}^2_{SCA}$)

$clone_{o(mn)}$ is the random variable clone within full-sib family ~ NIID(0, $\hat{\sigma}^2_{CLONE}$)

$t*gca_{im}$ and $t*gca_{in}$ are the random variables test by female general combining ability and test by male general combining ability interactions, respectively ~ NIID(0, $\hat{\sigma}^2_{TESTxGCA}$)

$t*fam_{imn}$ is the random variable test by full-sib family interaction ~ NIID(0, $\hat{\sigma}^2_{TESTxFAM}$)

$t*clone_{io(mn)}$ is the random variable test by clone within full-sib family interaction ~ NIID(0, $\hat{\sigma}^2_{TESTxCLONE}$)

$t*c*gca_{ijm}$ and $t*c*gca_{ijn}$ are the random variables test by culture by female general combining ability and test by culture by male general combining ability, respectively ~ NIID(0, $\hat{\sigma}^2_{TxCxGCA}$)

$t*c*fam_{ijmn}$ is the random variable test by culture by full-sib family ~ NIID(0, $\hat{\sigma}^2_{TxCxFAM}$)

$t*c*clone_{ijo(mn)}$ is the random variable test by culture by clone within full-sib family interaction ~ NIID(0, $\hat{\sigma}^2_{TxCxCLONE}$)

$r*gca_{k(ij)m}$ and $r*gca_{k(ij)n}$ are the random variables replication by female general combining ability and replication by male general combining ability, respectively ~ NIID(0, $\hat{\sigma}^2_{REPxGCA}$)

$r*fam_{k(ij)mn}$ is the random variable replication by full-sib family ~ NIID(0, $\hat{\sigma}^2_{REPxFAM}$)

$e_{ijklmnop}$ is the random error associated with each test ~ NIID(0, $\hat{\sigma}^2_{ERROR}$).

The single-site model for the clonal population is identical, except that all model factors with subscript *i* are removed (sources involving test).

All traits were also analyzed for the seedling population assuming a randomized complete block design (incomplete block dropped from model). Both single-trial

analyses and an across-trial analysis were performed again assuming heterogeneous errors across sites.

[3-2]

$$z_{ijklmn} = \mu + T_i + T*C_{ij} + R_{k(ij)} + gca_l + gca_m + sca_{lm} + t*gca_{il} + t*gca_{im} + t*\ fam_{ilm} + t*c*gca_{ijl} + t*c*gca_{ijm} + t*c*\ fam_{ijlm} + r*gca_{k(ij)l} + r*gca_{k(ij)m} + r*\ fam_{k(ij)lm} + e_{ijklmn}$$

where

z_{ijklmn} is the measured growth trait of the *n*th seedling from the *lm*th full-sib family in the *k*th replication of the *j*th cultural treatment in the *i*th test

μ is the seedling population mean and the other variables are defined as above (just adjusting the appropriate subscripts).

Genetic parameters were estimated and standard errors were calculated according to Foster and Shaw (1988) using the variance components from the appropriate model.

[3-3] $\hat{V}_A = 4\hat{\sigma}^2_{GCA} = V_A + \frac{1}{4}V_{AA} + \frac{1}{16}V_{AAA}\cdots$

is the estimate of additive genetic variance.

[3-4] $\hat{V}_D = 4\hat{\sigma}^2_{SCA} = V_D + \frac{1}{2}V_{AA} + \frac{1}{2}V_{AD} + \frac{1}{4}V_{DD}\cdots$

is the estimate of dominance genetic variance.

[3-5] $\hat{V}_I = \hat{\sigma}^2_{CLONE} - 2\hat{\sigma}^2_{GCA} - 3\hat{\sigma}^2_{SCA} = \frac{1}{4}V_{AA} + \frac{1}{2}V_{AD} + \frac{3}{4}V_{DD}\cdots$

is the estimate of epistatic genetic variance for the clonal population.

[3-6] $\hat{V}_{G_C} = 2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}$

is the estimate of total genetic variance for the clonal population.

[3-7] $\hat{V}_{G_S} = 4\hat{\sigma}^2_{GCA} + 4\hat{\sigma}^2_{SCA}$

is the estimate of total genetic variance for the seedling population (assuming no epistasis).

[3-8] $$\hat{V}_{P_C} = 2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + 2\hat{\sigma}^2_{TxCxGCA} + \hat{\sigma}^2_{TxCxFAM} + \hat{\sigma}^2_{TxCxCLONE} + 2\hat{\sigma}^2_{REPxGCA} + \hat{\sigma}^2_{REPxFAM} + \frac{\sum_{i=1}^{6} \hat{\sigma}^2_{ERROR_i}}{6}$$

is the estimated phenotypic variance for the across-trial model for the clonal population.

[3-9] $$\hat{V}_{P_S} = 2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{TESTxFAM} + 2\hat{\sigma}^2_{TxCxGCA} + \hat{\sigma}^2_{TxCxFAM} + 2\hat{\sigma}^2_{REPxGCA} + \hat{\sigma}^2_{REPxFAM} + \frac{\sum_{i=1}^{6} \hat{\sigma}^2_{ERROR_i}}{6}$$

is the estimated phenotypic variance for the across-trial model for the seedling population.

Individual tree narrow-sense heritability ($\hat{h}^2$) and broad-sense heritability ($\hat{H}^2$) were derived using the estimated variance components for all the growth traits and each propagule type at each site and across sites. Standard errors were calculated using Taylor series expansion (Kendall and Stuart 1963; Namkoong 1979; Huber *et al.* 1992; Dieters 1994). The following heritability formulae were used for the clonal data.

[3-10] $\hat{h}^2 = \frac{\hat{V}_A}{\hat{V}_{P_C}} \approx \frac{4\hat{\sigma}^2_{GCA}}{\hat{V}_{P_C}}$

is the across-trial estimate of individual tree narrow-sense heritability for the clonal population, where $\hat{V}_{P_C}$ is from [3-8].

[3-11] $\hat{H}^2 = \frac{\hat{V}_G}{\hat{V}_{P_C}} \approx \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}}{\hat{V}_{P_C}}$

is the across-trial estimate of individual tree broad-sense heritability for the clonal population, where $\hat{V}_{P_C}$ is from [3-8].

Heritability estimates were also obtained for the seedling data for each trial and across-trials.

[3-12] $$\hat{h}^2 = \frac{\hat{V}_A}{\hat{V}_{P_S}} \approx \frac{4\hat{\sigma}^2_{GCA}}{\hat{V}_{P_S}}$$

is the across-trial estimate of individual tree narrow-sense heritability for the seedling population, where $\hat{V}_{P_S}$ is from [3-9].

[3-13] $$\hat{H}^2 = \frac{\hat{V}_G}{\hat{V}_{P_S}} \approx \frac{4\hat{\sigma}^2_{GCA} + 4\hat{\sigma}^2_{SCA}}{\hat{V}_{P_S}}$$

is the across-trial estimate of individual tree broad-sense heritability for the seedling population, where $\hat{V}_{P_S}$ is from [3-9].

The various growth variables at each site were also analyzed with a bivariate mixed model with the growth of the clones and seedlings as two dependent variables. Type B genetic correlations for general combining ability ($\hat{r}_{B_{propGCA}}$) and full-sib family value ($\hat{r}_{B_{propFS}}$) between cuttings and seedlings were estimated in order to compare parental and full-sib family performance between propagule types. The genetic correlation between propagule types for additive effects, for example, gives us an indication of whether parental ranks are dependent upon whether their progeny are grown as cuttings or seedlings, while a type B genetic correlation at the full-sib family level measures the performance of full-sib families across propagule types.

[3-14] $\hat{r}_{B_{propGCA}} = \frac{Cov(GCA_{CUT}, GCA_{SEED})}{\sqrt{Var(GCA_{CUT}) \times Var(GCA_{SEED})}}$

is the type B genetic correlation of additive effects between propagule types using genetic variance component estimates from the bivariate analysis.

[3-15] $\hat{r}_{B_{propFS}} = \frac{2Cov(GCA_{CUT}, GCA_{SEED}) + Cov(SCA_{CUT}, SCA_{SEED})}{\sqrt{(2Var(GCA_{CUT}) + Var(SCA_{CUT})) \times (2Var(GCA_{SEED}) + Var(SCA_{SEED}))}}$

is the type B genetic correlation of full-sib family values between propagule types using genetic variance component estimates from the bivariate analysis.

The extent of genotype x environment interaction was investigated by analyzing data across trials for each propagule type using the variance components from the appropriate model. Type B genetic correlations were calculated for additive effects, full-sib family, and the total genetic or clonal value across the trials (Yamada 1962; Burdon 1977). Standard errors of type B correlations were calculated using the Taylor series expansion method.

[3-16] $\hat{r}_{B_{gxeGCA}} = \frac{\hat{\sigma}^2_{GCA}}{\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{TESTxGCA}}$

is the type B genetic correlation for additive effects across trials.

[3-17] $\hat{r}_{B_{gxeFS}} = \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA}}{2\hat{\sigma}^2_{GCA} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{TESTxFAM}}$

is the type B genetic correlation for full-sib families across trials.

[3-18] $\hat{r}_{B_{gxeCLONE}} = \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}}{2\hat{\sigma}^2_{GCA} + 2\hat{\sigma}^2_{TESTxGCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{CLONE} + \hat{\sigma}^2_{TESTxCLONE}}$

is the type B genetic correlation for total genetic or clonal value across trials for the clonal population.

Ranging from 0 to 1, a value of $\hat{r}_{B_{gxeGCA}}$ near one indicates little genotype x environment interaction and that the parents ranked the same across the trials, while a low $\hat{r}_{B_{gxeGCA}}$ (near zero) indicates that parental ranks were not stable across the sites and hence, genotype x environment interaction exists. A high $\hat{r}_{B_{gxeFS}}$ indicates that full-sib families performed similarly across the sites, while a high $\hat{r}_{B_{gxeCLONE}}$ indicates that the total genetic values of the clones were stable across the trials.

Results and Discussion

Overall Growth of Clones and Seedlings

Survival of both clonal and zygotic propagules was high across all of the field trials. Survival of the rooted cuttings ranged from 91.7-98.3% at the six field trials, while seedling survival ranged from 86.5-97.4%. At the time of planting, seedlings were generally taller than rooted cuttings, and this trend has continued through year two (Table 3-3). For example, after the first and second growing seasons seedlings were on average 11 cm and 10 cm taller than rooted cuttings, respectively. It has been suggested that propagule size differences at the time of planting may create difficulties in the analysis and interpretation of subsequent growth (Frampton and Foster 1993). However, these differences in initial propagule size may not be a problem in the current study because height increments were very similar between both propagule types, with site means ranging between 1.0-2.0 m and 1.0-1.9 m for rooted cuttings and seedlings, respectively (Table 3-3).

The initial and first year treatments were the same within a site during test establishment, and there were no cultural differences between propagule types for 1st year height. There were some differences in mean height due to the effects of cultural

intensity during the second growing season at some of the sites. However, these effects were more a function of scale. Although the overall means of the growth variables differed by cultural treatment, the ranks of parents, families, or clones were not affected. Type B genetic correlations exceeded 0.85 indicating little cultural treatment x genetic effect interaction, and therefore, cultural effects are ignored for the purposes of this study. This implies that the rankings of parents, families, and clones were robust across multiple management regimes through age two.

Table 3-3. Mean 1st year height, 2nd year height, height increment, and crown width by propagule type for each of the six field trials. Although means are expressed in meters, analyses were conducted using measured traits in centimeters.

		1st Year Height (m)	2nd Year Height (m)	Height Increment (m)	Crown Width (m)
Test A	*Clones*	1.0	2.1	1.2	0.9
	Seedlings	1.1	2.3	1.2	1.0
Test B	*Clones*	0.8	1.8	1.0	0.9
	Seedlings	0.9	1.8	1.0	0.9
Test C	*Clones*	1.0	2.1	1.1	1.2
	Seedlings	1.1	2.3	1.2	1.3
Test D	*Clones*	0.7	2.0	1.3	1.0
	Seedlings	0.8	2.1	1.3	1.1
Test E	*Clones*	1.2	3.3	2.0	2.0
	Seedlings	1.3	3.2	1.9	2.0
Test F	*Clones*	0.5	1.7	1.3	1.1
	Seedlings	0.6	1.8	1.3	1.1

Genetic Components of Variance

All of the early growth traits demonstrated genetic variation (Figure 3-1; Table 3-4; Appendix C). However, the genetic variation partitioned differently for the two propagule types. In all cases, the estimate of additive genetic variation was greater for

the clones than the seedlings. Within a single trial, for instance, the estimate of the additive genetic variation for 2nd year height based on seedlings was 0 to 0.58 that of additive genetic variation based on clonal data. Similar trends were observed from the across-trial analyses. The estimate of additive genetic variation for the clonal material was 4.7, 3.3, 2.9, and 2 times greater than the additive genetic variation for seedlings for 1st year height, 2nd year height, height increment, and crown width, respectively (Table 3-4).

Based on single-trial analyses, the majority of the genetic variation associated with all of the growth variables in the clonal population was additive, while in the seedling population the trend was towards nonadditive genetic variation (Figure 3-1). For example, at Test D all of the genetic variation associated with 2nd year height of clones was additive, while for the seedlings it was dominance genetic variation (Figure 3-1). When all of the data were analyzed together, then the estimates of dominance genetic variation were approximately equivalent for both propagules (Table 3-4) suggesting a large test by dominance interaction for the seedlings. Epistasis was negative for all growth variables. As a result, estimates of additive and dominance genetic variance for these traits might not be upwardly biased as indicated by the expected portions of epistatic interactions defined in Equations 3-3 and 3-4 (Foster and Shaw 1988). Isik *et al.* (2003) reported similar trends in the partitioning of the genetic variation for clones and seedlings including negative epistasis estimates for height, diameter, and volume through age six. They also reported that the additive genetic variation for growth traits was always greater for clones than the estimate for seedlings, while dominance genetic variation was greater in the seedling population (Isik *et al.* 2003).

Table 3-4. Genetic parameter estimates for 1st year height, 2nd year height, height increment, and crown width by propagule type across all six trials. Standard errors are given in parentheses.

	1st Year Height		**2nd Year Height**		**Height Increment**		**Crown Width**	
	Clone	Seed	Clone	Seed	Clone	Seed	Clone	Seed
$\hat{V}_A$	69.8 (21.3)	14.7 (7.8)	401.5 (122)	119.9 (50.8)	152.6 (47)	51.8 (24.2)	110.3 (34)	55 (20)
$\hat{V}_D$	12 (6.2)	17.5 (9.3)	86.4 (36.5)	83.3 (41.6)	30.3 (13.6)	41.9 (21.5)	19.7 (10.1)	20 (11.7)
$\hat{V}_I$	-11.5 (11.4)	---	-107.6 (64.3)	---	-39.7 (24.8)	---	-13.5 (18.1)	---
$\hat{V}_G$	70.3 (10.8)	32.1 (9.5)	380.3 (61.1)	203.1 (53.8)	143.2 (23.6)	93.65 (26.1)	116.5 (17.2)	75 (20.7)
$\hat{V}_P$	442.8 (11.3)	405.2 (7.6)	1806 (62.3)	1703 (36.2)	902.6 (24.5)	972 (19.4)	640.9 (17.8)	649.5 (14.3)
$\hat{h}^2$	0.16 (0.04)	0.04 (0.02)	0.22 (0.06)	0.07 (0.03)	0.17 (0.05)	0.05 (0.02)	0.17 (0.05)	0.08 (0.03)
$\hat{H}^2$	0.16 (0.02)	0.08 (0.02)	0.21 (0.03)	0.12 (0.03)	0.16 (0.02)	0.1 (0.02)	0.18 (0.02)	0.11 (0.03)
$\hat{r}_{B_{gxeGCA}}$	0.81 (0.06)	0.71 (0.2)	0.88 (0.04)	0.78 (0.12)	0.83 (0.05)	0.6 (0.15)	0.82 (0.06)	0.78 (0.12)
$\hat{r}_{B_{gxeFS}}$	0.8 (0.05)	0.5 (0.1)	0.88 (0.04)	0.66 (0.09)	0.83 (0.05)	0.58 (0.1)	0.82 (0.05)	0.67 (0.09)
$\hat{r}_{B_{gxeCLONE}}$	0.69 (0.04)	---	0.77 (0.03)	---	0.76 (0.04)	---	0.76 (0.03)	---

Genetic causes are not the only source for similarity among relatives. Non-genetic factors, such as C effects, can lead to upwardly biased estimates of total genetic and nonadditive genetic components of variance when analyzing clonal data (Libby and Jund 1962). C effects are often assumed negligible when estimating epistatic variance (Foster and Shaw 1988). If C effects are present then the total genetic variation associated with

clones will be overestimated (Libby and Jund 1962). Significant C effects are likely to occur in traits that are measured soon after propagation, but apparently lessen for traits measured at later times (Libby and Jund 1962). In the current experiment, if C effects exist, then estimates of epistatic genetic variation will be confounded with C effects because ramets of a clone came from a single, non-replicated hedge. However, the estimates for epistasis were negative for all of the growth traits (Table 3-4), which suggest that interloci interactions are not an important source of genetic variation for early growth traits, and that C effects may not be a major contributing factor. Further, estimates of additive and dominance should be free of confounding C effects in the current experiment since randomization of clones occurred at all stages including hedge establishment, propagation, and growth prior to and after test establishment.

As Falconer and Mackay (1996) point out, relatives of all sorts may resemble one another because of sharing a common environment. The variance attributed to a common environment occurs more frequently and contributes greater to the covariance of full-sibs than to the covariance of any other sort of relatives (Falconer and Mackay 1996). When a common environment effect exists, then the differences between means of families become greater than when these non-genetic factors are not present. In the case of full-sib families, then this will lead to biased or inflated estimates of dominance genetic variation.

The results seen in the current experiment for dominance genetic variance estimates in the seedling population appear to be a result of a partitioning problem in that estimates of the variance due to dominance are inflated at the expense of additive effects (Figure 3-1). Seeds are often sown and seedlings grown in full-sib family blocks in the greenhouse

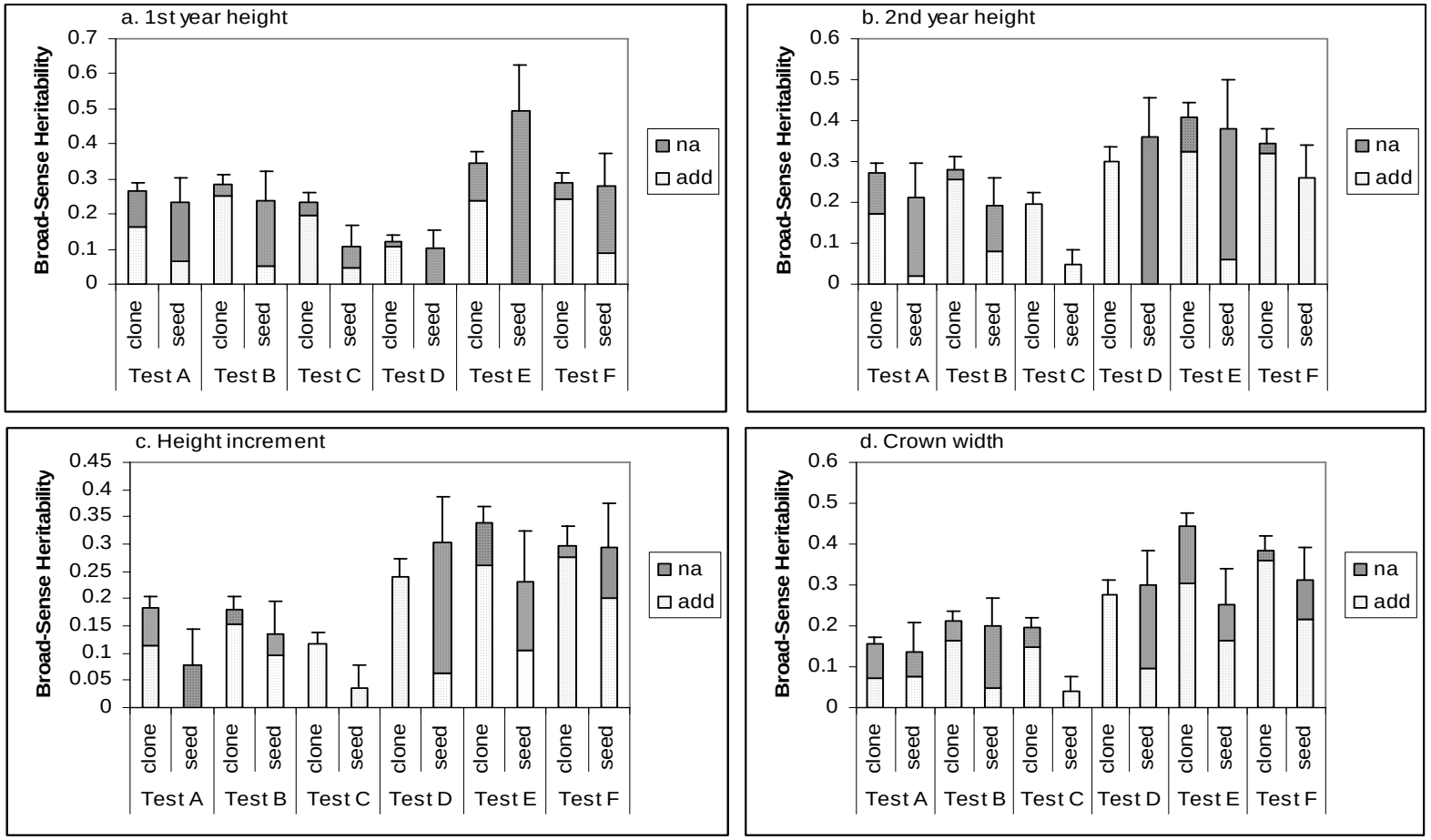


Figure 3-1. The proportion of additive (add) and nonadditive (na) genetic variance components for clones and seedlings across the six field trials, where h^2 = add and H^2 = add + na: a. 1st year height, b. 2nd year height, c. Height increment, and d. Crown width. Standard error bars for broad-sense heritability estimates are included.

or nursery for progeny testing or other field testing. In order to eliminate the common environment effect at the family level, then seedlings within a full-sib family should be randomized over the environment in which they are being grown and tested. Although rooted cuttings were randomized at all stages of propagation, the seedlings were in fact grown in full-sib blocks, and apparently a common environment effect has carried over to the field through age two measurements.

Heritability Estimates

First year height, 2nd year height, height increment, and crown width were all influenced by additive genetic variation. Individual tree narrow-sense heritability was always greater for the clones than for seedlings for all of the traits (Figure 3-1, Table 3-4). Generally, $\hat{h}^2$ estimates of total height increased from age one to age two for both clones and seedlings (Figure 3-1, Table 3-4). Narrow-sense heritability for total height increased from 0.16 at age one to 0.22 at age two for the clones, while in the seedling population, $\hat{h}^2$ increased from 0.04 to 0.07 (Table 3-4). In addition, $\hat{h}^2$ estimates for all of the growth traits from the seedling data had larger standard errors associated with them than the estimates from clones (Table 3-4). Yet, the differences in heritability estimates between propagule types may be related to C effects. If C effects are present, then H^2 estimates from the clonal population may be inflated. However, estimates of $\hat{h}^2$ from the clonal population may not be upwardly biased since clones within full-sib families were randomized in the hedge orchard and throughout the study, thus reducing spurious C effects at the parental and full-sib family levels.

Heritability estimates have been reported for several loblolly pine seedling and clonal populations. In a study reported by Isik *et al.* (2003), individual tree narrow-sense

heritability for volume at age six was 0.3 for clones, while for seedlings $\hat{h}^2$ was 0.06. In another loblolly pine clonal test, heritability estimates for total height growth through age five for two factorials were similar to the values reported here (Paul *et al.* 1997). They reported $\hat{h}^2$ for 1st and 2nd year height as 0.08 and 0.17, respectively (mean of two factorials). Unfortunately, they did not include seedlings in their study. Analogous results have been reported in *Eucalyptus globulus* Labill. for heritability estimates for diameter for both clones and seedlings in that $\hat{h}^2$ was always greater for the clonal population than the seedling population (Costa e Silva *et al.* 2004).

Broad-sense heritability estimates for the various growth traits were always larger for the clonal material than the seedlings except for the estimates of $\hat{H}^2$ for 1st year height at Test E and for all four variables at Test D (Figure 3-1). However, $\hat{H}^2$ estimates from the across-trial models were always greater for the clones than seedlings (Table 3-4). Because of the negative estimates of epistasis in the clonal population, $\hat{H}^2$ estimates were equivalent to the estimates for individual tree narrow-sense heritability for all traits. As was the case with $\hat{h}^2$, seedling estimates of $\hat{H}^2$ had higher standard errors associated with them (Figure 3-1).

Type B Genetic Correlations Between Propagule Types

In order to further compare the clonal rooted cuttings and zygotic seedlings, type B genetic correlations for general combining ability and full-sib family value between propagule types were estimated. For all growth traits measured, the type B genetic correlation between propagule types for additive effects was high with values exceeding 0.72 (Table 3-5). For example, $\hat{r}_{B_{propGCA}}$ for 1st year height ranged from 0.93-0.99, while

for height increment $\hat{r}_{B_{propGCA}}$ ranged from 0.72-0.99 (Table 3-5). These high genetic correlations imply that parental rankings for early growth traits are stable regardless of whether their progeny are being tested as zygotic seedlings or rooted cuttings (Figure 3-2a).

Table 3-5. Genetic correlations between propagule types for 1st year height, 2nd year height, height increment, and crown width at the parental ($\hat{r}_{B_{propGCA}}$) and full-sib family ($\hat{r}_{B_{propFS}}$) levels.

	1st Year Height		**2nd Year Height**		**Height Increment**		**Crown Width**	
Test	$\hat{r}_{B_{propGCA}}$	$\hat{r}_{B_{propFS}}$	$\hat{r}_{B_{propGCA}}$	$\hat{r}_{B_{propFS}}$	$\hat{r}_{B_{propGCA}}$	$\hat{r}_{B_{propFS}}$	$\hat{r}_{B_{propGCA}}$	$\hat{r}_{B_{propFS}}$
A	0.99	0.62	0.99	0.65	0.99	0.38	0.86	0.86
B	0.99	0.74	0.99	0.86	0.99	0.99	0.99	0.81
C	0.93	0.74	0.99	0.75	0.99	0.99	0.99	0.97
D	0.93	0.44	0.99	0.64	0.99	0.92	0.93	0.92
E	0.99	0.55	0.89	0.71	0.72	0.77	0.99	0.69
F	0.95	0.49	0.96	0.89	0.95	0.83	0.99	0.83

The type B genetic correlations for additive effects between propagule types observed in the current study were consistent with the expectations reported by Borralho and Kanowski (1995). In a simulation study comparing the performance of clones and seedlings from the same half-sib family, Borralho and Kanowski (1995) reported that the expected correlations between propagule types exceeded 0.8 when greater than 100 seedlings or propagules were tested. Additionally, in a field study comparing rooted cuttings and seedlings from four half-sib families, Foster *et al.* (1987) reported that family rank correlations between propagule types were positive and significant for 1st year height (0.52), 3rd year height (0.66) and 6th year height (0.70).

Full-sib families also ranked relatively similar regardless of propagule type at most of the sites (Table 3-5; Figure 3-2b). For example, type B genetic correlations at the full-sib family level ranged from 0.64-0.89 for 2nd year height. However, $\hat{r}_{B_{propFS}}$ was more variable from site to site for 1st year height and height increment. The results observed here are in accordance with those reported by Frampton *et al.* (2000) in that full-sib families or parental trees selected based on seedling genetic trials should also perform well as rooted cuttings.

Genotype x Environment Interaction

The stability of parents and full-sib families across sites was compared for the clonal and seedling populations (Table 3-4). The type B genetic correlation for additive effects across sites was always greater for the clones, although these estimates were moderately high for both populations. For example, $\hat{r}_{B_{gxeGCA}}$ for 2nd year height was 0.88 and 0.78 for the clonal and seedling populations, respectively (Table 3-4). Full-sib families also ranked comparably across sites for the clonal population with $\hat{r}_{B_{gxeFS}}$ values exceeding 0.8 for all of the early growth traits (Table 3-4). These across site genetic correlations were estimated with more precision using clonal replicates as evidenced by lower standard errors for the estimates from the clonal population.

An additional genotype x environment interaction between test and total genetic value can be estimated using clonal tests. The total genetic values of the clones were fairly stable when all of the data was considered from all sites, indicating that a good clone at one site is good at all the sites (Table 3-4). However, based on analyses from pairs of trials (data not shown), there appears to be a propagation effect relating to the season the cuttings were rooted. For example, the worst genetic correlations were

observed between the trial established with rooted cuttings from the winter setting and any of the other trials, while the best genetic correlations were obtained from the field trials that contained rooted cuttings originating from the spring setting. Similar results

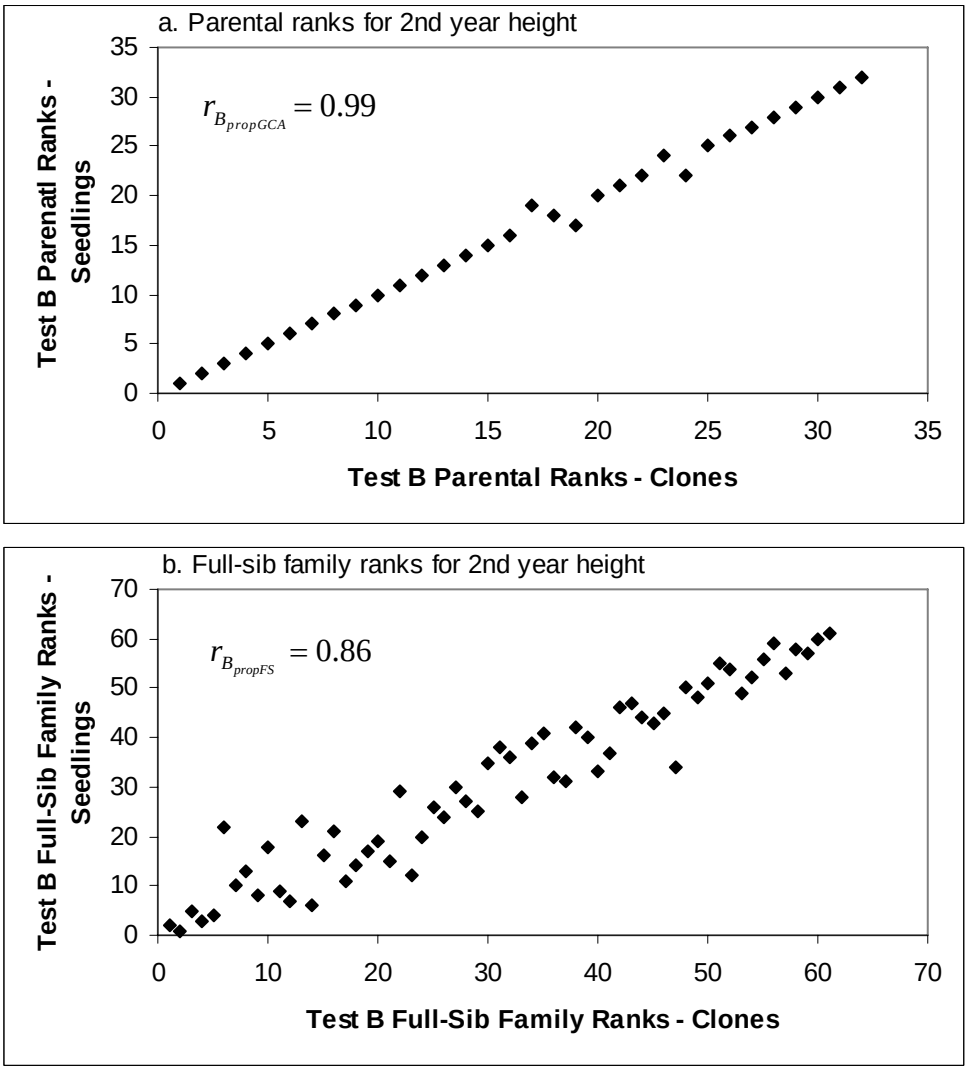


Figure 3-2. Rank-rank plots showing type B genetic correlations between clones and seedlings from Test B based on: a. Parental BLUP values, b. Full-sib family BLUP values, where full-sib BLUP values are equal to the sum of the predicted general combining ability for each of the two parents plus the predicted specific combining ability of the cross.

were also observed with rooting with this same population in that poor genetic correlations were observed between rooting ability in winter and spring (Chapter 2; Baltunis *et al.* 2005).

Conclusion

Several forest industries in the southeastern United States are deploying full-sib families of loblolly pine operationally. In addition, many of these companies are pursuing clonal forestry programs with loblolly pine. Genetic field trials established with clones and seedlings from the same full-sib families provide an opportunity for comparing both half-sib and full-sib family performances for both propagules. Based on the current study, several conclusions can be drawn. First, clonally replicated seedling trials of loblolly pine provide genetic information with greater precision than zygotic seedlings. Second, genetic correlations between estimates of the genetic effects associated with these growth traits between these propagule types were highly favorable. These high genetic correlations between propagule types reassure that parental and full-sib family rankings are stable regardless of propagule type. This implies that parental and full-sib family rankings based on existing seedling progeny trials could be used to select parents and families that perform well when they are deployed as rooted cuttings. Third, little genotype x environment interaction was observed across sites at the parental, family, and clonal level for all traits. However, there appears to be a carry-over effect relating to the season in which the cuttings were rooted for the clonal material. Finally, randomization is essential at all stages in testing when estimating genetic parameters. The lack of randomization for the seedling population apparently resulted in a problem with partitioning of the genetic variance, causing full-sib families to appear more different and inflating estimates of dominance genetic variation.

CHAPTER 4
GENETIC GAIN FROM SELECTION FOR ROOTING ABILITY AND EARLY GROWTH IN VEGETATIVELY PROPAGATED CLONES OF LOBLOLLY PINE

Introduction

Loblolly pine (*Pinus taeda* L.) is the most important commercial tree species in the southeastern United States (McKeand *et al.* 2003). Genetic improvement of loblolly pine has been occurring since the 1950's in several tree improvement programs. These programs have aimed to increase the population mean breeding value of a few key traits, such as stem volume, disease resistance, and wood properties, through breeding and selection of superior genotypes. Tree improvement programs are based on recurrent selection for general combining ability and capture only a portion of the additive genetic variation with open-pollinated seedlings for deployment. However, the nonadditive portion of genetic variation, dominance and epistasis, may be important components of variation for traits. For example, Stonecypher and McCullough (1986) reported that the estimates of nonadditive variance were approximately equal to those of additive variance for growth traits in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) through age six. Additionally, Paul *et al.* (1997) reported that both additive and dominance genetic variance increased from age one to five for height growth for loblolly pine clones. The only manner in which to capture the total genetic variation is through operational deployment of clonal propagules.

Based on current technologies, several forest industries in the southeastern United States are pursuing clonal forestry programs for loblolly pine using either somatic embryogenesis or rooted cuttings (Weber and Stelzer 2002). Two main criteria need to be met prior to operational deployment of loblolly pine clones. First, loblolly pine clones must perform well, *e.g.*, meet the selection criteria for the desired traits. This involves the accumulation of reliable data for the clones from greenhouse screening, field trials, etc. Second, the selected clones have to be propagated in large enough numbers for deployment. For a rooted-cutting-based clonal program, this involves bulking up the number of hedges (ramets) of a particular clone or group of selected clones through serial propagation and then producing reforestation stock efficiently from the bulked-up clones. Only those tested clones that can be propagated easily in sufficient numbers will be economically feasible for deployment.

Loblolly pine is considered a difficult to root species (Wise and Caldwell 1994). In populations that have not experienced any selection pressure for rooting ability, loblolly pine has been reported to root near 50% (Foster 1990; Baltunis *et al.* 2005). Previous rooting studies with loblolly pine have demonstrated substantial family and clonal variation for rooting ability (Foster 1990; Baltunis *et al.* 2005), indicating the potential for increasing rooting efficiency for both clonal deployment and through recurrent selection and breeding. Selection for both rooting ability and field growth will be necessary for a successful loblolly pine clonal forestry program based on rooted cutting technology (Foster *et al.* 1985; Foster *et al.* 2000; Baltunis *et al.* 2005).

The objectives of this study were to (i) determine the genetic correlation between rooting ability and 2nd year height, (ii) predict the genetic gain associated with selection

for rooting ability, (iii) predict the genetic gain associated with selection for 2nd year height, and (iv) predict the genetic gain from combined selection for rooting ability and 2nd year height using a Monte Carlo selection index.

Materials and Methods

Population

The parental population consisted of twenty first-generation and ten second-generation selections from the larger Loblolly Pine Lower Gulf Elite Population. Two additional first-generation, slow-growing parents were included. The parental selections represent the Atlantic Coastal Plain, Florida, and Lower Gulf provenances of loblolly pine. These thirty-two loblolly pine parents were mated in a partial diallel design and created 70 full-sib families from which approximately 2,200 seedling hedges were generated and given unique clonal identifications (Appendix A). On average, each parent was involved in about four crosses.

Rooting and Field Trials

The propagation of the rooted cuttings for this study has previously been described (Baltunis *et al.* 2005; Chapter 2). But briefly, the seedling hedges were repeatedly sheared to slow down the effects of maturation and increase the number of shoots available for collection. Cuttings were set in five rooting trials over two years in May 2001, July 2001, January 2002, April 2002, and June 2002 in trials Spring01, Summer01, Winter02, Spring02, and Summer02, respectively. Four to nine ramet clonal row plots were set in four to six replications depending on trial (Chapter 2). At the time of collection, the hedges were 13, 15, 22, 25, and 27 months old, respectively. Cuttings were assessed for rooting nine to eleven weeks after they were set (Baltunis *et al.* 2005; Chapter 2).

Six field trials (A, B, C, D, E, F) were established with rooted cuttings from the three latter rooting trials. Three field trials each were established in Georgia and Florida between October 2002 to April 2003 (see Table 3-1). The clonal propagules for each field trial, however, came from a single rooting trial. Single-tree-plots of a clone were established in four replications in each of two cultural treatments (high and low intensity) in each test, except for Test E where there was only one cultural treatment and four replications. The goal for the high intensity treatment was to push the trees to their utmost potential by reducing competition and providing a non-limiting supply of nutrients, while the low intensive culture provides insights into family and clonal performance under a less optimal cultural regime (FBRC 2000). Previous analyses of growth for this population had shown that type B genetic correlations exceeded 0.85 indicating little cultural treatment x genetic effect interaction, and therefore, cultural treatment effects were ignored for the purposes of this study (Chapter 3).

Statistical Analyses

A bivariate parental linear mixed-effects model was used to obtain estimates of variance and covariance components for rooting ability and 2nd year height using ASREML (Gilmour *et al.* 2002):

[4-1] $\mathbf{y}_i = \mathbf{X}_i\mathbf{b}_i + \mathbf{Z}_{n_i}\mathbf{n}_i + \mathbf{Z}_{u_i}\mathbf{u}_i + \mathbf{Z}_{f_i}\mathbf{f}_i + \mathbf{Z}_{c_i}\mathbf{c}_i + \mathbf{Z}_{o_i}\mathbf{o}_i + \mathbf{Z}_{p_i}\mathbf{p}_i + \mathbf{Z}_{q_i}\mathbf{q}_i + \mathbf{e}_i$,

where $\mathbf{y}_i$ is the vector of observations indexed (i) by rooting ability and 2nd year height, $\mathbf{b}_i$ is the vector of fixed effects (i.e. mean, trials and replications within trials) and $\mathbf{X}_i$ is the known incidence matrix relating the observations in $\mathbf{y}_i$ to the fixed effects in $\mathbf{b}_i$

where $\mathbf{X}_i\mathbf{b}_i = \begin{bmatrix} \mathbf{X}_{root} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{height} \end{bmatrix} \begin{bmatrix} \mathbf{b}_{root} \\ \mathbf{b}_{height} \end{bmatrix}$,

$\mathbf{n}_i$ is the vector of random incomplete blocks nested within replication and test effects

$$\sim\text{MVN}\left(\mathbf{0},\begin{bmatrix}\mathbf{I}_{root}\hat{\sigma}^2_{INC_{root}} & \mathbf{0}\\ \mathbf{0} & \mathbf{I}_{height}\hat{\sigma}^2_{INC_{height}}\end{bmatrix}\right)$$

$\mathbf{u}_i$ is the vector of random parent (female and male) general combining ability effects

$\sim\text{MVN}\left(\mathbf{0},\mathbf{G}\otimes\mathbf{A}\right)$ where $\mathbf{G}=\begin{bmatrix}\hat{\sigma}^2_{GCA_{root}} & \hat{\sigma}_{GCA_{rootheight}}\\ \hat{\sigma}_{GCA_{rootheight}} & \hat{\sigma}^2_{GCA_{height}}\end{bmatrix}$ and $\mathbf{A}$ = numerator relationship matrix,

$\mathbf{f}_i$ is the vector of random specific combining ability effects $\sim\text{MVN}\left(\mathbf{0},\mathbf{S}\otimes\mathbf{I}_\mathbf{s}\right)$ where $\mathbf{S}=\begin{bmatrix}\hat{\sigma}^2_{SCA_{root}} & \hat{\sigma}_{SCA_{rootheight}}\\ \hat{\sigma}_{SCA_{rootheight}} & \hat{\sigma}^2_{SCA_{height}}\end{bmatrix}$ and $\mathbf{I}_s$ is an identity matrix of size equal to the number of full-sib families,

$\mathbf{c}_i$ is the vector of random clones within full-sib family effects $\sim\text{MVN}\left(\mathbf{0},\mathbf{C}\otimes\mathbf{I}_c\right)$ where $\mathbf{C}=\begin{bmatrix}\hat{\sigma}^2_{CLONE_{root}} & \hat{\sigma}_{CLONE_{rootheight}}\\ \hat{\sigma}_{CLONE_{rootheight}} & \hat{\sigma}^2_{CLONE_{height}}\end{bmatrix}$ and $\mathbf{I}_c$ is an identity matrix of size equal to the number of clones,

$\mathbf{o}_i$ is the vector of random test by full-sib family effects

$$\sim\text{MVN}\left(\mathbf{0},\begin{bmatrix}\mathbf{I}_{root}\hat{\sigma}^2_{TESTxFAM_{root}} & \mathbf{0}\\ \mathbf{0} & \mathbf{I}_{height}\hat{\sigma}^2_{TESTxFAM_{height}}\end{bmatrix}\right),$$

$\mathbf{p}_i$ is the vector of random test by clone within full-sib family effects

$$\sim\text{MVN}\left(\mathbf{0},\begin{bmatrix}\mathbf{I}_{root}\hat{\sigma}^2_{TESTxCLONE_{root}} & \mathbf{0}\\ \mathbf{0} & \mathbf{I}_{height}\hat{\sigma}^2_{TESTxCLONE_{height}}\end{bmatrix}\right),$$

$\mathbf{q}_i$ is the vector of random replication within test by full-sib family effects

$$\sim \text{MVN}\left(\mathbf{0}, \begin{bmatrix} \mathbf{I}_{root}\hat{\sigma}^2_{REPxFAM_{root}} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_{height}\hat{\sigma}^2_{REPxFAM_{height}} \end{bmatrix}\right),$$

$\mathbf{e}_i$ is the random vector of residual terms $\sim \text{MVN}\left(\mathbf{0}, \begin{bmatrix} \mathbf{I}_{root}\hat{\sigma}^2_{ERROR_{root}} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_{height}\hat{\sigma}^2_{ERROR_{height}} \end{bmatrix}\right)$,

$\mathbf{Z}_{n_i}$, $\mathbf{Z}_{u_i}$, $\mathbf{Z}_{f_i}$, $\mathbf{Z}_{c_i}$, $\mathbf{Z}_{o_i}$, $\mathbf{Z}_{p_i}$, and $\mathbf{Z}_{q_i}$ are the known incidence matrices relating the observations in $\mathbf{y}_i$ to effects in $\mathbf{n}_i$, $\mathbf{u}_i$, $\mathbf{f}_i$, $\mathbf{c}_i$, $\mathbf{o}_i$, $\mathbf{p}_i$, and $\mathbf{q}_i$, respectively, and $\mathbf{I}_i$ is the identity matrix of dimension equal to the number of observations for rooting ability or 2nd year height.

Causal Components of Variance

Genetic parameters were estimated and standard errors were calculated for rooting ability and 2nd year height from the bivariate analysis according to Foster and Shaw (1988).

[4-2] $\hat{V}_A = 4\hat{\sigma}^2_{GCA} = V_A + \frac{1}{4}V_{AA} + \frac{1}{16}V_{AAA}\cdots$

is the estimate of additive genetic variance.

[4-3] $\hat{V}_D = 4\hat{\sigma}^2_{SCA} = V_D + \frac{1}{2}V_{AA} + \frac{1}{2}V_{AD} + \frac{1}{4}V_{DD}\cdots$

is the estimate of dominance genetic variance.

[4-4] $\hat{V}_I = \hat{\sigma}^2_{CLONE} - 2\hat{\sigma}^2_{GCA} - 3\hat{\sigma}^2_{SCA} = \frac{1}{4}V_{AA} + \frac{1}{2}V_{AD} + \frac{3}{4}V_{DD}\cdots$

is the estimate of epistatic genetic variance.

[4-5] $\hat{V}_G = 2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}$

is the estimate of total genetic variance.

[4-6] $\hat{V}_P = 2\hat{\sigma}_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + \hat{\sigma}^2_{REPxFAM} + \hat{\sigma}^2_{ERROR}$

is the estimate of the phenotypic variance.

[4-7] $\hat{V}_{P_{\overline{HS}}} = \hat{\sigma}^2_{GCA} + \frac{\hat{\sigma}^2_{SCA}}{f} + \frac{\hat{\sigma}^2_{CLONE}}{fc} + \frac{\hat{\sigma}^2_{TESTxFAM}}{tf} + \frac{\hat{\sigma}^2_{TESTxCLONE}}{tfc} + \frac{\hat{\sigma}^2_{REPxFAM}}{tr} + \frac{\hat{\sigma}^2_{ERROR}}{trfcn}$

is the phenotypic variance of half-sib family means, f = harmonic mean number of full-sib families per half-sib family, c = harmonic mean number of clones per full-sib family, t = number of trials, r = harmonic mean number of replications per test, and n = harmonic mean number of ramets per clone per replication per test (n = 1 for field trials).

[4-8] $\hat{V}_{P_{\overline{FS}}} = 2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \frac{\hat{\sigma}^2_{CLONE}}{c} + \frac{\hat{\sigma}^2_{TESTxFAM}}{t} + \frac{\hat{\sigma}^2_{TESTxCLONE}}{tc} + \frac{\hat{\sigma}^2_{REPxFAM}}{tr} + \frac{\hat{\sigma}^2_{ERROR}}{ctrn}$

is the phenotypic variance of full-sib family means.

[4-9] $\hat{V}_{P_{\overline{CL}}} = 2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + \frac{\hat{\sigma}^2_{TESTxFAM}}{t} + \frac{\hat{\sigma}^2_{TESTxCLONE}}{t} + \frac{\hat{\sigma}^2_{REPxFAM}}{tr} + \frac{\hat{\sigma}^2_{ERROR}}{trn}$

is the phenotypic variance of clonal means.

Heritability Estimates

Heritability estimates have previously been reported for this population for rooting ability (Baltunis *et al.* 2005; Chapter 2) and 2nd year height (Chapter 3). However, since rooting ability and 2nd year height were analyzed using a bivariate model in the current study, heritabilities were estimated based on the genetic parameter estimates from the bivariate model and differed slightly from those reported previously. Standard errors were calculated using the Taylor series expansion method (Kendall and Stuart 1963; Namkoong 1979; Huber *et al.* 1992; Dieters 1994).

[4-10] $\hat{h}^2 = \frac{\hat{V}_A}{\hat{V}_P} \approx \frac{4\hat{\sigma}^2_{GCA}}{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + \hat{\sigma}^2_{REPxFAM} + \hat{\sigma}^2_{ERROR}}$ is

the individual tree narrow-sense heritability.

[4-11] $\hat{H}^2 = \frac{\hat{V}_G}{\hat{V}_P} \approx \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}}{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE} + \hat{\sigma}^2_{TESTxFAM} + \hat{\sigma}^2_{TESTxCLONE} + \hat{\sigma}^2_{REPxFAM} + \hat{\sigma}^2_{ERROR}}$ is

the individual tree broad-sense heritability.

[4-12] $\hat{H}^2_{\overline{HS}} = \frac{\hat{\sigma}^2_{GCA}}{\hat{V}_{P_{\overline{HS}}}}$ is the half-sib family mean heritability.

[4-13] $\hat{H}^2_{\overline{FS}} = \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA}}{\hat{V}_{P_{\overline{FS}}}}$ is the full-sib family mean heritability.

[4-14] $\hat{H}^2_{\overline{CL}} = \frac{2\hat{\sigma}^2_{GCA} + \hat{\sigma}^2_{SCA} + \hat{\sigma}^2_{CLONE}}{\hat{V}_{P_{\overline{CL}}}}$ is the clonal mean heritability.

Type B Genetic Correlations

Although the same genotypes, or clones, were tested in the rooting and field trials, these measurements were not necessarily taken on the same ramet. Therefore, type B genetic correlations between rooting ability and 2nd year height for general combining ability, full-sib family value, and the total genetic value were calculated. Standard errors of these estimates were calculated using the Taylor series expansion method (Kendall and Stuart 1963; Namkoong 1979; Huber *et al.* 1992; Dieters 1994).

[4-15] $\hat{r}_{B_{GCA}} = \frac{\hat{\sigma}_{GCA_{rootheight}}}{\sqrt{\hat{\sigma}^2_{GCA_{root}} \times \hat{\sigma}^2_{GCA_{height}}}}$ is the type B genetic correlation between rooting ability

and 2nd year height for additive effects, and $\hat{\sigma}_{GCA_{rootheight}}$ is the covariance between general combining ability effects for rooting ability and 2nd year height..

[4-16] $\hat{r}_{B_{FS}} = \frac{2\hat{\sigma}_{GCA_{rootheight}} + \hat{\sigma}_{SCA_{rootheight}}}{\sqrt{\left(2\hat{\sigma}^2_{GCA_{root}} + \hat{\sigma}^2_{SCA_{root}}\right)\left(2\hat{\sigma}^2_{GCA_{height}} + \hat{\sigma}^2_{SCA_{height}}\right)}}$ is the type B genetic correlation between rooting ability and 2nd year height for full-sib families, and $2\hat{\sigma}_{GCA_{rootheight}} + \hat{\sigma}_{SCA_{rootheight}}$ is the covariance between the full-sib family effects for rooting ability and 2nd year height.

[4-17] $\hat{r}_{B_{TG}} = \frac{2\hat{\sigma}_{GCA_{rootheight}} + \hat{\sigma}_{SCA_{rootheight}} + \hat{\sigma}_{CLONE_{rootheight}}}{\sqrt{\left(2\hat{\sigma}_{GCA_{root}} + \hat{\sigma}^2_{SCA_{root}} + \hat{\sigma}^2_{CLONE_{root}}\right)\left(2\hat{\sigma}^2_{GCA_{height}} + \hat{\sigma}^2_{SCA_{height}} + \hat{\sigma}^2_{CLONE_{height}}\right)}}$ is the type B genetic correlation between rooting ability and 2nd year height for the total genetic value of clones, and $2\hat{\sigma}_{GCA_{rootheight}} + \hat{\sigma}_{SCA_{rootheight}} + \hat{\sigma}_{CLONE_{rootheight}}$ is the covariance between the total clonal value effects for rooting ability and 2nd year height.

Genetic Gain

Genetic gain was estimated for a number of deployment options based on various selection scenarios using the BLUP values from the bivariate analysis. All deployed populations were assumed to be propagated as rooted cuttings. An additional assumption for gain calculations was that the seventy full-sib families and all clones within families were available for deployment. The deployment strategies considered were 1) half-sib family deployment, 2) full-sib family deployment, and 3) clonal deployment. For a half-sib family deployment option, the predicted value for each parent (general combining ability) was used to select the best half-sib family for deployment as rooted cuttings. Full-sib family values were calculated by summing the predicted values for the female general combining ability, male general combining ability, and the full-sib family specific combining ability. The genetic gain over the trait mean was determined for deployment of the best full-sib family.

The total genetic value of a clone was determined by summing the predicted values for the female general combining ability, male general combining ability, the full-sib family specific combining ability, and clone within full-sib family. Several clonal deployment strategies were compared. First, the genetic gain was determined for deployment of the single best clone for each trait. A second clonal deployment strategy was considered by selecting the top ten full-sib families and then deploying the single best clone from each of these ten families. Two additional clonal deployment strategies were compared by selecting the top 10% and 1% of clones (out of 2200 possible clones) using an unrestricted selection index for 2nd year height and rooting ability. The total genetic values of the clones were weighted with the following weights: 1:0, 0.9:0.1, 0.8:0.2, ..., 0.2:0.8, 0.1:0.9, 0:1 for 2nd year height and rooting ability, respectively, in the Monte Carlo index (Cotterill and Dean 1990). All gains were expressed as the percentage gain over the mean of the trait:

[4-18] $\%Gain = \left(\frac{\bar{x}_i - \bar{y}_i}{\bar{y}_i} \right) \times 100\%$, where

$\bar{x}_i$ is the average predicted value for trait *i* of the selected population, and

$\bar{y}_i$ is the population mean for either rooting ability or height.

In addition genetic gain was calculated using theoretical gain formulae assuming an unrelated population (Falconer and Mackay 1996), and these estimates were compared with the predicted genetic gain based on BLUP values for the half-sib family, full-sib family, and best clone deployment options. The following formulae were used:

[4-19] $Gain = i_{hs}\hat{H}^2_{\overline{HS}}\sqrt{\hat{V}_{P_{\overline{HS}}}}$ is the genetic gain associated with selection of the best half-sib family, and i_{hs} is the selection intensity corresponding to selecting one out of thirty-two half-sib families (i = 2.07) and assuming all half-sib families are unrelated,

[4-20] $Gain = i_{fs}\hat{H}^2_{\overline{FS}}\sqrt{\hat{V}_{P_{\overline{FS}}}}$ is the genetic gain associated with selection of the best full-sib family, and i_{fs} is the selection intensity corresponding to selecting one out of seventy full-sib families (i = 2.38) and assuming all full-sib families are unrelated,

[4-21] $Gain = i_c\hat{H}^2_{\overline{CL}}\sqrt{\hat{V}_{P_{\overline{CL}}}}$ is the genetic gain associated with clonal selection, and i_c is the selection intensity corresponding to selecting one out of 2,206 clones (i = 3.58) and assuming all individuals are unrelated.

Results and Discussion

Causal Components of Variance

The genetic parameter estimates from the bivariate analysis of rooting ability and 2nd year height in loblolly pine clones were consistent with the estimates from their respective univariate analyses, and both traits showed genetic variation (Table 4-1; Appendix D). Additive genetic variation accounted for the majority of the genetic variation associated with rooting ability and 2nd year height (Table 4-1). Dominance genetic variation contributed a minor portion to the total genetic variation for both traits. Epistasis appears to be more important for rooting ability than 2nd year height as evidenced by a negative estimate of epistasis for 2nd year height (Table 4-1). However, estimates of epistasis may be confounded with C effects since rooted cuttings of a clone originated from a single ortet.

Type B Genetic Correlations

The bivariate analysis of five rooting trials and six field trials also allowed for estimation of the genetic covariance between rooting ability and 2nd year height for parental effects, full-sib family effects, and the total genetic value of clones within full-sib family. There was a positive genetic relationship between rooting ability and 2nd year height at all three genetic levels (Table 4-1). The genetic correlation at the parental level between rooting ability and 2nd year height ($\hat{r}_{B_{GCA}}$) was 0.32. At the full-sib family level, the genetic correlation between traits ($\hat{r}_{B_{FS}}$) was 0.39. The correlation of total genetic values of clones for rooting ability and 2nd year height ($\hat{r}_{B_{TG}}$) was 0.29.

Previous studies have also reported positive correlations between rooting traits and growth. For example, Paul *et al.* (1993) reported that 1st year and 5th year height growth had strong genetic correlations with rooting (0.61 and 0.69, respectively) in western hemlock clones (*Tsuga heterophylla* (Raf.) Sarg.). In another study, Foster *et al.* (1985) reported that the genetic correlation between rooting ability and growth of western hemlock clones in a growth chamber was 0.37. A weak, but positive, relationship between the number of roots on loblolly pine cuttings and subsequent field growth has been reported (Foster *et al.* 2000). On the other hand, Goldfarb *et al.* (1998) reported that the phenotypic correlation between the number of roots and first year field growth in loblolly pine rooted cuttings was negligible.

C effects relating to the season in which the cuttings were set has previously been discussed in this population (Chapter 3). Further evidence of the presence of C effects can be seen from the genetic correlations between the total genetic value of clones for rooting ability and 2nd year height based on analyses involving a single rooting and field

trial (Table 4-2). Rooted cuttings planted in each field trial originated from a single sticking date, and the highest genetic correlations ($\hat{r}_{B_{TG}}$) occurred between traits from these trials. For example, rooted cuttings from the Winter02 rooting trial were planted only in the Field A trial. The total genetic correlation between rooting ability from Winter02 and 2nd year height from Field A was 0.24, while 2nd year height from Field A had much lower correlations with rooting ability from any of the other sticking dates

Table 4-1. Means, variance component estimates, heritabilities, and genetic correlations from the bivariate analysis of rooting ability and 2nd year height. Standard errors are given in parentheses.

	Rooting Ability	2nd Year Height
$\bar{x}$	42 %	210 cm
$\hat{V}_A$	0.0135 (0.004)	399.3 (119.6)
$\hat{V}_D$	0.0014 (0.002)	77.3 (39.6)
$\hat{V}_I$	0.0085 (0.002)	-94.9 (63.7)
$\hat{V}_G$	0.0235 (0.002)	381.7 (59.9)
$\hat{V}_P$	0.2299 (0.002)	1836 (61.0)
$\hat{V}_{P_{\overline{HS}}}$	0.0038 (0.001)	108.1 (29.6)
$\hat{V}_{P_{\overline{FS}}}$	0.0085 (0.002)	231.1 (59.1)
$\hat{V}_{P_{\overline{CL}}}$	0.0286 (0.002)	434.1 (59.9)
$\hat{h}^2$	0.059 (0.02)	0.22 (0.06)
$\hat{H}^2$	0.102 (0.01)	0.21 (0.03)
$\hat{H}^2_{\overline{HS}}$	0.88 (0.04)	0.92 (0.03)
$\hat{H}^2_{\overline{FS}}$	0.84 (0.04)	0.95 (0.01)
$\hat{H}^2_{\overline{CL}}$	0.82 (0.01)	0.88 (0.02)
$\hat{r}_{B_{GCA}}$	0.32 (0.19)	
$\hat{r}_{B_{FS}}$	0.39 (0.17)	
$\hat{r}_{B_{TG}}$	0.29 (0.08)	

(Table 4-2). This indicates that the vigor of the hedge at the time of rooting may have influenced rooting and subsequent field growth.

The biological significance of positive correlations between rooting and 2nd year height in the current study is unclear. Goldfarb *et al.* (1998) reported that root morphological traits were not meaningful for subsequent field growth after one year of growth for loblolly pine rooted cuttings. However, it has generally been acknowledged that stock plant vigor is related to rooting. Perhaps the vigor of the stock plant is an indicator of the metabolic activity of cuttings during root initiation. The more metabolically active genotypes may have rooted quicker and formed better root systems. In the current study, rooting was assessed at nine to eleven weeks, and selection may have indirectly been for rate of rooting as opposed to strictly rooting ability. Clones that rooted quickly during the rooting period may have had increased metabolic activity. A positive genetic correlation between rooting and growth implies that some of the same genes are responsible for the expression of each trait. There may be genes in common for rooting and height relating to metabolic activity. Genotypes that have a tendency for increased rates of metabolic activity may root at higher frequencies and grow larger than genotypes that do not have these alleles.

Genetic Gain

The importance of selecting for rooting ability in a clonal forestry program based on rooted cuttings has long been recognized (Foster *et al.* 1984; Foster 1990; Baltunis *et al.* 2005). Genetic gains in field traits will not be realized if the clones can not be propagated efficiently for deployment. Therefore, increases in rooting ability could have broad economic impacts on a clonal forestry program (Foster *et al.* 1984). Positively correlated traits imply that selection for one trait should also lead to improvement in the

second trait. In the case of rooting ability and growth, positive genetic correlations can lead to substantial gains for both traits in a clonal forestry program based on rooted cuttings.

The genetic gains in rooting ability and 2nd year height based on BLUP values were compared for a number of deployment strategies. Selecting the top half-sib family for rooting ability would result in a gain of 36% in rooting ability (Figure 4-1). Deployment of the top half-sib family selected for rooting ability would result in a genetic gain of 5.4% in 2nd year total height (Figure 4-2). Selecting the highest ranking half-sib family for 2nd year height would result in a gain of 14.8% and 8.1% in rooting ability and 2nd year height, respectively (Figure 4-1 and Figure 4-2). The genetic gain predictions using theoretical calculations were slightly lower than the gain predictions based on BLUP values. The gain in rooting ability associated with selecting the best half-sib family for rooting was 26.7% (using Equation 4-19). The gain in height by selecting the top growing half-sib family was 9.4% (using Equation 4-19).

Slightly higher gains can be achieved by selecting the top full-sib family. Propagation of the top full-sib family selected for rooting ability results in genetic gains in rooting ability of about 43% over the population mean (Figure 4-1). Gains of nearly 9% in 2nd year total height could be expected by deploying the top rooting full-sib family (Figure 4-2). If the best growing full-sib family was propagated and deployed, then the genetic gains in rooting ability (Figure 4-1) and 2nd year height (Figure 4-2) would be 8.6% and 10.1%, respectively. The theoretical gain in rooting ability for full-sib selection was equivalent to the estimate based on BLUP values. However, the theoretical

gain for full-sib selection for 2nd year height was 16.3% which was higher than the estimate based on BLUPs.

Table 4-2. The total genetic correlation ($\hat{r}_{B_{TG}}$) between rooting ability and 2nd year height from analyses of a single rooting trial and field trial. Shaded values indicate the rooting trial in which cuttings originated from for their respective field trials. Standard errors are given in parentheses.

	Field A	**Field B**	**Field C**	**Field D**	**Field E**	**Field F**
Spring01	0.002 (0.07)	0.17 (0.07)	0.17 (0.07)	0.21 (0.07)	0.16 (0.07)	0.25 (0.07)
Summer01	0.08 (0.06)	0.07 (0.07)	0.17 (0.07)	0.21 (0.07)	0.14 (0.07)	0.12 (0.07)
Winter02	**0.24 (0.07)**	0.16 (0.07)	0.23 (0.07)	0.27 (0.08)	0.24 (0.08)	0.18 (0.08)
Spring02	0.06 (0.07)	**0.25 (0.07)**	**0.26 (0.08)**	**0.34 (0.07)**	**0.16 (0.07)**	0.24 (0.08)
Summer02	0.10 (0.07)	0.21 (0.07)	0.16 (0.08)	0.21 (0.08)	0.13 (0.08)	**0.28 (0.08)**

Clearly, the most gain for either trait would be achieved by selecting the single best clone (Figure 4-1 and Figure 4-2). For instance, by selecting the top clone for rooting ability, the genetic gain based on clonal predicted values in rooting was 96% (Figure 4-1) indicating that approximately 82% of the ramets would root from this clone (42% mean rooting plus 0.96 x 42% = 82%). However, selecting the top rooting clone from this population would result in a decrease (genetic loss) in overall 2nd year height (Figure 4-2). On the other hand, selecting the top clone for 2nd year height would result in a gain of nearly 27% in 2nd year height (Figure 4-2) and 43% in rooting ability (Figure 4-1) over the population mean for these traits. Genetic gain in rooting ability based on theoretical

calculations was greater than those based on BLUP values for selection of the single best clone and was 118%, while the gain in 2nd year height for the top growing clone was 31.3% (using Equation 4-21).

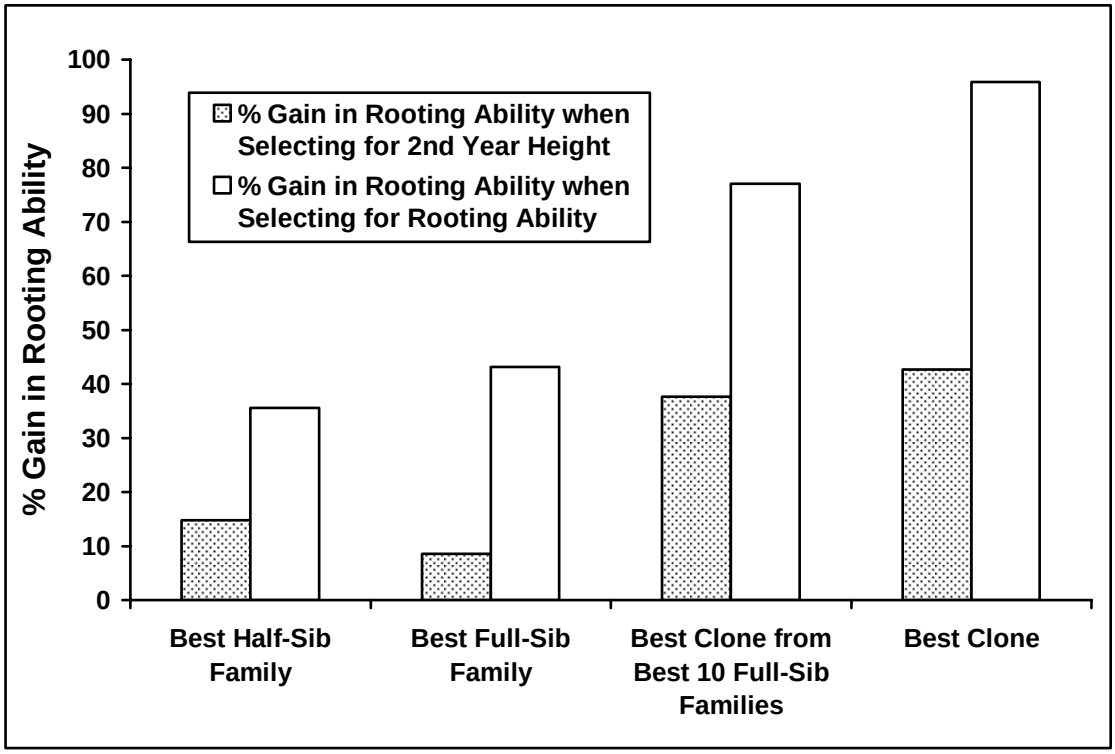


Figure 4-1. The genetic gain in rooting ability (%) over the population mean for deployment of the best half-sib family, full-sib family, best clone from the best ten full-sib families, and the single best clone when selecting for rooting ability or 2nd year height.

In order to address genetic diversity issues and the risk associated with deploying a single clone, a second clonal deployment option was considered by selecting the best clone from each of the ten highest ranking full-sib families. This strategy resulted in genetic gains nearly double that of the full-sib family deployment strategy. When the trait selected is rooting ability, the best clone in the top ten full-sib families would result in gains of 77% in rooting ability (Figure 4-1) and 5.6% in 2nd year height (Figure 4-2).

When the trait selected is for 2nd year height, then the genetic gains would be 37.6% in rooting ability (Figure 4-1) and 18.3% for 2nd year height (Figure 4-2).

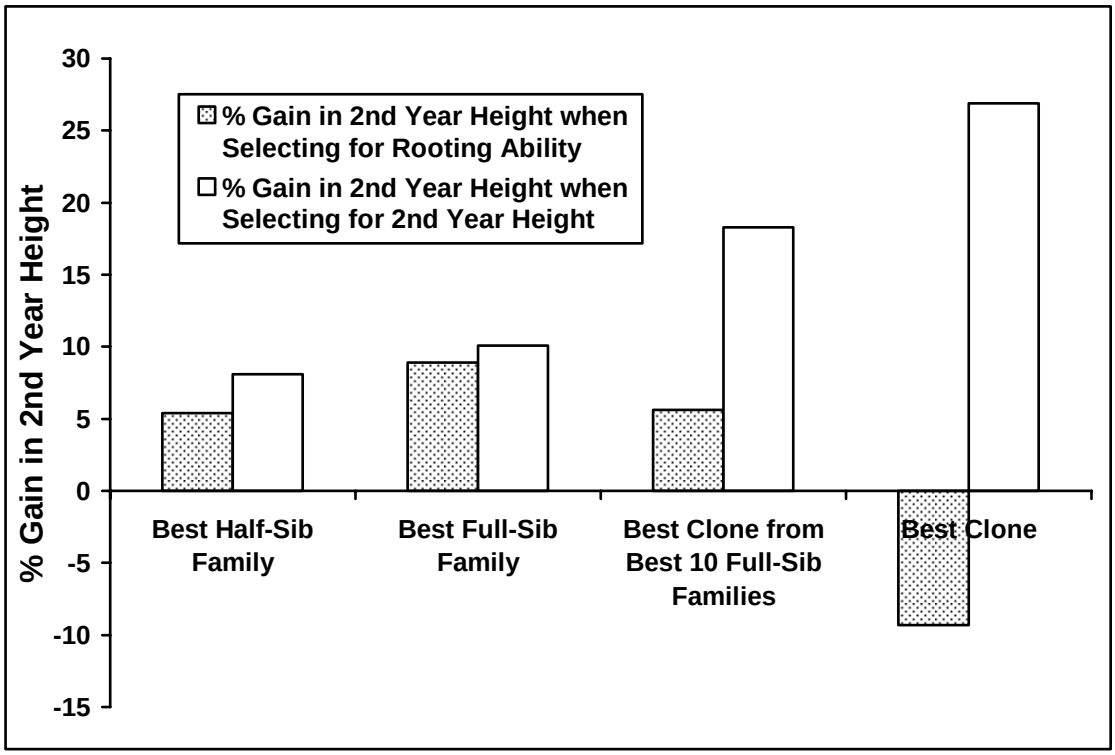


Figure 4-2. The genetic gain in 2nd year height (%) over the population mean for deployment of the best half-sib family, full-sib family, best clone from the best ten full-sib families, and the single best clone when selecting for rooting ability or 2nd year height.

Deployment of well-tested clones can result in genetic gains in both rooting ability and 2nd year total height. Both traits should be considered for a successful loblolly pine clonal forestry program based on rooted cutting technology. The responses to selection when considering both rooting ability and 2nd year height were compared for a number of selection indices (Figure 4-3). The gain in rooting ability associated with selecting the top 10% of clones ranged from 23.8% when only 2nd year height was considered to 57.6% when only rooting ability was considered (Figure 4-3). Higher gains can be

achieved by increasing the selection intensity. When only the top 1% of clones was selected, the gain in rooting ability ranged from 33.4% when only 2nd year height was considered to 80.6% when only rooting ability was considered (Figure 4-3). The genetic gain in 2nd year height associated with deployment of the best 10% of clones ranged from 4.8% when only rooting ability was considered to 12.6% gain when only 2nd year height was considered (Figure 4-3). Similarly, genetic gain in 2nd year height ranged from 3.8% to 18.5% when the best 1% of clones were selected (Figure 4-3).

Clonal forestry programs that consider multiple traits need to optimize their selection strategies for deployment populations. Arbitrarily setting the optimum selection weights on 2nd year height and rooting ability to 90% of the maximum genetic gain obtainable for a single trait, then the optimum selection weights can be compared. For example, if the top 10% of clones are selected and 90% of the maximum gain in rooting ability is considered, then the optimum weights on 2nd year height and rooting ability correspond to weights of 0.7 and 0.3, respectively. These weights result in genetic gains of 53.6% in rooting ability and 8.7% in 2nd year height (Figure 4-3). If 1% of the clones are selected, then the optimum weights are 0.6 and 0.4 on 2nd year height and rooting ability, respectively, and correspond to gains of 75.9% in rooting ability and 11% in 2nd year height (Figure 4-3).

If 90% of the maximum gain obtainable for 2nd year height is the criterion, then the optimum weights for both deployment options are 0.9 and 0.1 on 2nd year height and rooting ability, respectively. The genetic gains associated with selecting the top 10% of clones utilizing these weights results in gains of 40.4% in rooting ability and 11.9% in 2nd year height (Figure 4-3). If only the top 1% of clones are deployed using these selection

weights, then genetic gains of 54.2% in rooting ability and 17.4% in 2nd year height are obtainable (Figure 4-3). The maximum change in genetic gain of rooting ability results from increasing the weight on rooting ability from 0 to 0.1. In fact, an additional 17-21% gain in rooting ability can be obtained in the deployment population by increasing the weight on rooting ability from 0 to 0.1.

When 10% of the clones were selected (220), then the number of full-sib families represented in the deployment population ranged from 38 to 45 depending on selection index (Table 4-3). When only the top 1% of clones were selected (22), the number of full sib families represented in the deployment population varied from 9 to 15 (Table 4-3). Maximum genetic gains can be achieved by ignoring relatedness among selections. However, if no constraints are placed on the relatedness of selections, then there is a tendency to make many selections from the better families. Although the approximate average number of clones selected per full-sib family was five when 10% of the clones were selected, nearly 45% of these selections came from only five families when only rooting ability was selected. Similar trends can be seen when selecting 1% of the clones. For example, when all of the selection weight is on rooting ability, then there was an average of 2.44 clones per full-sib family selected (Table 4-3). However, 15 of the 22 selections came from three families. On the other hand, when considering the number of half-sib families represented in the selected population, 26 to 30 out of 32 possible parents are represented when 10% of the clones are selected. Thirteen to 17 parents are represented in the deployment population when 1% of the clones are selected (Table 4-3). Therefore, at least in this population, there may be sufficient genetic diversity in the deployment population even with higher selection intensities. Isik *et al.* (2005) reported

genetic gains in growth near 30% for loblolly clones from a different population irregardless of any restrictions on the relatedness of selections.

Conclusion

Rooting ability and 2nd year height are heritable traits in loblolly pine, and both traits showed substantial clonal variation. There was a positive genetic correlation between rooting and height at the parental, full-sib family, and clonal levels. Genetic gains in rooting ability and 2nd year height are possible as demonstrated by a number of deployment strategies. For hard-to-root species, like loblolly pine, a successful clonal

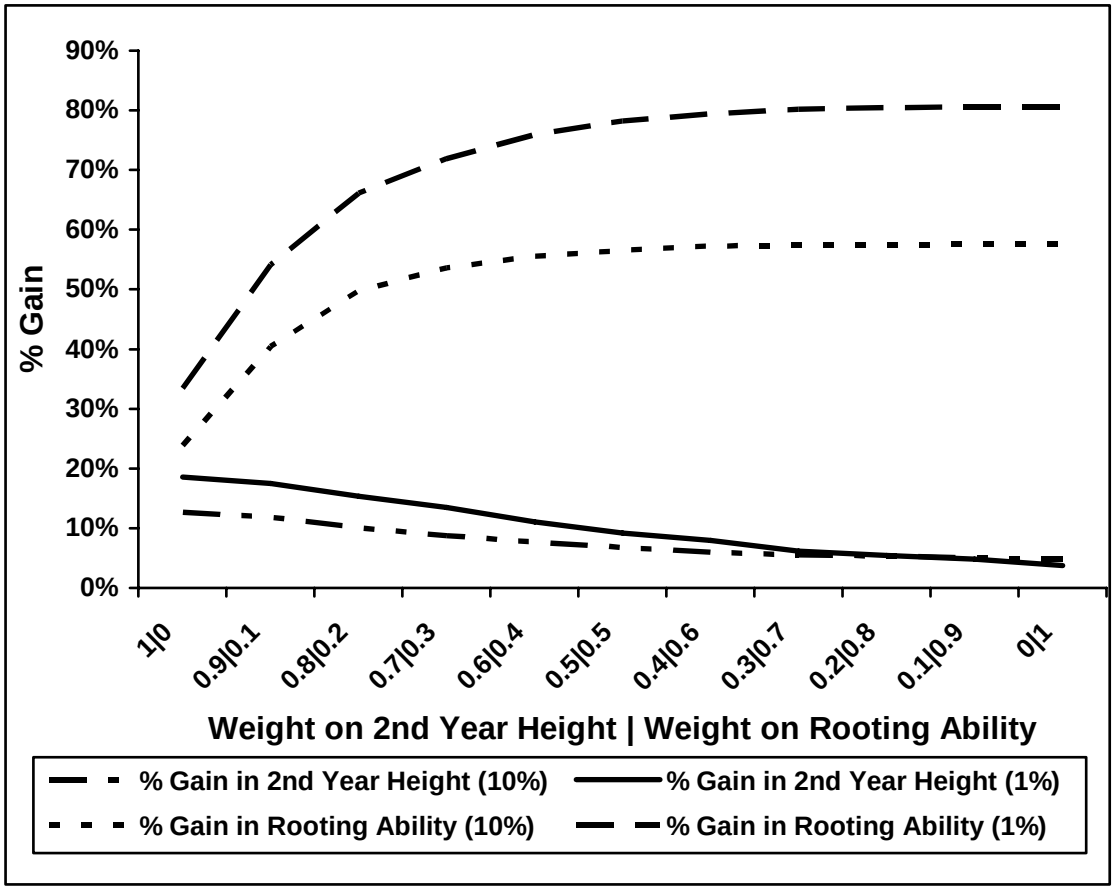


Figure 4-3. Responses to selection in rooting ability and 2nd year height with various selection indices for two clonal deployment options: 10% of clones selected and 1% of clones selected.

forestry program based on rooted cuttings must consider both rooting ability and growth when making selections. Because here these traits are positively correlated, selection for one trait should lead to positive gains in the other. Or, early culling of clones based on poor rooting will not negatively effect selection of clones for growth when field data becomes available.

Clonal forestry is not a breeding method to develop better genotypes. However, clonal forestry is a method to mass-produce well-tested genotypes. Short-term genetic gains may be maximized through deployment of well-tested clones, but long-term gains need to involve both clonal selection and recurrent selection for additive genetic variation through repeated selection and breeding. Restrictions on the relatedness of selections will be necessary when making selections for a breeding population or when deployment will be traditional zygotic seedlings from seed orchards in order to reduce detrimental effects of inbreeding depression.

Table 4-3. The number of full-sib families (half-sib families) and average number of clones per full-sib family (half-sib family) selected from selecting 10% or 1% of the top clones using the combined selection index.

	10% Clones Selected		**1% Clones Selected**	
Weight on 2nd Year Height: Weight on Rooting Ability	**# FS Families (# HS families)**	**Ave. # Clones per FS Family (HS Family)**	**# FS Families (# HS families)**	**Ave. # Clones per FS Family (HS Family)**
1:0	38 (26)	5.8 (16.9)	14 (17)	1.6 (2.6)
0.9:0.1	41 (28)	5.3 (15.7)	15 (17)	1.5 (2.6)
0.8:0.2	43 (28)	5.1 (15.7)	12 (15)	1.8 (2.9)
0.7:0.3	43 (30)	5.1 (14.7)	13 (16)	1.7 (2.8)
0.6:0.4	45 (30)	4.9 (14.7)	10 (12)	2.2 (3.7)
0.5:0.5	45 (29)	4.9 (15.2)	12 (15)	1.8 (2.9)
0.4:0.6	45 (29)	4.9 (15.2)	11 (15)	2.0 (2.9)
0.3:0.7	45 (29)	4.9 (15.2)	11 (15)	2.0 (2.9)
0.2:0.8	45 (29)	4.9 (15.2)	10 (14)	2.2 (3.1)
0.1:0.9	45 (29)	4.9 (15.2)	9 (13)	2.4 (3.4)
0:1	45 (29)	4.9 (15.2)	9 (13)	2.4 (3.4)

CHAPTER 5
CONCLUSION

Loblolly pine is the most important commercial tree species in the southeastern United States. Several forest industries in the southeastern United States are deploying full-sib families of loblolly pine operationally. In addition, many of these companies are pursuing clonal forestry programs with loblolly pine. Clones need to be well-tested before they can be deployed. This involves the accumulation of reliable data from propagation, growth traits, disease resistance, etc. Those well-tested clones that meet the selection criteria can lead to substantial genetic gain.

Several key results can be concluded from this research. With rooting data from 2,200 clones from 70 full-sib families, the current study gives better estimates of genetic components of variance for rooting than several previous studies. These results show a great deal of genetic variation for rooting among families and clones of loblolly pine. Combined with moderate to high estimates of family- and clonal-mean heritabilities and type B correlations for rooting ability in different rooting trials, these results indicate the potential for increasing rooting efficiency by selecting good rooting families and clones or culling poor rooters.

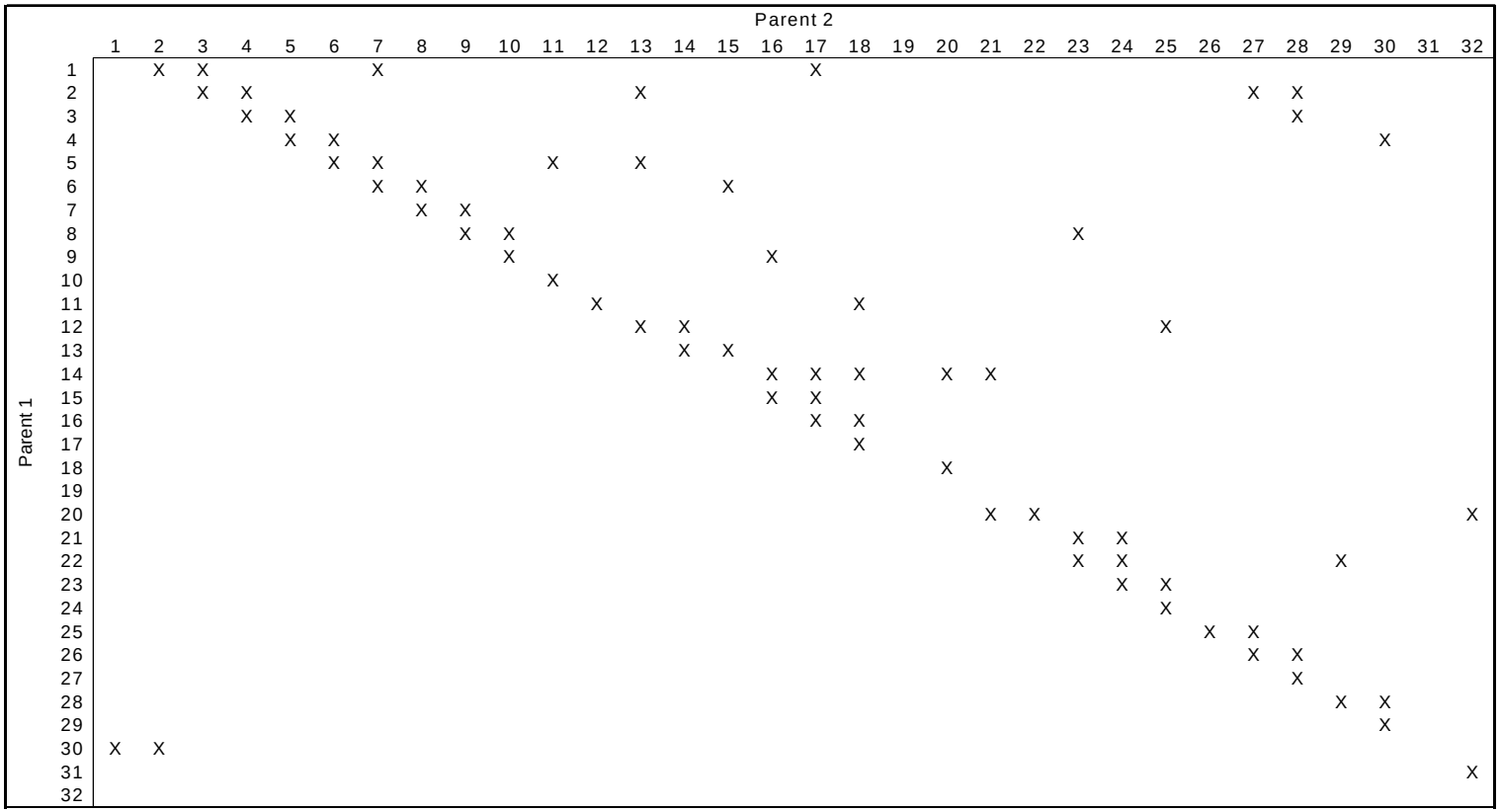
Field testing of clones is a necessary component to clonal forestry programs based on rooted cuttings. In addition, genetic field trials established with clones and seedlings from the same full-sib families provide an opportunity for comparing both half-sib and full-sib family performances for both propagules. Based on the results in Chapter 3, several conclusions can be drawn. First, clonally replicated seedling trials of loblolly

pine provide genetic information with greater precision than zygotic seedlings. Second, genetic correlations between propagule types for the growth traits were highly favorable. These high genetic correlations between propagule types reassure that parental and full-sib family rankings are stable regardless of propagule type. This implies that parental and full-sib family rankings based on existing seedling progeny trials could be used to select parents and families that perform well when they are deployed as rooted cuttings. Third, little genotype x environment interaction was observed across sites at the parental, family, and clonal level for all traits. However, there appears to be a carry-over effect relating to the season in which the cuttings were rooted for the clonal material. Finally, randomization is essential at all stages in testing when estimating genetic parameters. The lack of randomization for the seedling population apparently resulted in a problem with partitioning of the genetic variance, causing full-sib families to appear more different and inflating estimates of dominance genetic variation.

Rooting ability and 2nd year height are heritable traits in loblolly pine, and both traits showed substantial clonal variation. There was a positive genetic correlation between rooting and height at the parental, full-sib family, and clonal levels. Genetic gains in rooting ability and 2nd year height are possible as demonstrated by a number of deployment strategies. For difficult-to-root species, like loblolly pine, a successful clonal forestry program based on rooted cuttings must consider both rooting ability and growth when making selections. Because here these traits are positively correlated, selection for one trait should lead to positive gains in the other. Or, early culling of clones based on poor rooting will not negatively affect selection of clones for growth when field data become available.

Clonal forestry is not a breeding method to develop better genotypes. However, clonal forestry is a method to mass-produce well-tested genotypes. Short-term genetic gains may be maximized through deployment of well-tested clones, but long-term gains need to involve both clonal selection and recurrent selection for additive genetic variation through repeated selection and breeding. Restrictions on the relatedness of selections will be necessary when making selections for a breeding population or when deployment will be traditional zygotic seedlings from seed orchards in order to reduce detrimental effects of inbreeding depression.

APPENDIX A
LOBLOLLY PINE PARTIAL DIALLEL MATING DESIGN. THIRTY-TWO PARENTS WERE CROSSED TO GENERATE 70 FULL-SIB FAMILIES.


Parent 2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Parent 1
1 X X X X
2 X X X X X X X
3 X X X X X
4 X X X X
5 X X X X X X
6 X X X X X
7 X X X X
8 X X X X X
9 X X X X
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11 X X X X
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31 X X
32

APPENDIX B
VARIANCE COMPONENT ESTIMATES FOR ROOTING ABILITY

Table B-1. Observed variance component estimates for rooting of loblolly pine stem cuttings from single-trial analyses.

	Spring01	Summer01	Winter02	Spring02	Summer02
$\hat{\sigma}^2_{TRAY}$	0.003977	0.005357	0.002891	0.010084	0.004796
$\hat{\sigma}^2_{GCA}$	0.005018	0.004629	0.005383	0.004451	0.003817
$\hat{\sigma}^2_{SCA}$	0.002683	0.000771	0.001363	0.001868	0.00187
$\hat{\sigma}^2_{CLONE}$	0.041427	0.039236	0.024317	0.026364	0.023738
$\hat{\sigma}^2_{REPxFAM}$	0.000836	0.000559	0.000637	0.000661	0.001043
$\hat{\sigma}^2_{ERROR}$	0.190089	0.177991	0.204829	0.200698	0.142

Table B-2. Observed variance component estimates for rooting of loblolly pine stem cuttings from pair-wise-trial analyses.

	Spring01 Summer01	Spring01 Winter02	Spring01 Spring02	Spring01 Summer02	Summer01 Winter02	Summer01 Spring02	Summer01 Summer02	Winter02 Spring02	Winter02 Summer02	Spring02 Summer02
$\hat{\sigma}^2_{TRAY}$	0.004751	0.003637	0.007712	0.004474	0.004115	0.007929	0.005059	0.006905	0.003774	0.007824
$\hat{\sigma}^2_{GCA}$	0.003434	0.002923	0.00433	0.003929	0.003191	0.002844	0.002557	0.003065	0.002347	0.003915
$\hat{\sigma}^2_{SCA}$	0.001409	0.000417	0.001262	0.001876	0.00067	0.000654	0.001027	0.001107	0	0.001147
$\hat{\sigma}^2_{CLONE}$	0.021605	0.011304	0.017015	0.014235	0.012645	0.014791	0.014151	0.011209	0.010850	0.013135
$\hat{\sigma}^2_{TESTxGCA}$	0.001464	0.002034	0.000422	0.000688	0.001846	0.001380	0.001472	0.001609	0.002123	0.0004
$\hat{\sigma}^2_{TESTxFAM}$	0.000302	0.001758	0.001299	0.000539	0.000396	0.000572	0.000029	0.000423	0.001480	0.000485
$\hat{\sigma}^2_{TESTxCLONE}$	0.018862	0.021637	0.017991	0.022116	0.019564	0.018869	0.020504	0.014919	0.014083	0.012163
$\hat{\sigma}^2_{REPxFAM}$	0.000674	0.000764	0.000703	0.000956	0.000598	0.000613	0.000797	0.000646	0.000825	0.000767
$\hat{\sigma}^2_{ERROR}$	0.183321	0.198958	0.197306	0.162528	0.192458	0.192031	0.159335	0.202419	0.174684	0.177164

Table B-3. Observed variance components for rooting ability from the across-trial analysis using all five rooting trials.

Variance Component	Estimate
$\hat{\sigma}^2_{TRAY}$	0.005785
$\hat{\sigma}^2_{GCA}$	0.002921
$\hat{\sigma}^2_{SCA}$	0.001062
$\hat{\sigma}^2_{CLONE}$	0.01638
$\hat{\sigma}^2_{TESTxGCA}$	0.001364
$\hat{\sigma}^2_{TESTxFAM}$	0.000691
$\hat{\sigma}^2_{TESTxCLONE}$	0.017349
$\hat{\sigma}^2_{REPxFAM}$	0.000713
$\hat{\sigma}^2_{ERROR}$	0.184907

APPENDIX C
VARIANCE COMPONENT ESTIMATES FOR EARLY GROWTH TRAITS OF LOBLOLLY PINE CLONES AND SEEDLINGS FROM THE SAME FULL-SIB FAMILIES

Table C-1. Observed variance components for loblolly pine clones from the across-trial analyses of 1st year height, 2nd year height, height increment, and crown width. A separate error variance was modeled for each trial.

	1st Year Height	2nd Year Height	Height Increment	Crown Width
$\hat{\sigma}^2_{INC}$	53.4293	300.188	123.143	105.75
$\hat{\sigma}^2_{GCA}$	17.4525	100.363	38.141	27.5703
$\hat{\sigma}^2_{SCA}$	2.9992	21.601	7.5808	4.93
$\hat{\sigma}^2_{CLONE}$	32.3599	157.931	59.3416	56.4437
$\hat{\sigma}^2_{TESTxGCA}$	4.1225	13.3866	7.9053	5.9029
$\hat{\sigma}^2_{TESTxFAM}$	1.2003	3.7766	1.2451	1.3288
$\hat{\sigma}^2_{TESTxCLONE}$	22.4253	82.7545	29.0673	23.4036
$\hat{\sigma}^2_{TxCxGCA}$	0.4069	4.5692	2.942	2.2437
$\hat{\sigma}^2_{TxCxFAM}$	0.0000008	0.3873	0.0000005	1.1495
$\hat{\sigma}^2_{TxCxCLONE}$	0.0000008	17.1395	14.179	10.7818
$\hat{\sigma}^2_{REPxGCA}$	0.3275	1.0281	1.5638	0.6583
$\hat{\sigma}^2_{REPxFAM}$	1.9242	5.511	1.5806	0.8574
$\hat{\sigma}^2_{ERROR_A}$	512.142	1700.04	692.262	610.815
$\hat{\sigma}^2_{ERROR_B}$	279.24	853.321	427.247	306.51
$\hat{\sigma}^2_{ERROR_C}$	527.513	2061.14	928.903	843.627
$\hat{\sigma}^2_{ERROR_D}$	264.114	1110.76	864.549	329.026
$\hat{\sigma}^2_{ERROR_E}$	313.481	1124.11	682.649	423.413
$\hat{\sigma}^2_{ERROR_F}$	123.318	804.457	527.064	296.22

Table C-2. Observed variance components for loblolly pine seedlings from the across-trial analyses of 1st year height, 2nd year height, height increment, and crown width. A separate error variance was modeled for each trial.

	1st Year Height	2nd Year Height	Height Increment	Crown Width
$\hat{\sigma}^2_{GCA}$	3.6682	29.9688	12.9402	13.7566
$\hat{\sigma}^2_{SCA}$	4.3654	20.8173	10.4732	5.0053
$\hat{\sigma}^2_{TESTxGCA}$	1.5235	8.4371	8.6503	3.8956
$\hat{\sigma}^2_{TESTxFAM}$	8.5693	25.1585	8.5047	7.9082
$\hat{\sigma}^2_{TxCxGCA}$	0.0000007	0.0000004	0.0000002	0.5247
$\hat{\sigma}^2_{TxCxFAM}$	0.6036	0.0000001	1.375	1.0372
$\hat{\sigma}^2_{REPxGCA}$	0.0000001	0.0000001	0.0000001	1.2412
$\hat{\sigma}^2_{REPxFAM}$	6.3223	55.867	26.1737	15.4417
$\hat{\sigma}^2_{ERROR_A}$	599.229	1621.95	713.814	590.37
$\hat{\sigma}^2_{ERROR_B}$	361.489	1104.24	567.689	325.987
$\hat{\sigma}^2_{ERROR_C}$	508.95	2524.33	1246.59	1110.51
$\hat{\sigma}^2_{ERROR_D}$	294.534	1356.8	1059.94	501.614
$\hat{\sigma}^2_{ERROR_E}$	379.556	1766.73	1109.52	610.975
$\hat{\sigma}^2_{ERROR_F}$	101.715	755.187	585.714	341.353

APPENDIX D
VARIANCE COMPONENT ESTIMATES FROM THE BIVARIATE ANALYSES OF ROOTING AND 2ND YEAR HEIGHT

Table D-1. Observed variance component estimates from the bivariate analyses of rooting ability from Spring01 and 2nd year height from each of the field trials.

Spring01	Field A	Field B	Field C	Field D	Field E	Field F
$\hat{\sigma}^2_{INC_{root}}$	0.0039	0.0039	0.004	0.0039	0.004	0.004
$\hat{\sigma}^2_{INC_{height}}$	219.85	232.03	801.24	390.46	105.53	89.22
$\hat{\sigma}^2_{GCA_{root}}$	0.0051	0.0052	0.0052	0.0051	0.0052	0.0051
$\hat{\sigma}_{GCA_{rootheight}}$	0.0778	0.219	0.2515	0.3664	0.3268	0.1727
$\hat{\sigma}^2_{GCA_{height}}$	93.85	77.71	134.98	134.81	144.59	98.15
$\hat{\sigma}^2_{SCA_{root}}$	0.0027	0.0026	0.0026	0.0027	0.0026	0.0027
$\hat{\sigma}_{SCA_{rootheight}}$	0.0654	-0.1556	-0.0148	0.0203	0.0815	0.1294
$\hat{\sigma}^2_{SCA_{height}}$	28.34	3.7	8.13	28.18	43.77	30.08
$\hat{\sigma}^2_{CLONE_{root}}$	0.0415	0.0415	0.0414	0.0415	0.0414	0.0414
$\hat{\sigma}_{CLONE_{rootheight}}$	-0.21	0.3412	0.4084	0.3997	0.2865	0.7351
$\hat{\sigma}^2_{CLONE_{height}}$	386.08	191.46	250.52	230.24	426.68	207.67
$\hat{\sigma}^2_{REPxFAM_{root}}$	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008
$\hat{\sigma}^2_{REPxFAM_{height}}$	0.0996	5.31	23.68	15.47	24.33	15.44
$\hat{\sigma}^2_{ERROR_{root}}$	0.1901	0.1901	0.1901	0.1901	0.1901	0.1901
$\hat{\sigma}^2_{ERROR_{height}}$	1641.17	879.86	2003.72	1127.51	1072.05	834.69

Table D-2. Observed variance component estimates from the bivariate analyses of rooting ability from Summer01 and 2nd year height from each of the field trials.

Summer01	Field A	Field B	Field C	Field D	Field E	Field F
$\hat{\sigma}^2_{INC_{root}}$	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053
$\hat{\sigma}^2_{INC_{height}}$	220.06	232.4	801.8	389.37	105.55	89.37
$\hat{\sigma}^2_{GCA_{root}}$	0.005	0.005	0.005	0.005	0.005	0.0049
$\hat{\sigma}_{GCA_{rootheight}}$	0.243	0.1731	0.2933	0.3457	0.2579	0.0796
$\hat{\sigma}^2_{GCA_{height}}$	98.15	79.12	135.89	138.56	147.16	98.3
$\hat{\sigma}^2_{SCA_{root}}$	0.0007	0.0007	0.0007	0.0007	0.0007	0.0008
$\hat{\sigma}_{SCA_{rootheight}}$	0.0397	0.0141	-0.0125	0.0078	0.0616	0.1556
$\hat{\sigma}^2_{SCA_{height}}$	26.74	3.33	6.33	24.69	40.85	26.5
$\hat{\sigma}^2_{CLONE_{root}}$	0.0393	0.0392	0.0393	0.0393	0.0392	0.0393
$\hat{\sigma}_{CLONE_{rootheight}}$	-0.0944	-0.0803	0.3137	0.4131	0.2729	0.2437
$\hat{\sigma}^2_{CLONE_{height}}$	385.69	190.78	250.56	230.58	426.65	206.64
$\hat{\sigma}^2_{REPxFAM_{root}}$	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
$\hat{\sigma}^2_{REPxFAM_{height}}$	0.0757	5.3	23.92	15.69	24.4	15.71
$\hat{\sigma}^2_{ERROR_{root}}$	0.1779	0.1779	0.1779	0.1779	0.1779	0.1779
$\hat{\sigma}^2_{ERROR_{height}}$	1641.1	879.8	2003.35	1127.44	1072.03	834.0

Table D-3. Observed variance component estimates from the bivariate analyses of rooting ability from Winter02 and 2nd year height from each of the field trials.

Winter02	Field A	Field B	Field C	Field D	Field E	Field F
$\hat{\sigma}^2_{INC_{root}}$	0.003	0.0029	0.0029	0.0029	0.0029	0.0029
$\hat{\sigma}^2_{INC_{height}}$	220.76	232.19	803.25	391.48	106.31	89.66
$\hat{\sigma}^2_{GCA_{root}}$	0.0052	0.0053	0.0053	0.0052	0.0052	0.0052
$\hat{\sigma}_{GCA_{rootheight}}$	0.2043	0.1833	0.271	0.3	0.2017	0.0927
$\hat{\sigma}^2_{GCA_{height}}$	102.17	78.17	134.82	136.55	149.43	101.99
$\hat{\sigma}^2_{SCA_{root}}$	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014
$\hat{\sigma}_{SCA_{rootheight}}$	0.1209	0.0611	0.1015	0.1848	0.1924	0.1857
$\hat{\sigma}^2_{SCA_{height}}$	28.81	4.03	7.75	27.65	44.14	26.48
$\hat{\sigma}^2_{CLONE_{root}}$	0.0243	0.0243	0.0243	0.0243	0.0243	0.0243
$\hat{\sigma}_{CLONE_{rootheight}}$	0.6345	0.1392	0.3584	0.3934	0.6888	0.3461
$\hat{\sigma}^2_{CLONE_{height}}$	392.91	190.82	251.6	232.29	430.86	206.31
$\hat{\sigma}^2_{REPxFAM_{root}}$	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
$\hat{\sigma}^2_{REPxFAM_{height}}$	0.3872	5.3	23.62	15.68	24.36	15.49
$\hat{\sigma}^2_{ERROR_{root}}$	0.2048	0.2048	0.2048	0.2048	0.2048	0.2048
$\hat{\sigma}^2_{ERROR_{height}}$	1639.72	879.85	2003.09	1126.58	1071.65	834.4

Table D-4. Observed variance component estimates from the bivariate analyses of rooting ability from Spring02 and 2nd year height from each of the field trials.

Spring02	Field A	Field B	Field C	Field D	Field E	Field F
$\hat{\sigma}^2_{INC_{root}}$	0.0101	0.0997	0.01	0.01	0.01	0.01
$\hat{\sigma}^2_{INC_{height}}$	219.97	232.63	803.37	391.27	106.4	88.85
$\hat{\sigma}^2_{GCA_{root}}$	0.004	0.0041	0.004	0.004	0.004	0.0039
$\hat{\sigma}_{GCA_{rootheight}}$	0.1176	0.1904	0.1485	0.2154	0.1198	0.0669
$\hat{\sigma}^2_{GCA_{height}}$	97.42	78.53	136.8	140.76	148.65	97.39
$\hat{\sigma}^2_{SCA_{root}}$	0.0018	0.0018	0.0018	0.0018	0.0019	0.0019
$\hat{\sigma}_{SCA_{rootheight}}$	0.1254	0.1025	0.1448	0.242	0.3037	0.278
$\hat{\sigma}^2_{SCA_{height}}$	28.64	3.41	6.99	26.41	41.76	32.59
$\hat{\sigma}^2_{CLONE_{root}}$	0.0264	0.0265	0.0264	0.0265	0.0264	0.0264
$\hat{\sigma}_{CLONE_{rootheight}}$	-0.0583	0.4215	0.7019	0.8602	0.2998	0.5378
$\hat{\sigma}^2_{CLONE_{height}}$	385.33	192.98	256.21	23.29	427.8	205.39
$\hat{\sigma}^2_{REPxFAM_{root}}$	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007
$\hat{\sigma}^2_{REPxFAM_{height}}$	0	5.33	23.42	15.61	24.23	15.59
$\hat{\sigma}^2_{ERROR_{root}}$	0.2007	0.2007	0.2007	0.2007	0.2007	0.2007
$\hat{\sigma}^2_{ERROR_{height}}$	1641.16	879.54	2002.81	1127.07	1071.69	834.82

Table D-5. Observed variance component estimates from the bivariate analyses of rooting ability from Summer02 and 2nd year height from each of the field trials.

Summer02	Field A	Field B	Field C	Field D	Field E	Field F
$\hat{\sigma}^2_{INC_{root}}$	0.0048	0.0048	0.0048	0.0048	0.0048	0.0048
$\hat{\sigma}^2_{INC_{height}}$	219.68	232.5	802.51	390.45	105.24	88.7
$\hat{\sigma}^2_{GCA_{root}}$	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037
$\hat{\sigma}_{GCA_{rootheight}}$	0.1006	0.2194	0.1656	0.2104	0.0913	0.1656
$\hat{\sigma}^2_{GCA_{height}}$	96.96	78.41	135.51	138.45	145.59	99.21
$\hat{\sigma}^2_{SCA_{root}}$	0.0017	0.0017	0.0017	0.0018	0.0017	0.0017
$\hat{\sigma}_{SCA_{rootheight}}$	0.0439	0.0693	0.0831	0.101	0.2114	0.1497
$\hat{\sigma}^2_{SCA_{height}}$	27.33	3.68	7.906	26.24	41.0	29.59
$\hat{\sigma}^2_{CLONE_{root}}$	0.0238	0.0238	0.0238	0.0237	0.0238	0.0237
$\hat{\sigma}_{CLONE_{rootheight}}$	0.2082	0.1956	0.2738	0.3435	0.2526	0.5838
$\hat{\sigma}^2_{CLONE_{height}}$	386.1	190.66	249.78	229.44	426.75	206.54
$\hat{\sigma}^2_{REPxFAM_{root}}$	0.001	0.001	0.001	0.001	0.001	
$\hat{\sigma}^2_{REPxFAM_{height}}$	0.1938	5.25	23.88	15.48	24.3	
$\hat{\sigma}^2_{ERROR_{root}}$	0.142	0.142	0.142	0.142	0.142	
$\hat{\sigma}^2_{ERROR_{height}}$	1641.0	879.82	2003.21	1127.52	1072.29	

Table D-6. Observed variance component estimates from the bivariate analysis of rooting ability using all five rooting trials and 2nd year height using all six field trials.

Variance Component	Estimate
$\hat{\sigma}^2_{INC_{root}}$	0.0058
$\hat{\sigma}^2_{INC_{height}}$	331.45
$\hat{\sigma}^2_{GCA_{root}}$	0.0034
$\hat{\sigma}_{GCA_{rootheight}}$	0.1858
$\hat{\sigma}^2_{GCA_{height}}$	99.83
$\hat{\sigma}^2_{SCA_{root}}$	0.0004
$\hat{\sigma}_{SCA_{rootheight}}$	0.1155
$\hat{\sigma}^2_{SCA_{height}}$	19.33
$\hat{\sigma}^2_{CLONE_{root}}$	0.0164
$\hat{\sigma}_{CLONE_{rootheight}}$	0.3988
$\hat{\sigma}^2_{CLONE_{height}}$	162.72
$\hat{\sigma}^2_{TESTxFAM_{root}}$	0.0035
$\hat{\sigma}^2_{TESTxFAM_{height}}$	30.74
$\hat{\sigma}^2_{TESTxCLONE_{root}}$	0.0173
$\hat{\sigma}^2_{TESTxCLONE_{height}}$	103.55
$\hat{\sigma}^2_{REPxFAM_{root}}$	0.0007
$\hat{\sigma}^2_{REPxFAM_{height}}$	12.1
$\hat{\sigma}^2_{ERROR_{root}}$	0.1849
$\hat{\sigma}^2_{ERROR_{height}}$	1308.39

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BIOGRAPHICAL SKETCH

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