

VARIATION IN GROWTH, WATER RELATION, GAS EXCHANGE, AND STABLE
CARBON ISOTOPE COMPOSITION AMONG CLONES OF LOBOLLLY PINE
(*Pinus taeda* L.) UNDER WATER STRESS

By

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By

Maheteme T. Gebremedhin

Dedicated to my beloved parents, sisters and brothers who have always believed in me.

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TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS.....	iv
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
CHAPTERS	
ABSTRACT.....	x
1 INTRODUCTION.....	1
2 VARIATION IN MORPHOLOGICAL AND WATER RELATIONS TRAITS IN RESPONSE TO LIMITED SOIL WATER SUPPLY AMONG LOBLOLLY PINE (<i>Pinus taeda</i> L.) CLONES.....	8
Introduction.....	8
Materials and Methods.....	12
Genetic Material and Production of Hedges.....	12
Collection and Setting of Cuttings.....	12
Drought Stress Treatment.....	14
Morphological Measurements.....	14
Predawn Needle Water Potential.....	15
Pressure-Volume Curve Analysis.....	15
Stable Carbon Isotope Analyses.....	16
Statistical Analyses.....	17
Broad-Sense Heritability (H^2).....	18
Genetic Correlations: r_{B-TRT} and r_g	18
Results and Discussion.....	19
Effects of Drought on Growth and Morphology of Clones.....	19
Clone-by-Watering Treatment Interaction.....	20
Broad-Sense Heritability for Height and Biomass Components.....	21
Type-B Genetic Correlation.....	23
Type-A Genetic Correlation.....	23
The Effects of Drought on Water Relation Parameters.....	24
Pressure-Volume (P-V) Analyses.....	25
Heritability and Clone-by-Watering Treatment Interaction for P-V Parameters...	26
Stable Carbon Isotope Composition.....	27

Clonal Variation in Stable Carbon Isotope Composition.....	28
Broad-Sense Heritability for Stable Carbon Isotope Composition.....	29
Conclusions.....	29
3 STABLE CARBON ISOTOPE COMPOSITION AND STOMATAL LIMITATION TO PHOTOSYNTHESIS OF CLONES OF LOBLOLLY PINE (<i>Pinus taeda</i> L.) UNDER LIMITED SOIL WATER SUPPLY.....	41
Introduction.....	41
Materials and Methods.....	44
Plant Genetic Material.....	44
Greenhouse Culture for Seedling Hedge Production.....	44
Needle Gas-Exchange Measurements.....	45
Stomatal Limitation (L_s).....	46
Stable Carbon Isotope Composition.....	47
Statistical Analyses.....	48
Results and Discussion.....	48
Maximum Net Assimilation (A_{max}) and Stomatal Conductance (g_{max}).....	48
Clonal Differences in A_{max} and g_{max}	50
Stomatal Limitation to Light-Saturated Net Photosynthesis.....	52
Stable Carbon Isotope Composition.....	53
Clonal Differences in $\delta^{13}C$	54
Conclusion.....	55
4 CONCLUSION.....	63
APPENDIX	
A GENERAL STATISTICAL MODEL.....	68
B SUPPLEMENTAL FIGURES.....	69
REFERENCES.....	74
BIOGRAPHICAL SKETCH.....	83

LIST OF TABLES

<u>Table</u>	<u>page</u>
2.1. Geographical origins of superior parent hedges used to propagate the 30 clones used in the study.....	31
2.2. P-values of ANOVA on total biomass, percent needle, percent stem, percent root, root:shoot ratio, relative height (RGR1 and RGR2), (needle water potential (ψ_{pd}), relative water content at turgor loss point (RWC_{TLP}), osmotic potential at full saturation (Π_{SAT}), osmotic potential at turgor loss point (Π_{TLP}), and stable carbon isotope composition (^{13}C) for clones of loblolly pine under three watering treatments. RGR1 and RG2 are relative growth height (cm) calculated as the difference in height before and after the first (DT1) and the second (DT2) droughts, respectively.....	32
2.3. Variance component estimates (expressed as % of total for each trait) for growth traits of total biomass, percent needle, percent stem, percent root, root:shoot ratio, height at the end of the first (T6) and second drought (T7) cycles and stable carbon isotope composition ($\delta^{13}C$).....	33
2.4. Broad-sense heritability (H^2) and standard deviation (STD) estimates and type-B genetic correlation (STD) of loblolly pine clones for total biomass, percent needle, percent stem, percent root, root:shoot ratio and needle carbon isotope composition ($^{13}\delta C$).	34
2.5. Type-A genetic correlations among total aboveground biomass (g), height (at final harvest (cm), predawn water potential (ψ_{pd}) and needle stable carbon isotope composition ($\delta^{13}C$, ‰) for clonally propagated loblolly pines.....	35
2.6. Mean osmotic potential at full turgor (Π_{SAT}) and turgor loss (Π_{TLP}) points and relative water content at turgor loss point (RWC_{TLP}) of clones of loblolly pine in control, drought 1(DT1) and drought 2 (DT2) cycles..	36
3.1. Mean values (standard errors) for light saturated net photosynthesis rate (A_{max}), stomatal conductance (g_{max}), stomatal limitation (L_s), predawn needle water potential (Ψ_{pd}) and stable carbon isotope composition ($\delta^{13}C$). Lower panel contains P-values for watering treatment, clone and clone by treatment interaction for 12 clones of loblolly pine.....	57

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2.1. Trends of height growth of clones of loblolly pine across time before (Feb 27 - May 1) and after (May 9- May 20) drought. Height was measured at time T1, T2, T3, T4, T5, T6, and T7 corresponding to each measurement date. Height differences were not significantly different for time periods before drought (T1 to T5). Relative growth (RGR1 and RGR2), calculated as differences in height before and after drought, were significantly different at $P = 0.0059$ and $P = 0.0171$ during the first (DT1) and the second (DT2) drought cycles, respectively.....	37
2.2. Broad-sense heritability (H^2) estimates for incremental height before (Feb 27 - May 1) and after (May 9 - May 20) drought treatments.	38
2.3. Changes in needle predawn water potential (ψ_{pd}) across time for clones of loblolly pine subjected to water stress treatments	39
2.4. Broad-sense heritability (H^2) estimates for water relation parameters of predawn water potential, osmotic potential at saturation (OPSAT), osmotic potential at turgor loss point (OPTLP), and relative water content at turgor loss point (RWCTLP).....	40
3.1. Illustration of the use of A- C_i curve to estimate gas phase limitation to net photosynthesis using Method V of Jones (1985). The slope of the demand and supply functions are expressed as $(1/r^*)$ and $(-1/r_g)$, respectively. C_a and C_i are the ambient and intercellular CO_2 concentrations, respectively. A is net photosynthetic rate in $\mu mol m^{-2} s^{-1}$	58
3.2. Light-saturated net photosynthesis rate (A_{max}) as a function of light-saturated stomatal conductance (g_{max}) of single point measurements of plants under well-watered (open) and water-stressed (solid) treatments	59
3.3. Relationship between relative stomatal limitation (L_s) versus light-saturated photosynthesis (A_{max} , $\mu mol m^{-2} s^{-1}$) of single point measurements of plants under well-watered (open) and water-stressed (solid) treatments.....	60
3.4. Relationship between mean (\pm SE) stable carbon isotope composition ($\delta^{13}C$) and light saturated stomatal conductance ($mol m^{-2} s^{-1}$) (a), and light-saturated net photosynthesis ($\mu mol m^{-2} s^{-1}$) (b), for clones of loblolly pine under well-watered (open) and water-stressed (solid) treatments.. ..	61

3.5. Relationship between mean (\pm SE) and intercellular to ambient CO ₂ concentration ratio (C _i /C _a) and stable carbon isotope composition (d13) for clones of loblolly pine under well-watered (open) and water-stressed (solid) treatments.....	62
B.1. Mean monthly air temperature of greenhouse environment during study period (July 2001-June 2002).....	69
B.2. Relationship between light-saturated stomatal conductance (mmol m ⁻² s ⁻¹) and needle predawn water potential (MPa) of single point measurements for loblolly pine plants under well-watered (open) and water-stressed (solid) treatments.....	70
B.3. Mean predawn water potential (ψ_{pd}) for 12 clones of loblolly pine under three watering treatments of well-watered, drought-1 and drought-2. Clonal differences were not significant (P = 0.8395).....	71
B.4. Mean light-saturated photosynthesis rate (A _{max} , μ mol m ⁻² s ⁻¹), for clones of loblolly pine under well-watered and drought treatments. Measurement was taken four days after drought was imposed. Clonal differences were not significant (P= 0.2405).....	72
B.5. Mean carbon isotope composition (per mil) in well-watered (open), drought-1 (filled) and drought-2 (hatched) for clones of loblolly pine. Clonal differences were significant (P = 0.0034).....	73

ABSTRACT OF THESIS PRESENTED TO THE GRADUATE SCHOOL
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By

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Intermittent drought occurs in many parts of the natural range of *Pinus taeda* L. (loblolly pine), limiting its growth and performance. Clonal variation in growth, water relations, and isotopic carbon composition were examined for thirty clones of *P. taeda* in response to water stress treatments. The relative importance of stomatal limitation to light-saturated photosynthesis was determined under well-watered and water-stressed environment for a sub-set of the clones. Genetic parameters such as broad-sense heritability (H^2), clone-by-watering treatment interaction (gxe), and genetic correlations (type A and B) were estimated for growth, plant water relations, and stable carbon isotopic composition.

Drought significantly reduced total biomass accumulation and height in both drought cycles. There were no clonal differences in drought induced biomass allocation patterns, including the root: shoot ratio. Water stress significantly lowered predawn water potential (ψ_{pd}) in both drought cycles. There was no significant difference in osmotic potential at full turgor or osmotic potential at turgor loss point between the control and drought cycle treatments.

Estimated broad-sense heritabilities (H^2) for height and morphological traits were high. The type-B genetic correlation (r_{Bc}) was also high, ranging from 0.91 to 0.97, demonstrating the lack of a significant clone-by-watering treatment interaction. Estimated H^2 for needle ψ_{pd} , osmotic potential at full saturation, osmotic potential at the turgor loss point and relative water content at the turgor loss point were low. Consistent with the non-significant clone-by-watering treatment interaction, r_{Bc} for pressure-volume (P-V) parameters were high and close to one suggesting similar drought responses among clones. Carbon isotope composition ($\delta^{13}C$) of clones was strongly influenced by availability of water. Compared to well-watered clones, $\delta^{13}C$ of needles increased by 18 and 24‰ during the first and second drought cycles, respectively, suggesting higher discrimination against ^{13}C by clones in well-watered conditions. H^2 for $\delta^{13}C$ was low (0.079) regardless of the watering treatment.

Stomatal limitation to light-saturated net photosynthesis under well-watered conditions accounted for 20 to 30% on average, but increased to 40% under water stress, suggesting the control of net CO_2 assimilation was highly dominated by non-stomatal limitation during drought.

CHAPTER 1 INTRODUCTION

Loblolly pine (*Pinus taeda L.*) is an important timber species in the southeastern United States (Schultz, 1999). It grows naturally in southern and Mid-Atlantic States between the latitudes of 28^o N and 39^o N and longitudes 57^o W and 97^o W. Outside its native range, loblolly pine is extensively planted as an exotic species in subtropical regions of South America and in southern Africa (Bridgewater *et al.* 1997, Baez and White 1997). Because of its economic importance in the timber industry, loblolly pine is becoming the most preferred pine species throughout the southeastern United States. Recognizing its importance, considerable effort has been put into its genetic improvement since the early 1950s. As a result, significant genetic and economic gains have been obtained (Schultz, 1999).

Over the past 30 years, forest companies in the southeastern USA have been using genetically improved seedling stocks for loblolly pine regeneration. Genetically improved loblolly pine varieties comprise eighty percent of all of seedlings planted in the southeastern USA every year (Lantz and Kraus, 1987). However, with the ever-increasing demand for industrial timber wood and pressure on forestlands, the need to complement seedling based forestry with clonal forestry is becoming a necessity. In fact clonal reforestation is being considered as an alternative propagation technique for several important forest tree species (Foster and Shaw 1987, Ritchie 1994).

Clonal forestry is defined as large-scale deployments of a few clones that have proven their superiority in clonal tests (Libby and Ahuja 1993). Rooted cutting technology is a potential large-scale propagation technique for loblolly pine (Stelzer and Goldfrab 1997). While much progress has been made, true clonal forestry is not yet operational for loblolly pine.

At present, various forest companies and universities are conducting research in order to understand the biology of clones in relation to plantation site requirements before large-scale clonal deployment is initiated. To this end, the Forest Biology Research Cooperative (FBRC) program at the University of Florida has initiated a series of intensive greenhouse and field clonal screening programs designed to understand the growth, performance and stress physiology of clones of loblolly pine. Greenhouse and field clonal performance studies are currently underway to intensively screen 2,000 clones within the next few years. The purpose of this FBRC study was to characterize variation in drought response among a sub-set of clones selected from a population of clones that International Paper Company has been managing at its research facility near Jay, Florida.

Because variation in clonal drought response could be considerable, arrays of morphological and physiological measurements were performed on clones subjected to repeated drought cycles. Growth and performance could increase if clones are deployed according to site-specific conditions. Failure to match species to sites has been the cause of disappointing performance of many plantation projects (White *et al.* in press). One advantage clonal forestry offers is that it can exploit the genotype by

environment interaction by matching clones with sites to which they are adapted (Carson and Burdon 1991).

Across much of the loblolly pine range, moisture stress occurs periodically during the summer and fall growing seasons (Schultz 1997). Intermittent drought events during the summer months are becoming more frequent, limiting the establishment, growth and performance of loblolly pine. A combination of acute soil water deficits, (Schultz 1997) high summer temperatures (Ellsworth 2000) and steep diurnal evaporative demand could affect the survival and growth of trees in plantations. Past studies have shown that moisture stress, during the fall and spring growing season, limits growth and productivity of loblolly pine across many parts of the southeast, with more severe effects on well drained sites (Hacke *et al.* 2000).

In some cases, the species is planted on well-drained soils resulting in an increased risk of moisture and hydraulic conductivity loss (Sperry *et al.* 1998, Hacke *et al.* 2000). Climate change in the future may also lead to more frequent and severe drought, much like those that have occurred in the southeastern USA in the past few years. Considering the above circumstances, the selection of drought tolerant genotypes of loblolly pine is critical.

To determine whether drought tolerance regulating traits are favorably expressed under a limited water environment, this greenhouse study was designed to examine the influence of water stress on growth, gas exchange, water relations and stable carbon isotope composition among clones of loblolly pine. The second chapter of this thesis examines variation in morphological and water relations traits of 30 clones subjected to three watering treatments in a greenhouse. Biomass accumulation

and allocation patterns and incremental height measurements were determined for clones in three watering treatments. Because of its wide adaptation, genetic variation in loblolly pine can be considerable and previous studies have demonstrated differences in growth at provenances and family levels (van Buijtenen *et al.* 1976, Bongarten and Teskey 1987).

Little is known about the genetic variation in drought response among clonally propagated loblolly pine and knowledge about drought-response regulating mechanisms is very crucial. Morphological variation in water stress responses have been observed among clones of many forest species, including interior spruce (*Picea glauca* (Moench) Voss x *Picea engelmannii* Parry ex Engelm.) (Fan and Grossnickle 1998), white spruce (*Picea glauca* (Moench) Voss) (Lamhamedi *et al.* 2000), *Eucalyptus spp* (Le Roux *et al.* 1996, Osorio *et al.* 1998, Pita and Pardos 2001, Sasse and Sands 1996) and *Poplar spp* (Gebre *et al.* 1997, Souch and Stephens 1998, Tschaplinski and Blake 1998).

In general, plants cope with drought either by reducing the amount of water loss and/or by increasing water uptake. Broadly, mechanisms of drought resistance can be divided into two categories (Jones 1992): 1) traits that minimize the occurrence of damaging water deficit by delaying desiccation, such as stomatal closure, high cuticular resistance, extensive and deep root systems, and 2) traits that help the plant to continue normal physiological processes despite desiccation. These include turgor maintenance through osmotic adjustment and low cell wall elasticity. In areas where drought occurs for brief periods of time, such as the drought events

observed in southeastern USA, drought tolerance strategy could be more advantageous than the conservative drought avoidance strategy (Abrams 1988).

Needle predawn water potential (ψ_{pd}) and pressure-volume (P-V) curve analysis were used to identify clonal osmotic potential at the full turgor (π_{Full}) and turgor loss (π_{Zero}) points and relative water content at the turgor loss point (RWC_{TLP}). Previous studies have demonstrated that drought preconditioned seedlings of loblolly pine were able to osmotically adjust and continue physiological processes when subjected to drought (Hennesey and Dougherty 1984, Teskey *et al.* 1987). Similarly, Seiler and Johnsen (1988) reported lower osmotic potential in loblolly pine seedlings that had been through repeated drought than non-stressed seedlings. Further, genotypic variation in osmotic adjustments has been detected in loblolly pine (Bongarten and Teskey 1987). This variation is important in that it can be a potential source of selection for dehydration tolerant genotypes. It has also been suggested that genetic variation in osmotic adjustment can be used as selection criteria for improved dehydration tolerance (Morgan *et al.* 1991, Gebre *et al.* 1997).

Clonal selection for drought tolerance requires genetic knowledge of the water relation traits under different environmental conditions. Identifying traits such as low osmotic potential due to increased solute concentration and increased root growth are all traits that define the best genotypes for use in dry environments. However, past experience is that water relation traits have rarely been used in selection and breeding programs of forest species, partly because these physiological traits simply cannot be measured on all available trees in a large breeding population.

Because of its wide adaptation, genetic variation in loblolly pine can be considerable. Previous studies have demonstrated differences in growth among loblolly pine at provenance and family levels (van Buijtenen *et al.* 1976, Bongarten and Teskey 1987, Seiler and Johnsen 1988, Sierra *et al.* 1999). Accurate and precise genetic parameter estimates are also fundamental for determining breeding strategies and choosing genotypes for commercial propagation (White 1987). Estimation of variance components is important in providing useful information to breeders to estimate genetic parameters of interest. Genetic parameters including broad-sense heritabilities (H^2), clone x watering treatment interaction (gxe), type-A genetic correlation were estimated for both growth and water relation traits. Clonal variations in carbon isotope discrimination of needles sampled from the three watering regimes were also analyzed.

Chapter 3 discusses the effect of water stress on physiological processes when other environmental factors were kept optimal. Limitation to photosynthesis is mainly due to either decreased diffusion of carbon dioxide to the intracellular leaf space controlled by stomatal conductance (Hsiao 1973, Turner and Kramer 1980, Jones 1992) or biochemical processes (Wilson *et al.* 2000). Gas exchange techniques can provide useful information on the control of photosynthesis by stomata and intracellular processes (Jones 1992) and various approaches have been used to quantify the extent of stomatal as well as non-stomatal limitations (Jones 1985). To examine the effect of drought, light-saturated net photosynthesis (A_{\max}) and stomatal conductance (g_{\max}) were measured at five external CO_2 levels and drought-induced stomatal limitations to net photosynthesis were quantified.

Instantaneous measures of gas exchange processes at a single point in time may provide only limited insight into the overall physiological performance of a plant (Ehleringer and Cerling 1995). Measured physiological traits, such as gas exchange and water relations have been used in selection for drought tolerance. However, these traits, in most cases, fluctuate throughout the day and during the season and vary with the degree and intensity of drought. Thus, they may not provide an integrated measure of plant response. Naturally occurring variation in the abundance of stable isotopes of carbon in plants has been reported and is now used to understand various physiological processes in plants. Hence, carbon isotope composition ($\delta^{13}\text{C}$) of clones in response to drought was also examined in this project.

Overall, this study was conducted to understand the basic mechanisms involved in drought tolerance at the whole plant and foliage levels. Specifically, the study was designed with the following set of objectives:

1. To determine growth and plant water relation traits that contribute to clonal differences in drought tolerance,
2. To determine if shoot osmotic adjustment (OA) occurs among clones tested under two subsequent drought cycles and to see if OA is a viable selection criteria for drought tolerance for clonal selection,
3. To determine the extent of clone x watering treatment interaction for clones under varying soil water environments,
4. To determine the role of drought-induced stomatal limitation (L_s) to net photosynthesis among selected clones, and
5. To evaluate variation in needle stable carbon isotope composition ($\delta^{13}\text{C}$) among clones in response to drought.

CHAPTER 2
VARIATION IN MORPHOLOGICAL AND WATER RELATIONS TRAITS IN
RESPONSE TO LIMITED SOIL WATER SUPPLY AMONG LOBLOLLY PINE
(*Pinus taeda* L.) CLONES

Introduction

Loblolly pine (*Pinus taeda* L.), the most important commercial species in the southeastern United States, is planted widely on a range of soil and site conditions (Green and Mitchell 1992, Schultz 1999). According to an estimate, genetically-improved seedlings comprise eighty percent of all plantings each year (Lantz and Kraus 1987). However, with a rising demand for timber and pulp, the need to complement seedling based forestry with clonal forestry is becoming increasingly important. Although not operational yet, clonal reforestation is being considered as an alternative propagation technique by many forest industries (Ritchie 1994, Stelzer and Goldfarb 1997).

Rooted cutting technology, probably the most cost-effective large-scale propagation technique for loblolly pine, is currently being developed by large forest products companies (Stelzer and Goldfarb 1997). However, before the use of rooted cuttings becomes operational, we need to understand their physiological and morphological characteristics in order to assure their future use as potential propagation materials. Considering that severe planting season and growing season droughts are common in the range of loblolly pine, it is important to understand how these water limitations impact the water relations and growth parameters of newly planted rooted cuttings. Although morphological variation in water stress responses has been observed

among clones of many forest species including interior spruce (*Picea glauca* (Moench) Voss x *Picea engelmannii* Parry ex Engelm.) (Fan and Grossnickle 1998); white spruce (*Picea glauca* (Moench) Voss) (Lamhamedi *et al.* 2000); *Eucalyptus* spp (Le Roux *et al.* 1996, Osorio *et al.* 1998, Pita and Pardos 2001, Sasse and Sands 1999) and *Populus* spp (Gebre *et al.* 1997, Souch and Stephens 1998, Tschaplinski and Blake 1998), such information is non-existent for loblolly pine.

Clonal variation in morphological traits expressed under water stress is an important attribute of drought tolerance. This variation is often explained by genetic differences in drought tolerance (Cregg 1993, Blake *et al.* 1996 and Gebre *et al.* 1997). Traits conferring drought tolerance, such as increased rooting depth (Tschaplinski and Blake 1998), sensitivity to early leaf expansion (Osorio *et al.* 1998), and changes in biomass allocation pattern (Le Roux *et al.* 1996) have been identified among clones of many tree species. These morphological traits can be used routinely during selection, because they are easily recognized, inexpensive to measure and are more repeatable than physiological measurements (Cregg 1994).

Stable carbon isotope determinations provide time-integrated measures of plant physiological activities and plant interaction with the environment (Farquhar *et al.* 1989). Relationships between dry biomass accumulation and stable carbon isotope composition ($\delta^{13}\text{C}$) have been established for growth in C-3 plants (Farquhar and Sharkey 1982) and the association between $\delta^{13}\text{C}$ and the accumulation of plant dry mass may vary according to the environment (Prasolava *et al.* 2002). Correlation studies have also shown that $\delta^{13}\text{C}$ can be related to dry mass production under drought conditions (Osorio *et al.* 1998). Previous studies have indicated the presence of genetic variations in $\delta^{13}\text{C}$ to dry mass

accumulation (Zhang *et al.* 1993, Le Roux *et al.* 1996.). Low discrimination (Δ) for ^{13}C assimilation in water-limited environments has been used as criteria for improved water use efficiency (Osorio *et al.* 1998). Furthermore, the use of Δ as a screening tool in selection and breeding programs has been proposed (Sun *et al.* 1996, Prasolova *et al.* 2002).

Genetic variation in $\delta^{13}\text{C}$ has been reported for several tree species (Zhang *et al.* 1993, Osorio and Pereira 1994, Zhang and Marshall 1995, Zhang *et al.* 1997). Therefore, understanding of the genetic basis for variation in $\delta^{13}\text{C}$ can be very useful for ranking genotypes in that it may serve as a guide in tree breeding programs. Most of the studies reported previously on $\delta^{13}\text{C}$ have used very limited sets of genetic materials. This deficiency in experimental breadth makes it impossible to get reasonable genetic estimates such as broad sense heritability and genetic correlation. Therefore, there is limited information available on heritability, variance components, genotype-by-environment interaction and correlation estimates for $\delta^{13}\text{C}$ under water limited and non-limiting conditions. From the operational point of view, such information is crucial when introducing new genetic entries, such as untested clones, into the plantation scheme.

Although not specifically for rooted cuttings, past studies have shown that moisture stress during the fall and spring growing season limits growth and productivity in loblolly pine across many parts of the southeast with a more severe effect on well drained sites (Teskey *et al.* 1987). Climate change in the future may also lead to more frequent and severe droughts, much like the droughts of the past few years in the southeastern. Therefore the selection of drought tolerant genotypes of loblolly pine for

clonal deployment could be important. Hence, the present study was conducted with the following objectives.

1. Determine morphological and plant water relations traits that contribute significantly to clonal differences, if any, in dry mass accumulation and carbon allocation pattern,
- 2.
3. Estimate variance components and heritabilities of morphological, water relations and isotopic carbon composition traits in response to drought,
- 4.
5. Determine the extent, if any, of clone x environment interaction for a range of morphological and water relation traits under varying soil water environments.
- 6.
- 7.
- 8.

Materials and Methods

Genetic Material and Production of Hedges

The genetic material for the study was obtained from the Loblolly Pine Lower Gulf Elite Population (LPLGEP), which is a combined effort of the three tree improvement cooperatives in the southern USA. Parents were selected from a mix of tested first and second-generation selections originating from two different provenances, namely, Atlantic Coastal Plain and Florida (Table 2.1). These parents and clones are a subset of those being planted by the Forest Biology Research Cooperative in a field comparison study called CCLONES.

In January 2001 as part of the larger CCLONES study, seeds from 70 full-sib families were hand sown in Ray Leach SuperCell (Stuewe and Sons, Corvallis, OR) on a medium of peat, vermiculite and perlite (2:2:1). The seedlings were grown in a greenhouse maintained at 30°C /20°C day/night temperature and 60 % RH located at the International Paper facility near Jay, Florida. In October 2000, the seedlings (n=2100) were transplanted into 11.5-liter plastic pots containing a medium of bark:coir (1:1). Established hedges were periodically pruned and managed intensively to create 2100 clonal hedges.

Collection and Setting of Cuttings

In May 2001, approximately 18 uniform semi-lignified cuttings (ramets) were harvested from each of the 2100 established hedges (clones). The foliage of harvested stem cuttings consisted mostly of expanded primary needles and secondary needles that were just beginning to emerge from their fascicle sheaths. Cuttings were re-cut to a uniform length of 6 cm. The bottom cut surface was dipped in for 5 seconds in a

commercial solution of Dip-N-Grow (NAA 1mg /l, IBA 0.5mg/l) that was diluted with distilled water (1:10 v: v). The treated cuttings were set into a plastic trays containing a preformed rooting medium in a greenhouse maintained at 29/31°C max/min temperature and 75% RH.

In July 2001 (nine weeks after sticking), a total of 37,800 cuttings were assessed (presence and absence of roots) to calculate rooting percentage. A cutting was considered rooted if one or more roots were observed at the time of assessment. Three rooting classes were defined: good, intermediate and poor with >80, 60-80 and <30% rooting percentages, respectively. A total of 30 clones, 10 from each of the three rooting classes, were selected for this study.

In August 2001, a total of 540 cuttings (30 clones x 18 ramets/clone) were transported to a greenhouse at the University of Florida in Gainesville. Two days later, the 13-week-old cuttings were transplanted into plastic pots, 10cm wide x 36 cm deep, (Tall One Treepots™ FOB Oregon) containing 100% fine sand (acid washed).

The cuttings were then arranged in completely randomized blocks, split-plot design with six blocks. The whole plots were three levels of water treatment and the subplots were the 30 clones. The three watering treatments were randomly assigned within each block with one ramet per clone in each sub-plot; there were a total of 540 plants (6 complete blocks x 3 whole plot watering treatments x 30 sub-plot treatments). The cuttings were maintained in a greenhouse with ambient light and were hand-watered to pot water capacity every other day until the start of the experiment. Clones were fertilized every other week using Peters 24-12-12 (N-P-K plus micronutrients) at a rate of 2 gL⁻¹ N.

Drought Stress Treatment

Beginning on May 1, 2002 (10 months after transplanting the rooted cuttings), clones were subjected to three watering treatments to initiate the drought treatments. Because of the large sample size and the time needed for measurements to be taken, water stress treatments were initiated on a staggered block-by block basis. The three treatments were:

1. Single uninterrupted water stress treatment (DT1): 180 ramets (6 Blocks x 30 clones x x 1 ramet/clone) were subjected to drought by withholding water until the average (among-clone) predawn needle water potential (Ψ_{pd}) reached -1.75MPa . At this point, which occurred after 7 days without water, visible wilting of needles and non-lignified stem was apparent during the day. Drought gradually progressed until visible wilting (predetermined needle Ψ_{pd} of -1.75MPa) became apparent.
2. Two cycles drought treatment (DT2): a second set of 180 ramets were subjected to a first drought cycle simultaneous with and identical to the DT1 plants. The plants were then re-watered, and a second drought cycle, following the same protocols as the first, was initiated 48 hours after re-watering.
3. Well watered (control group): 180 ramets were watered every other day to pot field capacity, which maintained at needle Ψ_{pd} of $-0.3\text{MPa} \pm 0.1$.

Morphological Measurements

Height measurements were taken periodically beginning February 27, 2002 until the final harvest. To assess the effect of drought on stem elongation, relative height

growth (RGR1) was calculated as $(T6-T5)/T5$ and RGR2 $(T7-T6)/T6$, where T5, T6 and T7 are height measurements (cm) taken before the start of the drought, at the end of DT1 and DT2, respectively.

At the end of each drought cycle, plants were harvested and biomass was separated into needles and stem. Roots were carefully excavated and washed for dry weight measurements and root to shoot ratio was then calculated. Harvested biomass was dried in a forced-air oven for 48 h at 70 °C for dry weight determination.

Predawn Needle Water Potential

During the initial drought cycle treatment, Ψ_{pd} was assessed every other day on a randomly-selected subset of clones in each block to monitor the progression of drought. On the sixth day of each drought cycle, Ψ_{pd} was measured on every clone in the drought treatments (DT1 and DT2) and a subset (one third) of clones in the well-watered control treatment in all 6 blocks. For Ψ_{pd} determination, a fascicle with three fully-expanded needles was excised from the dominant terminal shoot. Predawn water potential was measured using a pressure chamber (PMS Instruments, Corvallis, OR), according to the standard technique (Schulte and Hinckley 1985).

Pressure-Volume Curve Analysis

Pressure – volume (PV) curves were developed for clones in each treatment cycle after seven days of drought. A total of 476 curves (4 blocks x 1 watering treatment x 29 clones x 1 ramet per clone for DT1 and 6 blocks x 2 watering treatments (control and DT2) x 30 clones x 1 ramet per clone) were constructed, using three pressure bombs, each measuring 10-15 clones per assessment day, using the techniques described by Tyree and Richter (1982).

The afternoon before each measurement day, 30 plants (15 control and 15 droughted) were removed from the greenhouse and watered to full saturation, covered with black plastic bag, before transferring them to a dark room for overnight saturation. Seven hours after saturation, a plant was removed from the bag and one of its lateral branches in the upper section of the shoot was severed and placed in a pressure chamber. Pressure-volume curves were constructed by simultaneous measurements of bulk shoot water potential (ψ_x) and gradual weight loss from cut surface as cuttings dehydrated from full saturation to wilting point. A PV- curve analysis program (Schulte 1992) was used to define osmotic potential at saturation ($\psi_{\pi\text{full}}$), turgor loss point ($\psi_{\pi\text{TLP}}$), and relative water content at turgor loss point (RWC_{tlp}) (Schulte and Hinckley 1985). The change in osmotic potential at turgor loss point ($\Delta\psi_{\pi\text{TLP}}$) was calculated as:

$$\Delta\psi_{\pi\text{TLP}} = \psi_{\pi\text{TLP drought}} - \psi_{\pi\text{TLP control}}$$

Stable Carbon Isotope Analyses

For carbon isotope analysis, fully expanded current year needles from the upper part of terminal leader and branches were used. For most part, sampled needles were adjacent to needles used for gas exchange determination. Harvested needles were dried at 70 °C in a forced-air drying room. The dried needles were then finely ground using a grinder. Six replicates (ramets) from each clone in each water treatment were sampled for a total of 540 sampled rooted cuttings (6 blocks x 3 watering treatments x 30 clones x 1 ramet per clone).

Stable carbon isotope ratio ($\delta^{13}\text{C}$) of foliage was measured using gas mass spectrometer (Europa Scientific ANCA-SL Stable Isotope Analysis System, Europa Scientific, Crewe, U.K) at Cornell Boyce Thompson Institute Stable Isotope Laboratory.

The isotope composition of foliage relative to PeeDee Belemnite was expressed following (Farquhar *et al.* 1989):

$$\delta^{13} \text{ (‰)} = (R_{\text{sample}} / R_{\text{standard}}) - 1$$

where R_{sample} is the molar abundance ratio of ^{13}C to ^{12}C of the dried needle tissue, and R_{standard} is the ^{13}C to ^{12}C ratio of the international PDB (Pee Dee Belemnite) standard). Then Carbon isotope discrimination (Δ) was calculated as:

$$\Delta = (\delta^{13} \text{C}_{\text{air}} - \delta^{13} \text{C}_{\text{plant}}) / (1 + \delta^{13} \text{C}_{\text{plant}}/1000),$$

where $\delta^{13} \text{C}_{\text{air}}$ and $\delta^{13} \text{C}_{\text{needle}}$ are the isotope composition of the air taken to be -8.0 ‰ (Craig 1957) and needle tissue material, respectively.

Statistical Analyses

The analyses of variance for all traits were conducted for a split plot design with watering treatments as the whole plot and clones as subplots. A mixed linear model was used to estimate variance components for data pooled from all six blocks. The model included watering treatment, as fixed effects and all other terms as random effects (blocks, clones and all interactions). Statistical analysis was conducted with the MIXED procedure of SAS (SAS Institute Inc.1996). The LSMEANS option of the GLM procedure was used to calculate least-square means for clones in each watering treatment. Preliminary ANOVA indicated that rooting class had no significant effect on all traits measured, thus, it was dropped from the model for all the analyses reported here. As indicated in Table 2.1, some of the clones shared either female or male parents or both as a result the statistical model used in this study may not be adequate in quantifying the various variance components necessary to calculate the various genetic parameters.

Broad-Sense Heritability (H^2)

The mixed procedure of SAS (SAS Institute 1996) was used to estimate variance components for each trait with restricted maximum likelihood method, REML (Littell *et al.* 1996). Broad-sense heritability, H^2 , was calculated according to Falconer and Mackay (1989):

$$H^2 = \sigma^2_C / \sigma^2_P, \text{ where } \sigma^2_C = \text{total among clones genetic variance;}$$

$$\sigma^2_P = \text{total phenotypic variance which includes } \sigma^2_C \text{ (clonal variance), } \sigma^2_{CT} \text{ (clonal x treatment interaction variance), and } \sigma^2_e \text{ (error variance)}$$

Genetic Correlations: r_{B-TRT} and r_g

Type B genetic correlation (r_{B-TRT}), which measures clone x watering treatment interaction was estimated for each trait as follows (Yamada 1962, Burdon 1977):

$r_{B-TRT} = \sigma^2_C / (\sigma^2_C + \sigma^2_{C*TRT})$ where, σ^2_C = clonal variance component of the total phenotypic variance; σ^2_{C*TRT} = clone by watering treatment interaction variance.

The type-B genetic correlation was used to examine the stability of clonal performance under the three watering regimes. A value of r_{B-TRT} near one indicates negligible interaction between clone and watering treatment suggesting stable clonal ranking under non-water stressed and stressed conditions. On the other hand, a value substantially less than one, (say, $r_{B-TRT} \leq 0.70$), implies significant clone by watering treatment interaction suggesting clonal ranking changes in response to watering treatments.

A bivariate restricted maximum likelihood (REML) procedure was used to estimate genetic correlation (r_g) and co-variance components among pairs of traits. The

broad-sense genetic correlation between traits arises mainly from pleiotropy which means that a given gene locus influences the expression of multiple traits (Falconer and Mackay 1989). It is defined for the true clonal values for the two traits 1 and 2 as:

$$r_g = \text{COV}_{1,2} / (\sigma^2_{c1} \cdot \sigma^2_{c2})^{1/2}$$

where, r_g is the genetic correlation between trait 1 and 2, $\text{COV}_{1,2}$ is the genotypic covariance between the two traits, σ^2_{c1} , the clonal genetic variance of trait 1, and σ^2_{c2} the clonal genetic variance of trait 2.

Results and Discussion

Effects of Drought on Growth and Morphology of Clones

The effect of water stress on stem elongation was statistically significant when relative growth rate in height was measured for 2 periods spanning DT1 and DT2 (Table 2.2). Compared to height measurement of control plants, drought caused reduction in relative height growth, by 11 and 7 % at the end of the first (RGR1) and second (RGR2) drought cycles, respectively (Figure 2.1). Across measurement times, a wide range of clonal height variation ($P < 0.0001$) was detected (Table 2.2).

Total biomass accumulation was reduced significantly by water stress in all 30 clones (Table 2.2). At final harvest, reduction in total biomass accumulation was 17 and 11 % for DT1 and DT2, respectively compared to controls. Previous studies have also reported soil water deficit affecting biomass accumulation and growth for many woody species (Pereira and Pallardy 1989).

Biomass allocation to needles and roots exhibited significant differences among treatments (Table 2.2), but it was not clear if the difference were caused by drought. Percent stem fraction was consistently lower than percent needle fraction and accounted

for 25 %, 27%, and 29 % of the total biomass in control, DT1, and DT2 treatments, respectively, but differences were not significant (Table 2.2). Most clones tended to have a high proportion of aboveground biomass (65%) regardless of the treatment. Similar patterns of above ground component allocation to stem (28%) and needle (39%) were evident for clones in well watered and drought conditions. Although there is no previous work done specifically on drought response of clones of loblolly pine, observations in this study are in agreement with previous studies in which loblolly pine seedlings exhibited reduced root growth under limited soil environment (van Buijtenen 1976, Seiler and Johnson 1988). Similarly, Torreano and Morris (1998) reported lack of differences in biomass allocation among watering treatments of loblolly pine seedlings.

Deep and extensive root systems, which allow access to water deep in soil profile is considered an important morphological trait for plants in drier environments (Le Roux *et al.* 1996). The ability to produce a wide-spreading and prolific rooting system has been associated with water stress resistance in clones of *Populus* (Tschaplinski and Blake 1989).

Plants respond to shifts in resources supply by allocating carbon to the organ involved in capturing the limited resources (Dewar 1993). It has been suggested that high root to shoot ratio is a more desirable trait when selecting clones for drier environments (Grossnickle 2000). The result of this study also indicates a significant treatment effect in root: shoot dry matter ratio.

Clone-by-Watering Treatment Interaction

Clonal variation for all growth and biomass traits was highly significant and resulted in the largest source of variation (Table 2.3). The clone by water treatment interaction was small for root to shoot ratio and was of little importance. It must be noted,

however, that large inter-clonal differences existed even before the drought treatment was imposed. The non-significant ($P = 0.08$) interaction between clone and water stress treatment for root: shoot ratio suggests consistent clonal responses in biomass allocation pattern. The absence of the interaction can be explained partly by the short duration of the drought period. Longer drought periods may have revealed clonal differences in carbon allocation patterns.

Despite large clonal variations in height, repeated measure analysis did not reveal significant clone by watering treatment interaction (Table 2.2). The relative rankings of clones, as measured by height growth, were consistent when tested under well-watered and drought conditions, which may suggest that fast growing clones can be identified under both well-watered and stressed conditions.

Broad-Sense Heritability for Height and Biomass Components

Broad-sense heritability (H^2) estimates for incremental height were high, ranging from 0.55 to 0.64 in different measurement days (Figure 2.2). These broad sense heritability estimates are generally higher than previously reported values of non-clonal genetic materials (Megraw 1997, Hodge *et al.* 1999, Sierra-Lucero 1999) of loblolly pine. For total biomass, percent needle percent stem and percent root, estimated broad-sense heritabilities (H^2) were 0.37, 0.44, 0.35, 0.40, respectively (Table 2.4). These high heritability estimates may suggest that selection for increased biomass is possible among clones provided sufficient clonal variability for drought tolerance exists.

Because both the additive and non-additive genetic components are included in the computation of broad sense heritability, values are normally expected to be higher than narrow sense heritability (Becker 1992). Furthermore, the uniform greenhouse-growing environment under which the clones were growing reduced environmental

“noise”. Fehr (1987) suggested that any effort intended to reduce experimental noise or plant variability would certainly improve the estimate of heritability of traits. Height could be a useful trait to utilize in indirect selections for drought tolerance because it is easily recognizable and can be measured non-destructively.

High productivity environments usually permit greater genotypic expression allowing selection for a given trait to be more effective (Blum 1988). Estimates of H^2 under optimum and stressed conditions were similar suggesting that genetic expressions of morphological traits were not affected by drought. However, at this stage of the experiment, accepting or rejecting these estimates may be hasty, as there is no published estimate of H^2 for clonally propagated loblolly pine plant materials. As previously stated, there is evidence that loblolly pine has great genetic variability for drought tolerance across its range. Because the water stress treatment applied was rapid, the attempt was to obtain informative drought regulation mechanisms at organ and whole plant level. However, in this study, it appears that the drought period was not long enough to affect morpho-physiological mechanisms regulating drought responses. This was clearly observed with the quick resumption of growth after plants were re-watered for only 48 hrs.

In general, estimates of broad sense heritability for clones are fewer than narrow sense heritability primarily due to the relatively small number of tree species planted commercially as vegetative propagules (Shelbourne and Thulin 1974, Foster and Shaw 1987). Similar heritability values under stressed and non-stressed environments may also indicate equivalent selection advantage if selection for fast growing clones were made in either stressed and non-stressed conditions.

Type-B Genetic Correlation

Type-B genetic correlation can be defined as the genetic correlation for a trait measured under different growing environments (Yamada 1962). In this case, it measures the interaction between clone and the different watering treatments. For all morphological traits, type B genetic correlation was high (Table 2.4) ranging from 0.91 to 0.97 in line with the non-significant clone by watering treatment interaction. This may suggest the absence of unique clonal response to drought, or may be due to the short durations of the drought treatment. A type-B (r_{B-Tt}) genetic correlation near one indicates that interaction between clone and watering treatment is negligible, and so clonal ranking remains stable when measured under various watering regimes.

Type-A Genetic Correlation

Total biomass accumulation was significantly associated with height measurements taken across time regardless of the watering treatments. There was a high (0.80) positive genetic correlation between biomass and height, indicating close genetic association between the two traits (Table 2.5). Genetic correlation can result from pleiotropy which means that a given gene locus influences the expression of multiple traits (Mode and Robinson 1959). Although increased biomass may be an important trait under optimum soil water conditions, increased carbon allocation to below ground root biomass under drought condition is a more desirable morphological attribute.

Type-A genetic correlations between carbon isotope discrimination (Δ) with total aboveground biomass and height were - 0.86 and - 0.11, respectively (Table 2.5). The strong but negative genetic correlation between biomass accumulation and Δ suggest that differences in plant isotope composition is largely due to differences in physiological characteristics under varying soil moisture environment. The strong genetic correlation

between Δ and biomass accumulation suggests that the potential of using needle $\delta^{13}\text{C}$ for assisting in selection of clones better adapted varying soil moisture conditions. On the other hand, there was no significant correlation in the majority of the traits compared, particularly between growth and plant water relation traits, including Ψ_{pd} , and for all PV parameters suggesting the genetic bases of these traits is complex.

The Effects of Drought on Water Relation Parameters

Beginning on the second day, Ψ_{pd} values began to diverge from the controls and on day 5, they reached a mean of -1.75 MPa. Water stress significantly lowered predawn water potential (Ψ_{pd}) in both drought cycles (Figure 2.3). Throughout the experiment, clones in the control group maintained higher needle water potential, averaging -0.46 MPa. Clones in DT2 were subjected to a second drought cycle after being re-watered to a predetermined Ψ_{pd} value of -1.75 MPa. During the second drought cycle (DT2), predawn needle water potential on Day 4 was -1.55 MPa, higher by 0.20 MPa than in DT1. On Day 6, Ψ_{pd} reached -1.45 MPa, higher by 0.30 MPa.

During the recovery period, all clones returned to control Ψ_{pd} values 48 hrs after re-watering (data not shown). The ability to resume growth following relief of water stress is an important characteristic for early seedling establishment and growth in drought prone environments (Osorio *et al.* 1998). Significant clonal variation in predawn water potential was detected during both drought cycles (Table 2.5) and clonal variance was the second largest variance component of the total phenotypic variation. However, the clone x water treatment interaction for Ψ_{pd} was small and insignificant.

Pressure-Volume (P-V) Analyses

Significance values and LS means for bulk needle predawn water potential and pressure volume variables are summarized in Table 2.6. There were no significant differences in osmotic potential at full turgor (Π_{SAT}) or osmotic potential at turgor loss point (Π_{TLP}) between control and the drought cycle treatments.

Although differences were not significant, drought preconditioning tended to cause osmotic potential at full turgor (Π_{SAT}) and zero turgor (Π_{TLP}) to be lower (more negative) compared to clones in the control treatment (Table 2.2). The change in osmotic potential at saturation Π_{FULL} was significant for 13 clones out of the 30 clones tested ranging -0.1 to -0.48MPa (data not shown). Past research has also indicated that progressive drought cycles had no effect on shoot water relation parameters including Π_{FULL} , Π_{TLP} , and RWC_{TLP} for clones of interior spruce (*Picea glauca* x *Picea engelmannii*) (Fan and Grossnickle 1998). Relative water content at turgor loss point (RWC_{TLP}) was significantly different between treatments. By the end of the first drought treatment, relative water content at turgor loss point (RWC_{TLP}) reached 83.4%. On the other hand, RWC_{TLP} for well-watered clones was 80.1%. The maintenance of high RWC at lower water potentials is one means of avoiding desiccation (Abrams 1988). Maintenance of high relative water content at low water potential in both drought cycles could be the result of either a change in drought induced osmotic potential or cell wall extensibility or both.

Clonal variation in osmotic potential at full turgor (Π_{SAT}), osmotic potential at the turgor loss point (Π_{TLP}), and relative water content at turgor loss point (RWC_{TLP}) have been observed in *E. globulus* clones (Pitta and Pardos 2001). However, differences were

detected only when the clones were subjected to the mild stress level. It has been demonstrated that drought preconditioning could be important in enhancing osmotic adjustment in loblolly pine (Hennessey and Dougherty 1984, Bongarten Teskey 1987).

Previous studies have demonstrated that drought preconditioned seedlings of loblolly pine were able to osmotically adjust and continue physiological processes when subjected to drought (Hennessey and Dougherty 1984, Meier *et al.* 1992). Seiler and Johnson (1985) reported lower osmotic potential in loblolly pine seedlings that had been through repeated drought than in non-stressed seedlings. Furthermore, genotypic variation in osmotic adjustments has been detected in loblolly pine (Bongarten and Teskey 1987). This genetic variation could be important as a potential source of selection for dehydration tolerance.

This study finds no apparent osmotic adjustment (OA) among clones suggesting the absence of active solute accumulation in response to drought, although an increased osmotic potential at full saturation was evident in some clones. The lack of true OA for the clones could partly be explained by the short length of the drought period in this study. The rate and the frequency at which drought is imposed can also influence the osmotic potential component of the total water potential and hence the degree of osmotic adjustment (Turner *et al.* 1987). Rapid drought conditions (as is the case in most greenhouse drought studies) and root growth restriction due to smaller pot sizes are often mentioned for not allowing plants to adjust their osmotic potential enough for OA to occur (Fan and Grossnickle 1998).

Heritability and Clone-by-Watering Treatment Interaction for P-V Parameters

Analyses of variance revealed significant clonal variation in P-V parameters (Table 2.2), but there was no evidence for strong clone by treatment interaction for any of

the parameters. As a result, no important association among water relation parameters emerged. The negligible clone by watering treatments interaction indicates that clonal ranking remained consistent in all treatments. Estimated broad-sense heritability (H^2) for predawn water potential and derived P-V parameters were small (Figure 2-4). Small H^2 values may indicate limited importance of these traits as predictors of drought tolerance or may result from high experimental error of these traits. Measurements of water relation parameters such as P-V are prone to experimental errors due to operators, time of measurements and sampling period, which in turn increases the error variance. In line with the non-significant clone x watering treatment interaction, the type B genetic correlation for Ψ_{pd} (r_{B-TRT}) was high and close to one suggesting similar clonal drought responses irrespective of watering treatments (data not shown).

Stable Carbon Isotope Composition

The effect of water stress on carbon isotope composition ($\delta^{13}C$) of needles was significant (Table 2.2). Averaged over all clones, $\delta^{13}C$ in water stressed treatments were significantly higher (less discrimination, Δ) than in well-watered treatments with values -30.46, -29.92 and -29.70 ‰ for control, DT1 and DT2, respectively (Figure 2.5). The less negative needle $\delta^{13}C$ values of clones in the drought treatment was related to drought induced stomatal closure restricting gas exchange under severe drought conditions (Schultz 1986, Franks *et al.* 1997). A significant and negative genetic correlation was observed between $\delta^{13}C$ and total dry biomass accumulation (Table 2.5). The negative association indicated that faster growth was genetically associated with higher discrimination values.

Published works on growth traits and carbon isotopic composition for tree species under limited soil water are inconsistent, sometimes no correlation (MacFarlane and Adams 1998), or negatively correlated (Zhang *et al.* 1993, Flanagan and Johnsen 1995). The result of this study agrees with that found by (Bond and Stock 1990, Le Roux *et al.* 1996 and Pita *et al.* 2001) for Douglas fir, *Eucalyptus grandis*, *Eucalyptus grandis* x *Eucalyptus camaldulensis* in which it was found clones with superior growth had lower $\delta^{13}\text{C}$ values.

Clonal Variation in Stable Carbon Isotope Composition

There was a significant ($P = 0.0034$) variation in foliar $\delta^{13}\text{C}$ values among clones studied (Table 2.2). However, the clone x watering treatment interaction was not significant ($P = 0.2563$) with very minimal contribution of clone by treatment interaction variance (σ^2_{CT}) to the total phenotypic variance (data not shown). Genetic variation in leaf carbon isotope composition has been found in a number of tree species including *Pseudotsuga menziesii* (Mirb.) Franco (Zhang *et al.* 1993), *Picea glauca* (Monench) Voss (Sun *et al.* 1996) and for a hybrid of *Picea spp* (Fan and Grossnickle 1999).

Broad-Sense Heritability for Stable Carbon Isotope Composition

Broad-sense heritability (H^2) estimate for $\delta^{13}\text{C}$ was low (0.007) for clones in all three watering treatments (Table 2.4). Although the variance component due to clones was relatively higher (with the exception of the residual variance) than the other variance component, the broad sense heritability values for $\delta^{13}\text{C}$ remained low, which suggest that selection for drought tolerance using $\delta^{13}\text{C}$ may need more thorough understanding of the mechanism underlying genetic differences. The clone by watering treatment interaction for $\delta^{13}\text{C}$ was not significant and resulted in high (0.79) type-B genetic correlation (Table 2.4), which suggests consistent clonal ranking (performance) in both well watered and water stressed soil environment.

Conclusions

It was apparent the clone x watering treatment for all traits was not significant, which, to a great extent, is attributed to the short and duration of the drought period. In this study, the goal was to characterize growth and water relation traits that are important for conferring drought tolerance. Genetic parameter estimates of traits involved in drought tolerance can provide important information for future loblolly pine clonal studies. Future work should identify better and efficient methods of identification of drought regulating traits in loblolly pine clones under water stressed environments. Based on the study results the following conclusions are drawn:

1. Drought treatments reduced total biomass accumulation significantly in both drought cycles. Aboveground biomass comprised the largest fraction of the total dry matter in all three treatments. Drought accounted for 16 and 11 % reduction in total aboveground dry biomass in DT1 and DT2, respectively, compared to the control.
2. Clonal ranking on the basis of the measured growth traits remained the same across treatments. The non-significant interaction between clone and water stress treatment means that drought morphological responses of trees in control group was as much the same as in the stressed groups.
3. For all growth traits, the type-B genetic correlation was high, ranging from 0.91 to

- 0.97. The high r_{B-TT} suggests an absence of unique clonal response to drought, which suggests that clonal selection for growth under water-limited environment may be as effective as selection under optimum soil water environment.
4. Estimated broad-sense heritability for height and morphological traits were high. The values are higher than any of the previously reported narrow-sense heritability values. The high H^2 values found for all the morphological (growth and height) traits indicate more repeatable expression of additive and non-additive gene actions.
 5. Analyses of variance revealed significant clonal variation in P-V parameters, but there was no evidence indicating a clone by watering treatment interaction for any of the parameters. The negligible clone by watering treatments interactions for the parameter indicates the rankings were consistent. A practical interpretation of this non-significant interaction is that P-V parameters may not be useful in clonal selection programs.
 6. The stable carbon isotope composition ($\delta^{13}C$) of foliage sampled from the three watering treatments varied significantly with clones in the control treatment exhibiting lower $\delta^{13}C$. Clones under drought treatment discriminate ^{13}C significantly lower than clones in the control group. Negative correlation between total aboveground biomass and carbon isotope composition $\delta^{13}C$ was observed.
 7. Broad-sense heritability estimate for $\delta^{13}C$ was low (0.079) regardless of the watering treatment. The type-B genetic correlation for $\delta^{13}C$ was high (0.79) suggesting consistent clonal ranking in both well watered and water stressed soil environment.
 8. Estimated broad-sense heritability for osmotic potential at full saturation (Π_{100}), osmotic potential at turgor loss point (Π_{TLP}) and relative water content at turgor loss

point (RWC_{TLP}) were 0.07, 0.09 and 0.06, respectively. In general, water relation traits are subject to measurement error. As a result, estimates of H^2 can be less accurate because of the large experimental error associated. Lower heritability values indicate that the trait is not amenable to selection.

9. Consistent with non-significant clone x watering treatment interaction, the type-B genetic correlation (r_{B-TRT}) for P-V parameters were high and close, to one suggesting similar clonal drought responses irrespective of watering treatments. However, this generalization should be considered with caution, as there is no published similar work to validate the results.

Table 2.1. Geographical origins of superior parent hedges used to propagate the 30 clones used in the study.

Clone ID	Female parent	Source ¹	Male parent	Source ¹
40048	1	AC	2	FL
40191	1	AC	5	AC
40913	2	FL	18	AC
41224	3	FL	20	FL
41914	4	AC	10	AC
41927	4	AC	10	AC
41972	4	AC	10	AC
42212	5	AC	2	FL
42660	6	GC	21	GC
42836	4	AC	22	AC
43048	7	FL	2	FL
43115	8	AC	23	AC
43480	9	AC	24	AC
43523	10	AC	20	FL
43619	11	AC	1	AC
43892	11	AC	15	FL
44205	12	AC	15	FL
44621	4	AC	19	AC
45159	13	FL	25	FL
45325	14	FL	3	FL
45357	14	FL	3	FL
45883	9	AC	25	AC
46009	15	FL	2	FL
46017	15	FL	2	FL
46054	15	FL	2	FL
46101	16	FL	26	AC
46365	14	FL	9	AC
46440	17	FL	27	FL
46628	18	AC	4	AC
47007	19	AC	15	FL

Source¹ FL = Florida, AC = Atlantic Coastal, and GC = Georgia Coastal

Table 2.2. P-values of ANOVA on total biomass, percent needle, percent stem, percent root, root to shoot ratio, relative height (RGR1 and RGR2), (needle water potential (ψ_{pd}), relative water content at turgor loss point (RWC_{TLP}), osmotic potential at full saturation (Π_{SAT}), osmotic potential at turgor loss point (Π_{TLP}), and stable carbon isotope composition (^{13}C) for clones of loblolly pine under three watering treatments. RGR1 and RGR2 are relative growth height (cm) calculated as the difference in height before and after the first (DT1) and the second (DT2) droughts, respectively.

	Total biomass (g)	Percent needle (%)	Percent stem (%)	Percent root (%)	Root: shoot	RGR1 (height)	RGR2 (height)	ψ_{pd} (MPa)	RWC_{TLP} (%)	Π_{SAT} (MPa)	Π_{TLP} (MPa)	^{13}C composition (‰)
Block	0.0016	0.0923	0.0004	0.0007	0.0005	0.0008	0.0122	0.3672	0.4205	0.4052	0.3670	0.1181
Block*Treatment	0.3084	0.0036	0.0245	0.0167	0.0019	0.9327	0.8431	0.0001	0.0002	0.0123	0.0354	0.0001
Treatment	0.0001	0.0101	0.5666	0.0023	0.0019	0.0059	0.0171	0.0454	0.0158	0.2667	0.1120	0.0008
Clone	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0132	0.0009	0.0003	0.0103	0.0034
Clone*Treatment	0.0001	0.0416	0.0337	0.0967	0.0823	0.6163	0.5156	0.4496	0.9416	0.5764	0.1794	0.2563

Table 2.3. Variance component estimates (expressed as % of total for each trait) for growth traits of total biomass, percent needle, percent stem, percent root, root to shoot ratio, height at the end of the first (T6) and second drought (T7) cycles and stable carbon isotope composition ($\delta^{13}\text{C}$).

Source of variation	Total Biomass	Percent Needle	Percent Stem	Percent Root	Root to shoot	Height (T6)	Height (T7)	$\delta^{13}\text{C}$
Block	6.08	0.0	0.0	0.0	3.96	0.0	0.0	4.8
Block*Treatment	0.43	0.0	0.0	0.0	0.0	3.6	3.3	8.01
Clone	35.0	0.0	0.0	0.0	7.0	5.0	54.6	6.4
Clone*Treatment	2.90	43.5	41.3	36.2	33	0.0	0.0	1.7
Error	55.5	56.5	58.6	63.8	56	46.3	42.1	79

Table 2.4. Broad-sense heritability (H^2) and standard deviation (STD) estimates and type-B genetic correlation (STD) of loblolly pine clones for total biomass, percent needle, percent stem, percent root, root to shoot ratio and needle carbon isotope composition ($^{13}\delta C$).

Trait	H^2 (STD)	Type-B (STD)
Total Biomass	0.37 (0.071)	0.92 (0.063)
Percent needle	0.43 (0.073)	0.94 (0.050)
Percent stem	0.35 (0.069)	0.98 (0.061)
Percent root	0.39 (0.072)	0.94 (0.056)
Root: shoot ratio	0.39 (0.072)	0.94 (0.055)
$^{13}\delta C$ composition	0.079 (0.124)	0.79 (0.09)

Table 2.5. Type-A genetic correlations among total aboveground biomass (g), height (at final harvest (cm), predawn water potential (ψ_{pd}) and needle stable carbon isotope composition ($\delta^{13}C$) for clonally propagated loblolly pines.

	Above ground biomass	Height	Predawn water potential	$\delta^{13}C$
Aboveground biomass	-	0.80	- 0.04	- 0.86
Height		-	0.09	- 0.11
Predawn water potential			-	-
$\delta^{13}C$				-

Table 2.6. Mean osmotic potential at full turgor (Π_{SAT}) and turgor loss (Π_{TLP}) points and relative water content at turgor loss point (RWC_{TLP}) of clones of loblolly pine in control, drought 1(DT1) and drought 2 (DT2) cycles.

Treatment	Π_{SAT} (MPa)	Π_{TLP} (MPa)	RWC_{TLP} (%)
Control	-2.16(0.027) ¹	-1.62(0.022)	80(0.004)
Drought 1(DT1)	-2.28(0.036)	-1.70(0.029)	84(0.055)
Drought 2(DT2)	-2.29(0.027)	-1.69(0.022)	84(0.004)

¹ Mean values (n=6) are given with standard errors in parenthesis.

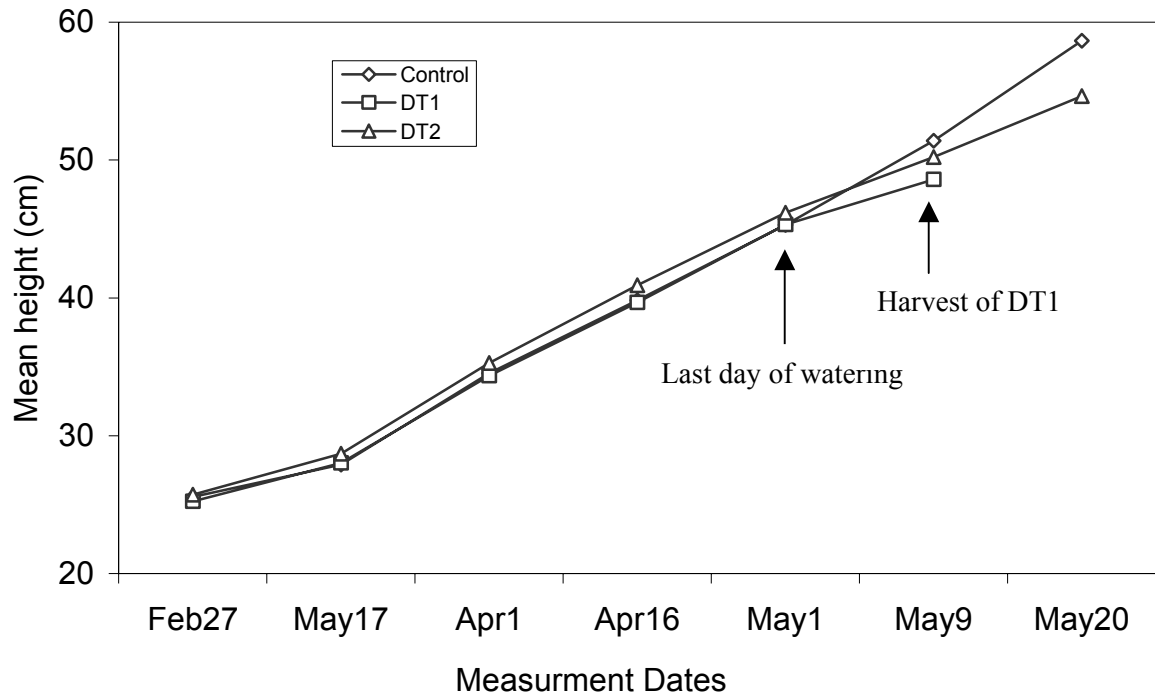


Figure 2.1 Trends of height growth of clones across time before (Feb 27- May 1) and after (May 9-May 20) drought. Height was measured at time T1, T2, T3, T4, T5, T6, and T7 corresponding to each measurement date. Differences for height were not significantly different for measurement periods before drought (T1 to T5). Relative growth height, (RGR1 and RGR2) calculated as relative height growth differences before and after drought, were significantly different at $P = 0.0059$ and $P = 0.0171$ during the first (DT1) and second (DT2) droughts, respectively.

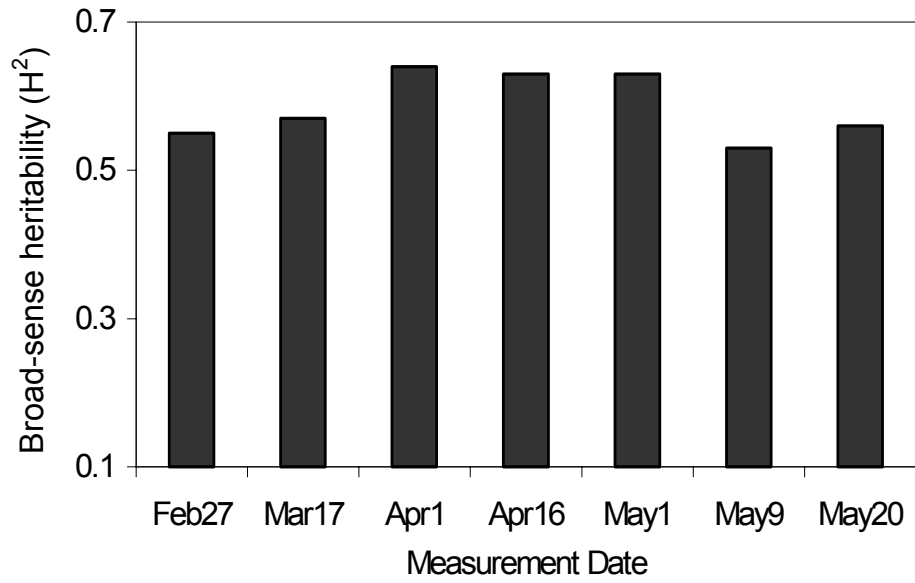


Figure 2.2. Broad-sense heritability (H^2) estimates for incremental height before (Feb27- May1) and after (May9- May 20) drought treatments.

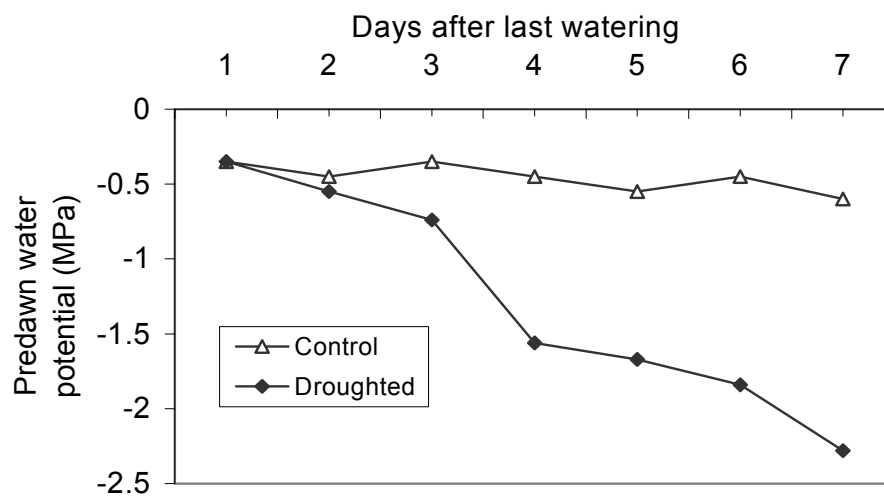


Figure 2.3. Changes in needle predawn water potential (Ψ_{pd}) across time for clones of loblolly pine subjected to rapid water stress treatments.

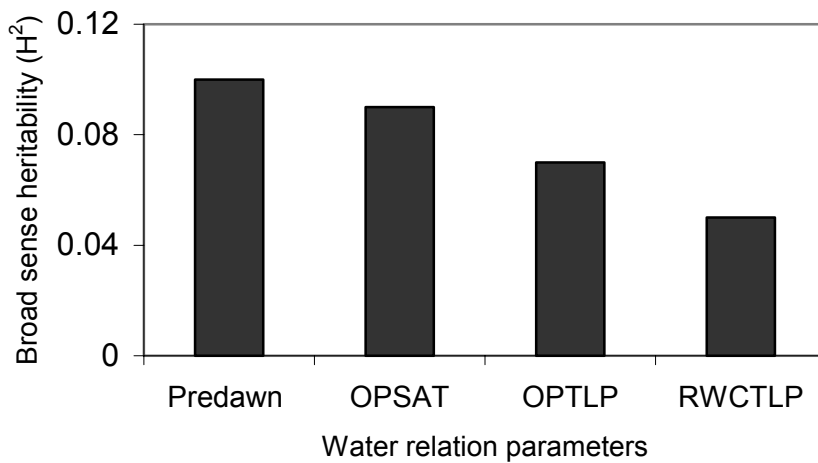


Figure 2.4. Broad-sense heritability (H^2) estimates for water relation parameters of predawn water potential, osmotic potential at saturation (OPSAT), osmotic potential at turgor loss point (OPTLP), and relative water content at turgor loss point (RWCTLP).

CHAPTER 3
STABLE CARBON ISOTOPE COMPOSITION AND STOMATAL LIMITATION TO
PHOTOSYNTHESIS OF CLONES OF LOBLOLLY PINE (*PINUS TAEDA* L.) UNDER
LIMITED SOIL WATER SUPPLY

Introduction

Water stress inhibits plant growth by disrupting physiological processes through reductions in stomatal conductance that results in limitations of carbon fixation (Hsiao 1973, Turner and Kramer 1980, Jones 1992). Plants can tolerate or avoid the onset of cellular dehydration using a range of physiological strategies. One of the most important desiccation-avoidance mechanisms is stomatal closure (Levitt 1980, Kozłowski *et al.* 1991). Although stomatal closure is one of the earliest responses to soil drying in preventing water loss from plant surfaces, it is also the main cause for drought-induced reduction in photosynthesis (Salisbury and Ross 1991, Davies and Zhang 1991 Kramer and Boyer 1995).

Because of the central role stomata play in the exchange of CO₂ and water vapor, emphasis is put on stomatal conductance and its impact on gas exchange in response to water stress (Seiler 1985, van den Driessche 1991). A series of physical and biochemical processes are involved in transferring CO₂ from surrounding air to the intercellular space. Stomatal conductance and leaf water potential are highly related and plant species display different patterns of stomatal response to decreasing leaf water potential. These responses to water stress depend on a number of genetic and environmental factors (Pezeshki and Chambers 1986, Sala and Tenhunen 1994). Stomata respond to soil water depletion in

advance of measurable change of leaf water status (Blackman and Davies 1985), but the precise mechanisms controlling the processes are not clearly understood (Pallardy *et al.* 1995, Bray 1997).

Native to the southeastern United States, loblolly pine (*Pinus taeda* L.) is the most extensively planted and important commercial timber species in the southeastern USA (Borders and Harrison 1989). Across its range, seasonal and year-to-year fluctuation in soil water availability may limit net CO₂ assimilation rate (Teskey *et al.* 1987). During the growing season, drought can have significant impacts on cumulative seasonal CO₂ assimilation (Ellsworth 2000). Thus, it may be desirable to select loblolly pine genotypes that can maximize carbon gain relative to the amount of water lost.

Genetic variation in physiological mechanisms related to drought tolerance has been observed in several tree species including loblolly pine (Bongarten and Teskey 1987, Teskey *et al.* 1987, Seiler and Johnson 1988), interior spruce (*Picea glauca* (Moench) Voss x *Picea englemannii* Parry ex Engelm (Grossnickle and Fan 1998), and ponderosa pine (*Pinus ponderosa* Dougl. Ex Laws) (Cregg 1993, 1994). This variation, for the most part, can be viewed as genetically controlled variation in either stomatal control or photosynthesis rates under water stress.

Gas exchange techniques can provide useful information on the control of photosynthesis by stomata and intercellular processes (Jones 1992) and various approaches have been used to quantify the extent of stomatal, as well as non-stomatal, limitations (Jones 1985). Limitations to photosynthesis are mainly due to decreased diffusion of carbon dioxide to the intercellular leaf space controlled by stomatal conductance or other biochemical processes (Wilson *et al.* 2000).

Physiological processes such as photosynthesis and stomatal conductance are phenotypic traits (Kramer 1986) that can be selected for in tree breeding programs. Previous studies have demonstrated the use of physiological measurements for selection of drought tolerance clones (Fan and Grossnickle 1998) or in other early clonal screening trails (Ceulemans and Impens 1983). Clonal variation in physiological traits expressed under water stress is an important component of drought tolerance and these differences largely are genetic differences in drought adaptation in some species (Cregg 1993).

Gas exchange techniques can rapidly assess the relationships between atmospheric CO₂ uptake and water loss. However, these values may not reflect integrated gas exchange responses over a growing period. Carbon isotope ($\delta^{13}\text{C}$) measurements provide an integrated measure of water use efficiency of plants (Ehleringer *et al.* 1993, Zhang *et al.* 1993). During photosynthesis, plants discriminate against the heavier ¹³C relative to ¹²C (O' Leary 1988). As a result, the ratio of ¹³C to ¹²C in plant tissue is lower than the ratio in the atmosphere.

Because carbon isotope ratio (¹³C/¹²C) is a time integrated average of the ratio of C_i/C_a , it provides information about the physiological activity of leaf tissue over its growing period. For plants, the isotope effect is due to the rapid diffusion of ¹²CO₂ into the mesophyll of leaves and the more rapid carboxylation action of Rubisco of ¹²CO₂ relative to ¹³CO₂ (Farquhar *et al.* 1982, Farquhar *et al.* 1989).

The primary objective of this study was to examine the effect of severe moisture stress on loblolly pine clones using water relations, gas exchange and stable carbon composition measurements. Specifically, the study had the following objectives:

1. To determine the relationship between needle water potential (Ψ_{needle}) and light-saturated photosynthetic rate and stomatal conductance for clones of loblolly pine,

2. To quantify the amount of drought-induced stomatal limitation (L_s) to light-saturated CO_2 assimilation rate, and
3. To determine how carbon isotope composition of foliage collected from droughted clones varies from well-watered clones.

Materials and Methods

Plant Genetic Material

The genetic material was obtained from the Loblolly Pine Lower Gulf Elite Population (LPLGEP). Parents were selected from a mix of tested first and second-generation populations in three different provenances, namely, Atlantic Coastal Plain, Georgia Coastal and the Florida sources (Table 2.1). For intensive physiological study, rooted cuttings from a subset ($n=12$, four from each rooting class as described in chapter 2) of clones were selected from the 30 clones on the basis of a range in height growth in the greenhouse.

Greenhouse Culture for Seedling Hedge Production

In January 2001, seeds from 70 full-sib families were hand sown. The culture and greenhouse environmental requirements for seedling growth are as described in chapter 2. Briefly, in October 2000, the seedlings ($n=2100$) were transplanted into 11.5-liter plastic pots until seedling hedges were established. Then, in May 2001, cuttings were harvested from the hedges. A total of 33,600 cuttings, arranged in a randomized block design, were set for rooting in a double-shell polyethylene covered greenhouse maintained at 29/31°C max/min temperature and 75% RH. Nine weeks later, cuttings were assessed for rooting.

In August 2001, a total of 540 cuttings (30 clones x 18 ramets/clones) were transported to a greenhouse in Gainesville, FL. Two days later, the cuttings were potted

in a sand medium, maintained in a greenhouse with ambient light. Immediately after transplanting, the cuttings were arranged in randomized complete block (n=6), for a split-plot design watering treatments as whole plot and clones as subplots.

Beginning on May 1, 2002, three levels of water treatments were applied. The three watering treatments, (i.e., single uninterrupted water stress treatment (DT1), two cycles drought treatment (DT2) and well watered) were randomly assigned within each block (18 whole plots = 3 watering treatments x 6 blocks). Each treatment was replicated six times requiring 180 plants.

Needle Gas-Exchange Measurements

Light-saturated photosynthesis (A_{\max}) rates were measured on the 4th day of drought on a subset of clones (n=12) in both well-watered and water-stressed treatments using a portable photosynthesis open-flow gas exchange system (Li-6400, LiCor Inc., Lincoln, Nebraska, USA). Partial light-saturated A-C_i curves were generated at a photosynthetic photon flux density (PPFD) of 2000 $\mu\text{ mol m}^{-2} \text{ s}^{-1}$.

Reference carbon dioxide was set at five concentration points: 225, 300, 370, 400 and 450 ppm. The first measurement was taken at external CO₂ of 370 ppm. Then, the concentration was lowered in a step-wise fashion to 300 and 225 ppm and finally increased to 400 and 450 ppm. Before each measurement, the initial CO₂ was sustained in the leaf chamber for 3-4 minutes. Three fully expanded current year fascicles (9 needles) in the upper third of the terminal leader were used for measurement. Light and carbon dioxide were provided by internally mounted LED light source (Li-Cor 6400-02B) and CO₂ injector, respectively. Throughout measurements, block temperature inside the

cuvette was maintained at 25 °C with a constant airflow rate of 200 mol sec⁻¹. A_{\max} , g_{\max} and C_i were calculated according to von Caemmerer and Farquhar (1981).

All A-C_i data points were recomputed on a total surface needle area basis. For area determination, needle radius was measured using an optical micrometer and total surface area was computed using the formula (Grace 1987):

$$\text{Surface area (in cm}^2\text{)} = 2rl (\pi + n) / 100,$$

where:

r = average needle radius (mm)

l = length of needle in cuvette (mm)

n = number of needles in the fascicle ($n=3$)

Stomatal Limitation (L_s)

Jones (1985) outlined several methods for utilizing measurements of the A-C_i function to calculate the relative limitation to photosynthesis by stomatal and non-stomatal factors. Jones' (1985) Method V was used:

$$L_s = \frac{r_g}{r_g + r^*}$$

where L_s is the relative stomatal limitation to photosynthesis, r_g is the total gas phase (stomatal plus boundary layer) resistance, r^* is the mesophyll or non-stomatal resistance. r_g was calculated from A_{\max} , the photosynthesis measurement at the operating point ($C_a = 370 \mu \text{ mol mol}^{-1}$) as:

$$r_g = \frac{C_i - C_a}{-A_{\max}}$$

r^* was calculated as the reciprocal of the slope of the line tangent to the A-C_i curve at the operating point. This slope was obtained by linear regression through the five net photosynthesis measurements taken at the closely spaced values of C_i (Figure 3.1). The calculation of stomatal limitation assumes negligible cuticular conductance and absence of stomatal patchiness, both of which result in overestimation of C_i (Ni and Pallardy 1992).

Stable Carbon Isotope Composition

For carbon isotope analysis, fully expanded current year needles from the upper part of terminal leader and branches were used. For the most part, sampled needles were adjacent to needles used for A-C_i determination. Harvested needles were dried at 70 °C in a forced-air drying room. The dried needles were then finely ground using a coffee grinder. Six replicates (ramets) from each clone in each water treatment were sampled, one from each block.

Stable carbon isotope composition ($\delta^{13}\text{C}$) of needle was measured using a gas mass spectrometer (Europa Scientific ANCA-SL Stable Isotope Analysis System, Europa Scientific, Crewe, U.K) at Cornell Boyce Thompson Institute Stable Isotope Laboratory. The isotope composition of foliage relative to PeeDee Belemnite was expressed following Farquhar *et al.* (1989):

$$\delta^{13} \text{ (‰)} = (R_{\text{sample}} / R_{\text{standard}}) - 1$$

where R_{sample} is the molar abundance ratio of ^{13}C to ^{12}C of the dried needle tissue, and R_{standard} is the ^{13}C to ^{12}C ratio of the international PDB (Pee Dee Belemnite) standard.

The carbon isotope discrimination (Δ) was calculated from the stable carbon composition as (Farquhar *et al.* 1989):

$$\Delta = \frac{\delta_{air} - \delta_{plant}}{1 + \delta_{plant} / 1000}$$

where $\delta^{13}C_{air}$ and $\delta^{13}C_{needle}$ are the isotope composition of the air taken to be -8.0 ‰ (Craig 1957) and needle tissue material, respectively.

Statistical Analyses

The analyses of variance (ANOVA) for all gas exchange parameters (A_{max} , g_{max} , C_i and L_s) and stable carbon isotope composition were evaluated for a split-plot design, with three watering treatments as the whole plots and clones as subplots. Significant levels for the fixed effects of clones and watering treatments were obtained from PROC MIXED procedure of SAS using LSDMEAS option (SAS 1996). The F-tests were used to determine if significant differences existed among clones and among watering treatments.

Results and Discussion

Maximum Net assimilation (A_{max}) and Stomatal Conductance (g_{max})

Drought resulted in significant reductions in A_{max} and g_{max} (Table 3.1). Averaged over all clones, A_{max} was greater in well-watered than in the drought treatments with mean rates of $2.17 \mu\text{ mol m}^{-2} \text{ s}^{-1}$ in the well-watered and 0.79 and $0.65 \mu\text{ mol m}^{-2} \text{ s}^{-1}$ on the 4th day of the first and second drought cycles, respectively.

Similarly, drought lowered g_{max} significantly in both drought treatments. Mean g_{max} for well-watered clones was $56 \text{ mmol m}^{-2} \text{ s}^{-1}$. As drought progressed, g_{max} decreased to 9.6 and $9.0 \text{ mmol m}^{-2} \text{ s}^{-1}$ on the 4th day of the first and second drought cycles, respectively (Table 3.1). The decline in g_{max} was expected as A_{max} and g_{max} usually decrease simultaneously in response to drought. Stomatal closure is often considered as the primary physiological response to drought (Kubiske *et al.* 1996).

Rapid decline in stomatal conductance was observed below needle Ψ_{pd} of -1.25MPa. Light-saturated stomatal conductance (g_{max}) tended to decline with decreased Ψ_{pd} (Figure B.2.). In loblolly pine, stomata tend to close at needle water potential threshold value of -1.0 MPa with complete closure occurring at approximately -2.0 MPa xylem water potential (Teskey and Hinckley 1986).

Average values of needle Ψ_{pd} of clones were lower in both drought cycles (-1.36 and -1.35MPa) than in the well-watered treatments (-0.57MPa). The relationship between the initiation of stomatal closure and water potential is important in determining how plants respond to drought. Regression of Ψ_{pd} on stomatal conductance is one method of estimating the degree of stomatal control (Jones 1992), although this study did not find strong correlation between the two variables (Figure B2.)

The response of A_{max} and g_{max} followed the same trends in both drought cycles (Figure 3.2). Both variables declined in response to the second drought treatment suggesting increased sensitivity to drought, rather than an acclimation response treatment. This suggests that drought preconditioning had no role in enhancing or maintaining A_{max} . The lack of photosynthetic acclimation in this study could be due to the rapid and short duration nature of drought treatment. Previous studies have reported increased capability of photosynthesis at lower Ψ_{pd} under subsequent droughts for clones of hybrids of *Picea glauca* (Monench) Voss x *Picea engelmannii* Parry ex Engelm (Fan and Grossnickle 1998). Photosynthetic acclimation was also observed for black spruce seedlings exposed to repeated droughts (Zine El Abidine *et al.* 1994b).

In this study, Ψ_{pd} of drought treated clones returned to their pre-drought values in only 24 hrs after re-watering; however, photosynthesis of recovered clones was slow and

did not reach control values at the same rate as in Ψ_{pd} (data not shown). This indicates lower dehydration tolerance, probably due to damage to the photosynthetic apparatus (Pallardy *et al.* 1995, Stewart *et al.* 1995).

In a related study, P-V analyses indicated that Ψ_{π} at the turgor loss point did not decline after exposure to two drought cycles for most clones, indicating lack of drought-induced osmoregulation (Chapter 2). Higher pressure potential (Ψ_p) and lower osmotic potential at turgor loss and saturated points are indicative of drought hardening processes. It is possible that lack of differences in osmotic adjustment could partly explain the lack of significant clonal differences in gas exchange parameters.

Intercellular CO_2 concentrations (C_i) were lower for plants in drought treatments (Table 3.1). Mean C_i was $280 \mu \text{molmol}^{-1}$ for well-watered clones, while drought caused C_i to decline to 233 and $240 \mu \text{molmol}^{-1}$ in DT1 and DT2, respectively. As stomatal conductance declined, the limitation to CO_2 diffusion increased, causing C_i to decline, suggesting that net photosynthesis was inhibited by drought induced-stomatal closure. If C_i remained unchanged during drought, it would indicate that stomatal and non-stomatal limitations were equally important in affecting photosynthesis (Ni and Pallardy 1992). With drought, stomata impose a diffusional resistance to the entry of CO_2 into the intercellular space, thereby reducing C_i . This suggests that photosynthetic rates of loblolly pine clones were closely related to stomatal behavior during drought periods.

Clonal Differences in A_{max} and g_{max}

Differences in A_{max} and g_{max} among the clones tested were not significant (Table 3.1). Likewise, the interaction between clone and watering treatment was not significant

for A_{\max} and g_{\max} . However, notable differences were observed in gas exchange variables among a few clones, and deserve some explanation.

In the well-watered condition, clones 41972, 42660 and 43048 maintained the highest A_{\max} ($\geq 2.5 \mu \text{ mol m}^{-2} \text{ s}^{-1}$) of the 12 clones studied (Figure B.4). These clones also exhibited increased above ground biomass accumulation and an extensive root system under the well-watered condition suggesting a strong relationship between growth and gas exchange rates. On mesic sites, these clones may be preferred for planting, because they may have competitive advantage over other clones as a result of increased carbon gain when conditions are favorable for growth (Zhang *et al.* 1997). Previous studies attempting to relate photosynthetic rate and growth have resulted in conflicting results of negative (Samuleson and Seiler 1994) and positive (Ceulemans and Impens 1993) correlations between A_{\max} and growth. For example, Major and Jonhsen (1996) found strong positive correlation between A_{\max} and growth rate for two full-sib families of black spruce. Similar results were also found in populations of conifer species where increased biomass accumulation was observed when water was not limited (Zhang *et al.* 1996). Genetic differences in drought tolerance could arise from a number of factors, including genetic characteristics (Kubiske and Abrams 1992) and pre-existing environmental conditions (Seiler and Johnson 1988, Maier *et al.* 1992).

Despite its superior performance in well-watered conditions, clone 41972 was sensitive to water stress (Figure B.3). As a result, it had the lowest mean Ψ_{pd} of -1.58 MPa. In fact this clone exhibited signs of reversible mid-day wilting in well-watered conditions. This may be due to the extensive root system of this clone capable of exhausting the available soil water quickly. Although this study was not intended to test

xylem cavitation, its occurrence under severe soil water stress and acute daytime vapor pressure deficit is a possibility. In general, plants growing in sand operate at higher water potential (Hacke *et al.* 2000) and for fast growing plants under such conditions; there is a greater risk of xylem cavitation. The low A_{\max} value observed for clone 41972 may suggest damage to xylem conducting elements or the photosynthetic apparatus during drought (Kirschbaum 1987).

On the other hand, clones 45325 and 45357 maintained high A_{\max} values after two cycles of drought (Figure B.4). Consistent with high A_{\max} , these clones also maintained high g_{\max} as evidenced by a steeper slope in the linear region of the A- C_i curve (data not shown), which suggests greater photosynthetic capacity for a given C_i . These clones could be more appropriate for planting on sites prone to frequent and mild drought.

The ability to recover rapidly from drought could be an important drought tolerance characteristic when considering planting clones in drier areas (Fan and Grossnickle 1998). When water becomes limited, stomatal conductance decreases because of stomatal closure. But plants that use water efficiently under drying soil are more likely to survive and grow relatively better than drought sensitive genotypes (Wright *et al.* 1993).

Stomatal Limitation to Light-Saturated Net Photosynthesis

Stomatal limitation (L_s) occurred even for plants growing in optimal conditions (Figure 3.3). Averaged over all clones, L_s in control plants was 25%, which is within the range of 20 to 30 % reported for loblolly pine under optimum conditions (Teskey *et al.* 1986). Drought significantly increased stomatal limitation to 45 and 38% in the first and second drought cycles, respectively (Table 3.1). Because A- C_i was measured in the linear

portion of the curve, the reduction is primarily due to drought-induced stomatal closure. As drought increased, net assimilation decreased with decreasing C_i .

Net photosynthesis is not only limited by stomatal closure, but by non-stomatal factors as well. In this study non-stomatal limitations ($1-L_s$) were high. The results of this study also support previous findings (Teskey *et al.* 1986) that, in loblolly pine, stomatal limitation under drought is less than non-stomatal limitation.

Previous studies on a range of forest tree species have also indicated the occurrence of non-stomatal limitations to photosynthesis (Teskey *et al.* 1986, Ni and Pallardy 1992, Kubiske and Abrams 1992). In those studies it was reported that water deficit affected photosynthesis mainly through a direct effect on mesophyll cells rather than on stomatal conductance for CO_2 , suggesting a higher role of non-stomatal limitations. For example, Stewart *et al.* (1995) reported that non-stomatal limitation to photosynthesis became increasingly more important than stomatal limitations for black spruce seedling treated with repeated drought cycles. The increase in mesophyll limitation with increasing water stress has been described as an increase in resistance of mesophyll to CO_2 diffusion (Beadle and Jarvis 1977).

Stable Carbon Isotope Composition

Carbon isotope composition ($\delta^{13}C$) of clones was strongly influenced by availability of water (Table 3.1). Compared to well-watered clones, needle $\delta^{13}C$ increased by 18 and 24 ‰ during the first and second drought cycles, respectively (Table 3.1), suggesting higher discrimination against ^{13}C by plants in the well-watered conditions. These results agree with previous greenhouse experiments on a number of

conifer species showing similar enrichment in ^{13}C in needles with increase in drought (Zhang and Marshall 1995, Picon *et al.* 1996, Zhang *et al.* 1997).

Averaged over all 12 clones examined, mean $\delta^{13}\text{C}$ was negatively related to both A_{max} and g_{max} (Figure 3.4a and b) and the associations were described by a linear function. Less negative $\delta^{13}\text{C}$ composition of tissue can be either the result of stomatal closure or high photosynthesis rate (Farquhar *et al.* 1989). The less negative needle ^{13}C for plants growing under water stress condition could be explained to a large extent by stomatal closure (Zhang and Marshall 1995, Flanagan and Johnsen 1995, Sun *et al.* 1996). As drought intensifies, stomatal closure restricts CO_2 diffusion into the intercellular space, resulting in lowered C_i/C_a (Figure 3.5). Further decrease in stomatal conductance increases the relative concentration of $^{13}\text{CO}_2$, thereby reducing the ability of Rubisco to discriminate against $^{13}\text{CO}_2$. This resulted in decreased discrimination (Δ), as water deficit increased.

Clonal Differences in $\delta^{13}\text{C}$

Clonal differences in isotopic carbon composition were detected ($P = 0.0204$). The clone x watering treatment interaction was not significant ($P = 0.4794$) for $\delta^{13}\text{C}$, as was true for gas exchange parameters (Table 3.1), suggesting that clonal ranking for $\delta^{13}\text{C}$ did not change with drought treatment. In well-watered plants, values of $\delta^{13}\text{C}$ ranged from a low of -30.79‰ for clone 44205 to a high of -30.09‰ for clone 45357 (Figure B 5.), with the largest difference in foliar $\delta^{13}\text{C}$ in the treatments among the 12 clones being 0.7‰ . Significant genetic (clonal) differences in isotopic discrimination have also been found in other tree species (Olbrich *et al.* 1993, Aitken *et al.* 1995, Flanagan and Johnsen 1995 and Prasalova *et al.* 2000). Greater discrimination for ^{13}C values of some clones,

such as 41972, supports the hypothesis that genotypes with greater stomatal conductances are more prone to drought when soil water is limited, as evidenced by the low ψ_{pd} and A_{max} (Figures B3 and B4).

Conclusion

1. The results of this study showed that, under water stress, the relative limitation of net CO_2 assimilation by stomata increased from 25 % to 38-45%. These calculated values of stomatal limitations for clones of loblolly pine are within the range suggested for stomatal factors.
2. The results of the study showed that preconditioned clones did not exhibit acclimation to photosynthesis during the relatively rapid drought treatments.
3. Because all clones of loblolly pine maintained positive light-saturated photosynthetic rate (A_{max}) under drought (although differences in were not significant), selection for increased drought tolerance may be possible by identifying clones with superior photosynthetic capability. Similarly, there were no drought-regulated changes in osmotic potential or carbon allocation patterns that can account for differences in either A_{max} and g_{max} (Chapter 2).
4. Although significant clone x watering treatment was not detected, some important growth performance differences were observed. Clone 41972 seemed to be the most promising clone for sites with optimum soil water, while clones 43048 and 42660, appeared to be drought tolerant clones.
5. Carbon isotope composition ($\delta^{13}C$) of clones was influenced by availability of water. Compared to well-watered clones, needle carbon isotope composition $\delta^{13}C$ of needles increased by 18 and 24 ‰ during the first and second drought cycles, respectively

suggesting higher discrimination against ^{13}C by clones in well-watered condition.

Clonal differences in isotopic carbon composition were detected ($P = 0.0034$).

However, the clone x watering treatment interaction was not significant ($P = 0.2563$).

Table 3.1. Mean values (standard errors) for light saturated net photosynthesis rate (A_{\max}), stomatal conductance (g_{\max}), stomatal limitation (L_s), predawn needle water potential (Ψ_{pd}) and stable carbon isotope composition ($\delta^{13}C$). Lower panel contains P-values for watering treatment, clone by treatment interaction for 12 clones of loblolly pine.

Treatment	A_{\max} ($\mu \text{ mol m}^{-2} \text{ s}^{-1}$)	g_{\max} ($\text{mol m}^{-2} \text{ s}^{-1}$)	L_s (%)	C_i ($\mu \text{ MOL MOL}^{-1}$)	Ψ_{pd} (MPA)	$\delta^{13}C$ (‰)
Well-watered	2.17(0.077)	0.056(0.002)	0.25(0.014)	280(9.8)	-0.54(0.016)	-30.52 (0.1246)
DT1	0.79(0.078)	0.0093(0.002)	0.45(0.012)	233(11.2)	-1.37(0.030)	-30.06 (0.1246)
DT2	0.65(0.071)	0.0099(0.006)	0.38(0.01)	240(8.5)	-1.36(0.038)	-29.76 (0.1246)
P-Values						
Treatment	0.0007	0.0003	0.0197	0.0359	0.0001	0.0008
Clone	0.2405	0.4953	0.3992	0.1723	0.8395	0.0034
Clone*Treatment	0.2367	0.0304	0.0001	0.2024	0.4322	0.2563

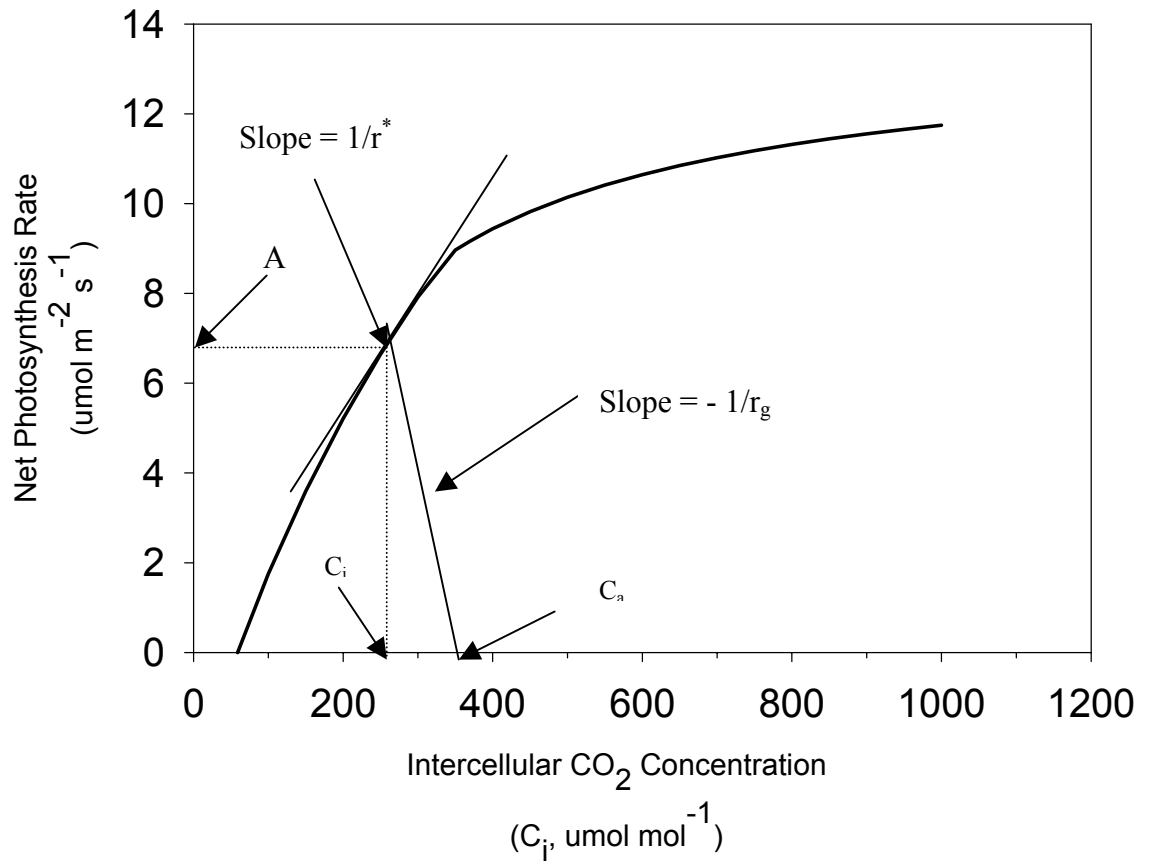


Figure 3.1. Illustration of the use of A-C_i curve to estimate gas phase limitation to net photosynthesis using Method V of Jones (1985). The slope of the demand and supply functions are expressed as $(1/r^*)$ and $(-1/r_g)$, respectively. C_a and C_i are the ambient and intercellular CO₂ concentrations, respectively. A is net photosynthetic rate in $\mu\text{mol m}^{-2} \text{s}^{-1}$.

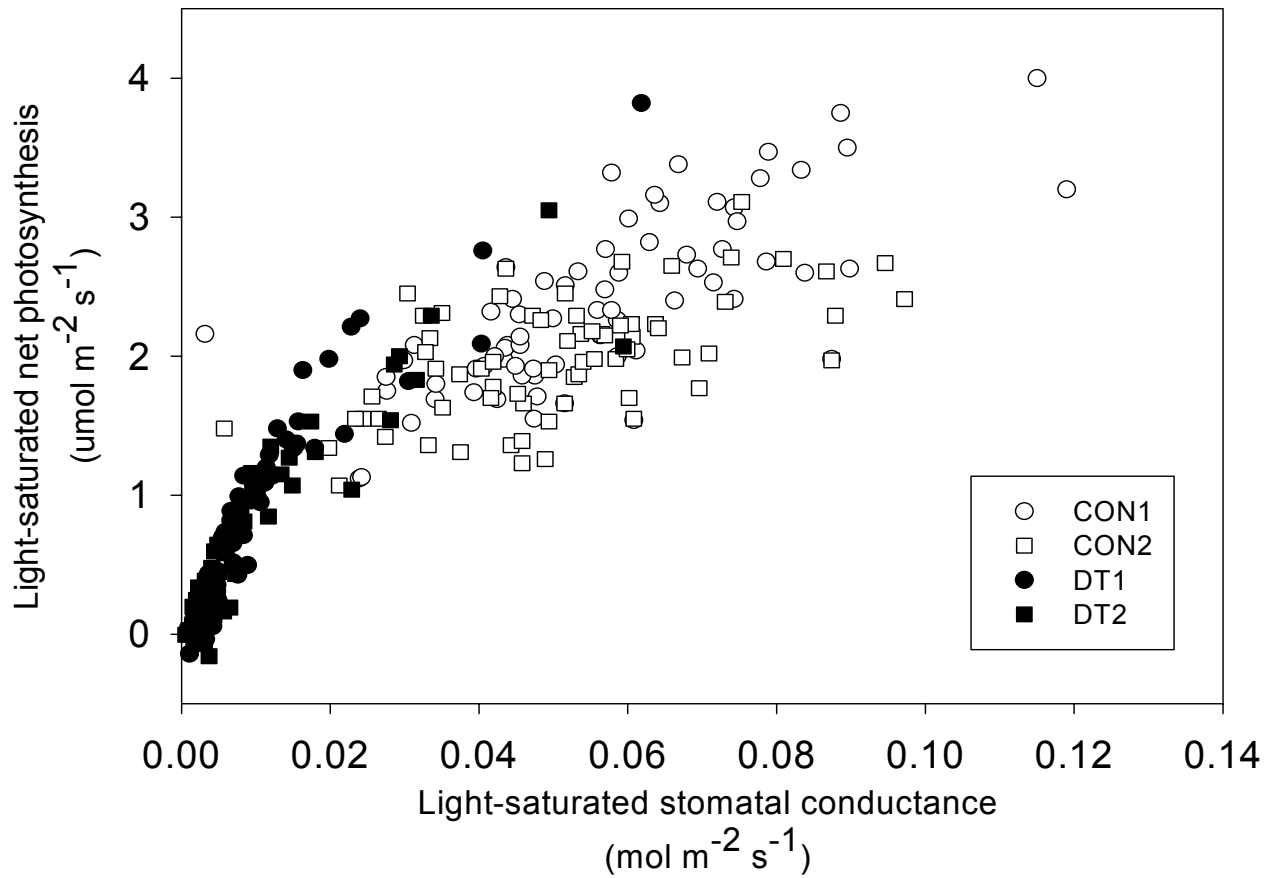


Figure 3.2 Light-saturated net assimilation rate (A_{\max}) as a function of light-saturated stomatal conductance (g_{\max}) of single point measurements of plants under well-watered (open) and water-stressed (solid) treatments.

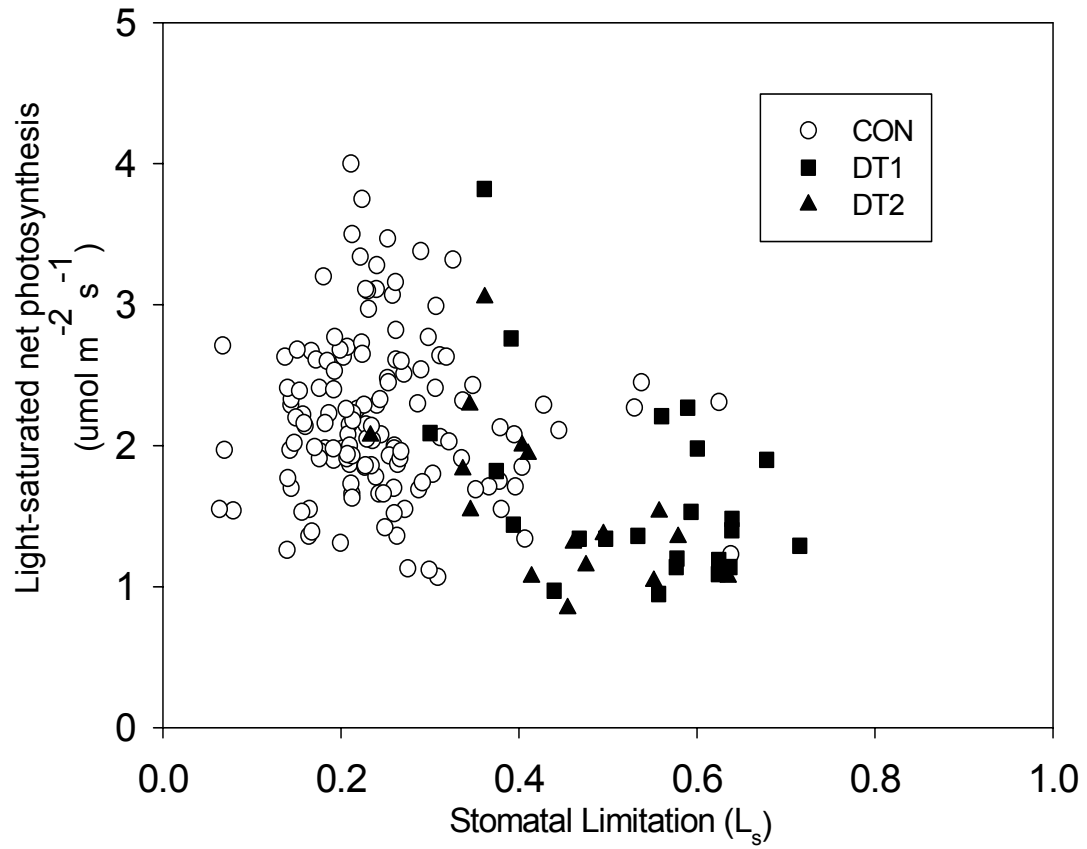
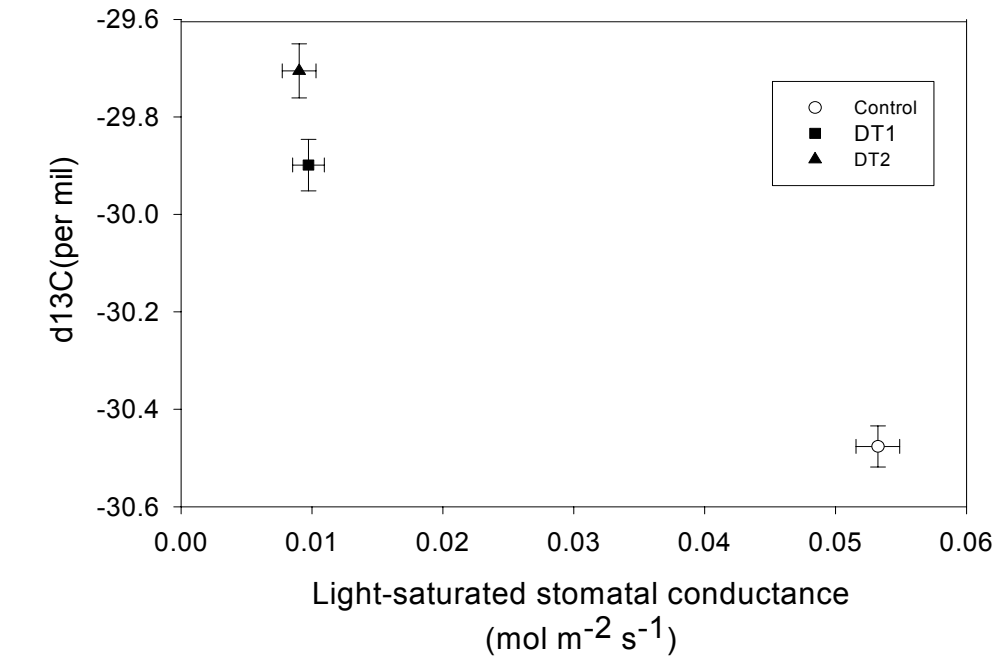
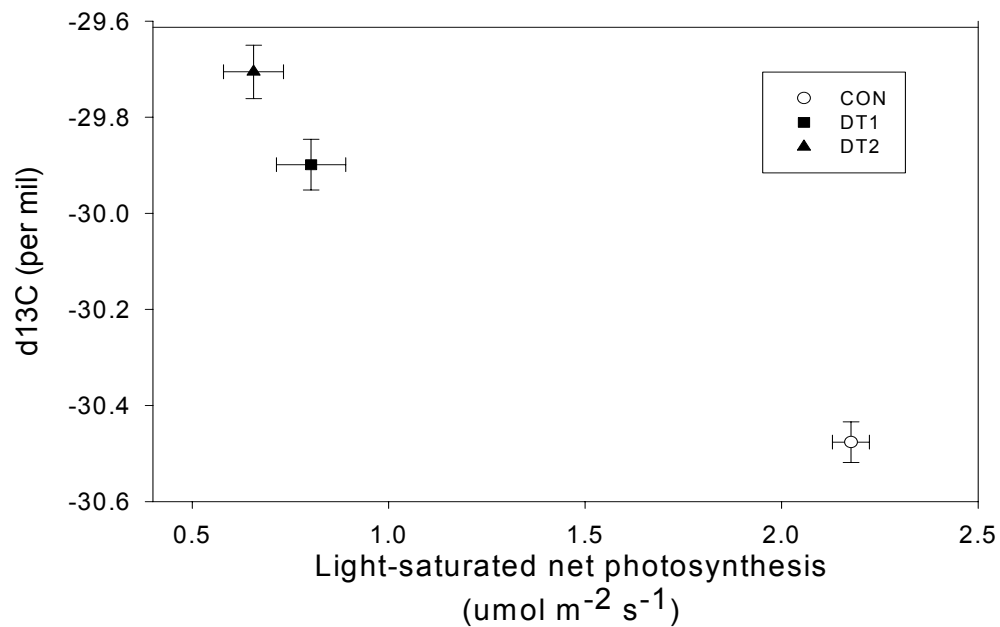


Figure 3.3 Relationship between relative stomatal limitation (L_s) versus light-saturated net photosynthesis (A_{\max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) of single point measurements of plants under well-watered (open) and water-stressed (solid) treatments.



a)



b)

Figure 3.4 Relationship between mean (\pm SE) stable carbon isotope composition ($d_{13}C$) and light-saturated stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) (a), and light-saturated net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (b), for clones of loblolly pine under well-watered (open) and water-stressed (solid) treatments.

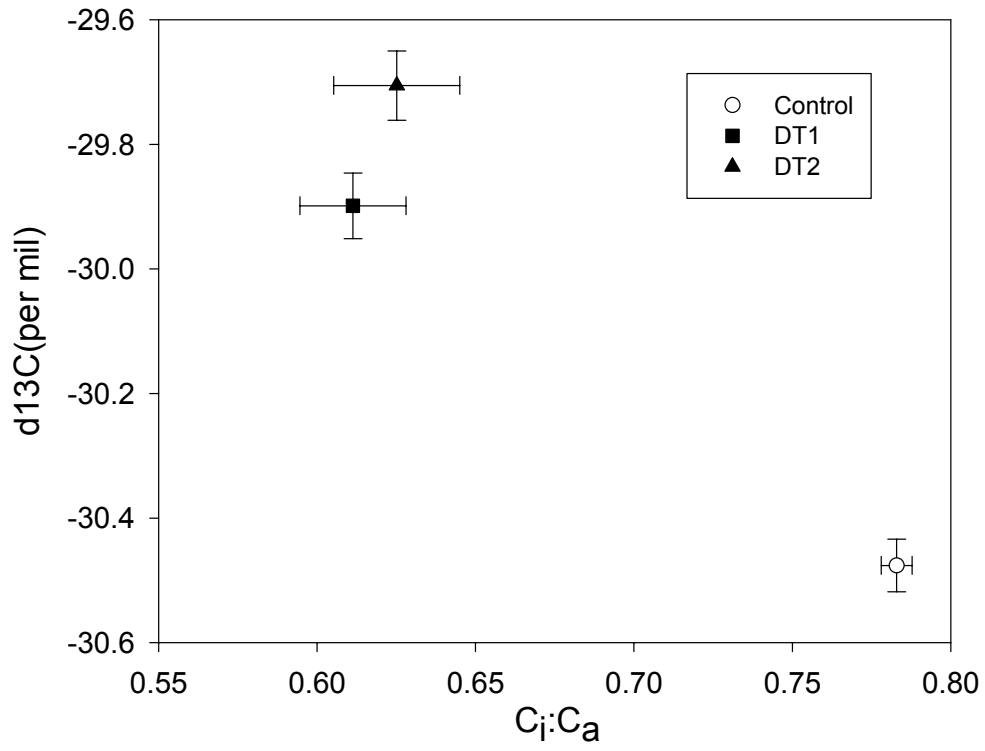


Figure 3.5 Relationship between mean (\pm SE) intercellular to ambient CO_2 concentration ratio (C_i/C_a) and needle stable carbon isotope composition ($d^{13}C$) for clones of loblolly pine under well-watered (open) and water-stressed (solid) treatments.

CHAPTER 4 CONCLUSION

Across the range of loblolly pine, intermittent drought events occur during the growing season, affecting growth and yield. Thus, knowledge about mechanisms underlying drought tolerance is very crucial when selecting genotypes for areas prone to higher risk of drought. Because most physiological and morphological traits involved in drought tolerance are quantitative, it is important that the relative contribution of the genetic and environmental influences traits be estimated.

In forestry, the use of drought regulating traits in selecting genotypes has rarely been used and it is an area worth of exploitation. Although many techniques are available to evaluate morphological and physiological characteristics related to drought adaptations, few have been successfully used in forest tree improvement programs. The difficulty is that many of the physiological measurements are time requiring, expensive and cannot be applied to a large population. Furthermore, no single morphological or physiological trait can be the sole indicator of drought tolerance. The use of carbon isotope composition in selecting clones for improved water use efficiency is promising. It provides information about gas exchange processes integrated over a growing season of a plant. In this respect, the use of carbon isotope discrimination is particularly useful for explaining genetic differences.

Identifying individual clones with favorable combination of traits for growth and drought tolerance is a difficult task. However, when selection focuses on only a few

specific traits related to drought tolerance, it might be possible to find clones that perform well under limited soil water environment. A statistically significant $g \times e$ interaction would suggest performance differences in the measured trait in question. However, soil type and water availability can vary considerably and this may generate different $g \times e$ results for different sets of environments requiring further testing under a wide variety of site conditions.

On the bases of the results of this study, the following general conclusions can be drawn.

1. The non-significant interactions between clone and water stress treatment for morphological traits suggest that growth response of clones in control group was as much the same as in the stressed groups. Clonal ranking on the basis of the measured morphological traits remained the same across treatments. Similarly, the pattern of clonal response in biomass accumulation to the treatments was consistent for clones in each watering treatments. This was shown by the high type B genetic correlation.
2. Despite the various morphological measurements taken, there was no evidence that indicated a single trait directly contributing to drought resistance. In fact, many of the early drought response mechanisms that drought tolerant species display, such as higher root:shoot ratio, were not clearly detected in this study.
3. Estimated broad-sense heritabilities for height and morphological traits were high. The values were higher than any of the previously reported narrow-sense heritabilities. The high H^2 values found for all the morphological (growth and height) traits indicate better expression of additive and non-additive gene actions.

4. Analyses of variance revealed significant clonal variation in P-V parameters (osmotic potential at saturation and turgor loss points, and relative water content at turgor loss point), but there was no evidence indicating a clone by treatment interaction for any of the parameters. The negligible clone by watering treatment interaction for these parameters indicates the presence of consistent clonal ranking. A practical interpretation of this non-significant interaction is that P-V parameters may not be useful in clonal selection programs.
5. Estimated broad-sense heritability for osmotic potential at full saturation (Π_{100}), osmotic potential at turgor loss point (Π_{TLP}) and relative water content at turgor loss point (RWC_{TLP}) were very low. In general, water relation traits were subject to higher measurement errors compared to growth traits. As a result, estimates of H^2 could be less accurate, because of the large experimental error associated. Low heritability values indicate that the trait is not amenable to selection.
6. Consistent with the non-significant clone x watering treatment interaction, the type B genetic correlation (r_{B-TRT}) for P-V parameters were high and close to one, suggesting similar clonal drought responses irrespective of watering treatments. However, this generalization should be considered with caution, as there is no other published work to validate the result.
7. The result of this study supports previous observations that, under water stress, the control of net CO_2 assimilation is dominated by non-stomatal limitation in loblolly pine. In addition, drought preconditioned clones of loblolly pine did not exhibit acclimation to drought stress to maintain photosynthesis.

8. Carbon isotope composition ($\delta^{13}\text{C}$) of clones was strongly influenced by availability of water. Compared to well-watered clones, needle carbon isotope composition $\delta^{13}\text{C}$ of needles increased by 18 and 24 ‰ during the first and second drought cycles, respectively, suggesting higher discrimination against ^{13}C by clones in well-watered conditions.
9. A significant and negative correlation was observed between $\delta^{13}\text{C}$ and total dry biomass accumulation when phenotypic Pearson Correlation coefficient (r^2) was computed on treatment bases ($r^2 = -0.38, -0.29, -0.14$ for control, DT1 and DT2, respectively). The negative correlation indicated that faster growth was genetically associated with higher discrimination values.
10. There was significant ($P = 0.0034$) variation in foliar $\delta^{13}\text{C}$ values among the clones studied. However, the clone x watering treatment interaction was not significant ($P = 0.2563$), with very minimal contribution of clone by treatment interaction variance (σ^2_{CT}) to the total phenotypic variance.
11. Broad-sense heritability (H^2) estimate for $\delta^{13}\text{C}$ was low (0.007) for clones in all three watering treatments. Although the variance component due to clones was higher, H^2 values for $\delta^{13}\text{C}$ remained low, which suggests that selection for drought tolerance using $\delta^{13}\text{C}$ may need a more thorough understanding of the mechanism underlying genetic differences. The clone by watering treatment interaction for $\delta^{13}\text{C}$ was not significant and resulted in a high (0.79) type-B genetic correlation, which suggests consistent clonal ranking (performance) in both well watered and water stressed soil environments.

12. Because all clones of loblolly pine maintained positive light saturated photosynthetic rate, (none of the 12 clones had negative A_{\max} values), selection for increased drought tolerance would be more effective by identifying clones with superior photosynthetic capability under more extreme drought conditions.

In this study, the goal was to characterize some of the most important morpho-physiological traits, which are known to confer drought tolerance, and an attempt has been made to estimate the most important genetic parameters commonly applied in clonal screening programs. It was apparent that the clone x watering treatment for all traits was not significant, which, to a great extent, could be attributed to the length and duration of the drought period. The water stress treatment applied was short in an attempt to obtain informative drought regulation mechanisms at the organ and whole plant level. Thus, it is important that a follow up study be done under more natural conditions. Furthermore, as shown in Table 2.1, some of the clones shared either female or male parents or both and as a result the statistical model used in this study may not be adequate in quantifying the various variance components necessary to calculate the genetic parameters.

Selection for drought tolerance can be accomplished by testing clones under a wide range of testing sites. Evidence from other clonal forestry programs, particularly large-scale eucalyptus and poplar clones plantations in USA, Australia, and in some countries of South America, show that clonal forestry has brought substantial financial as well as genetic gains over conventional forestry. Forest product companies in the Southeast could also benefit from loblolly pine clonal plantations. However, proper clonal screening and deployment strategies need to be developed, to ensure success of such large-scale clonal plantations.

APPENDIX A
GENERAL STATISTICAL MODEL

A mixed linear model was used to estimate variance components. The model included fixed and random factors and their interactions. Because preliminary analyses of variance showed no significant effect of rooting class for all the traits measured, rooting class was dropped from the model. The reduced model was:

$$Y_{ijkl} = \mu + B_i + B_i T_j + T_j + C_k + T_j C_k + \varepsilon_{ijkl}$$

where

Y_{ijkl} = phenotypic (trait) observation on the l^{th} ramet of the k^{th} clone in j^{th} water treatment group in i^{th} block;

μ = the overall phenotypic (trait) mean;

B_i = the random effect of the i^{th} block ($i = 1,2,3,4,5,6$), $\sim \text{NID}(0, \sigma^2_B)$;

T_j = the fixed effect of j^{th} whole plot (water stress) treatment ($j = 1,2,3$)

C_k = the random effect of the clone ($k = 1,2,3 \dots 30$) $\sim \text{NID}(0, \sigma^2_C)$;

$B_i T_j$ = the random effect of the block by treatment interaction, $\sim \text{NID}(0, \sigma^2_{BC})$;

$T_j C_k$ = the random effect of clone by water treatment interaction, $\sim \text{NID}(0, \sigma^2_{TC})$;

ε_{ijkl} = the effect of random error, $\sim \text{NID}(0, \sigma^2_e)$.

Effects of the watering treatments and clones were tested against the whole plot and sub-plot error, respectively. Significant levels for fixed effects (watering treatments) were obtained from PROC GLM of SAS procedure. The random factor effects of the model were block, block x watering treatment interaction, clone and the clone x watering treatment interaction.

APPENDIX B
SUPPLEMENTAL FIGURES

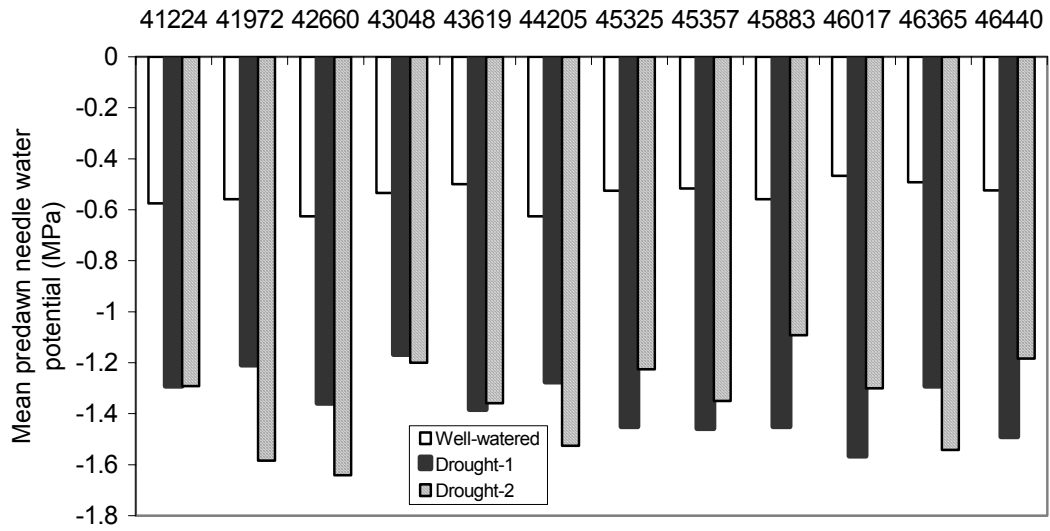


Figure B.1. Mean monthly air temperature of greenhouse environment during study period (July 2001- June 2002).

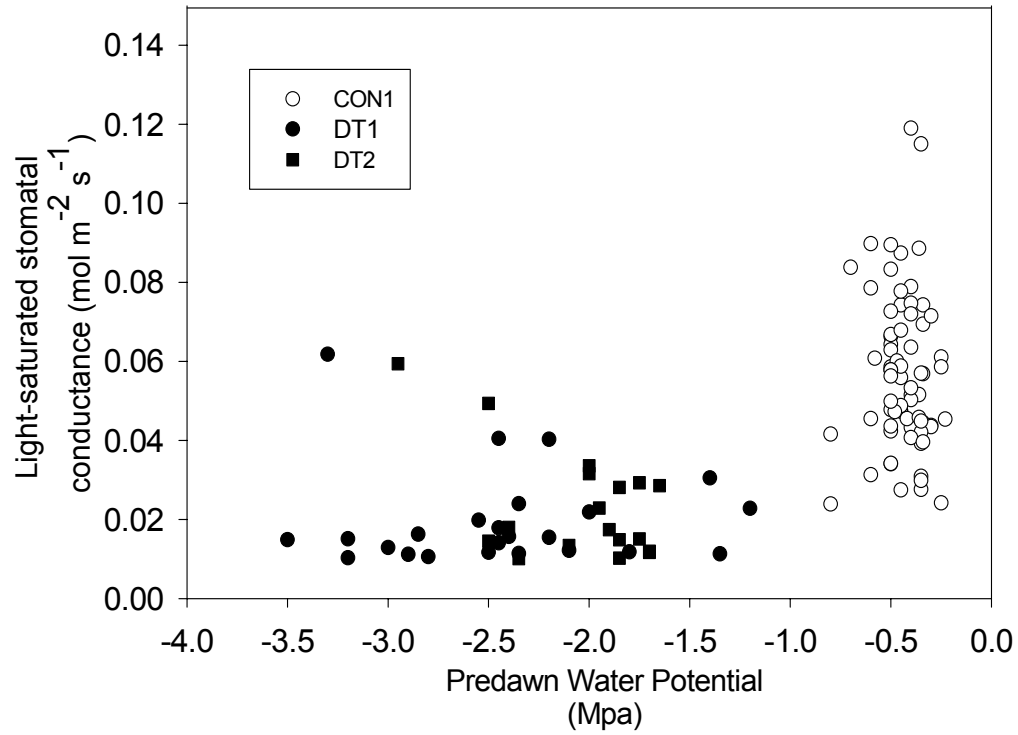


Figure B.2. Relationship between light-saturated stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) and needle predawn water potential (MPa) of single point measurements for loblolly pine plants under well-watered (open) and water-stressed (solid) treatments.

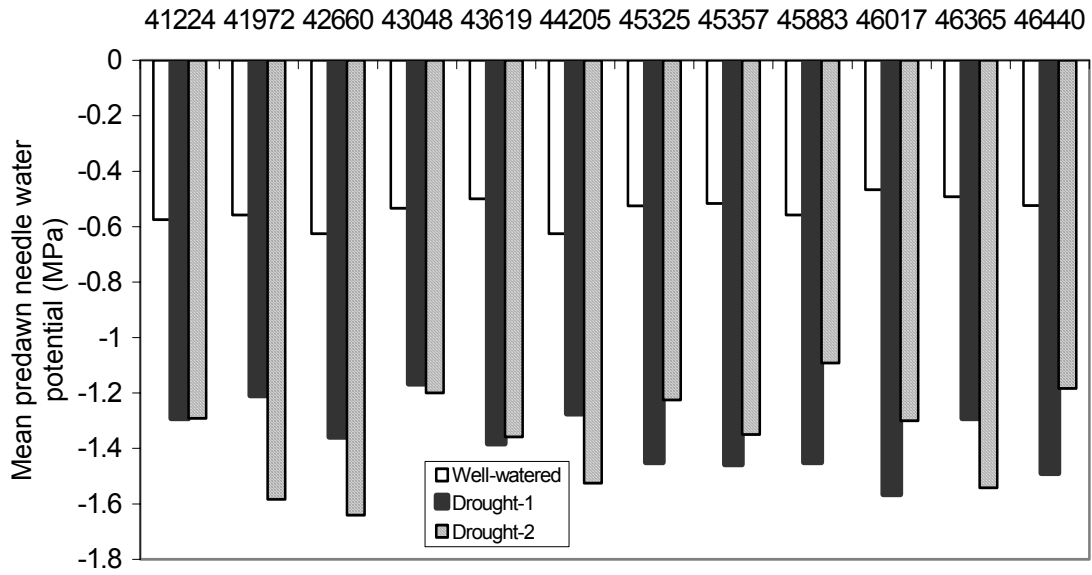


Figure B.3. Mean predawn water potential (ψ_{pd}) for 12 clones of loblolly pine under three watering treatments of well-watered, drought-1 and drought-2. Clonal differences were not significant ($P = 0.8395$).

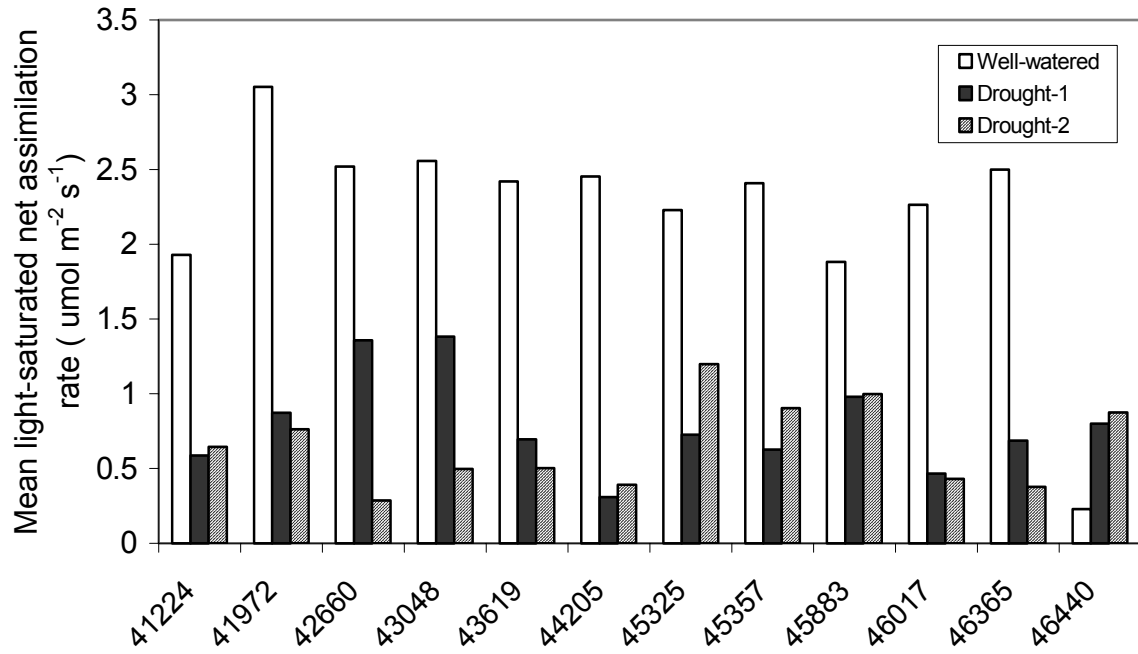


Figure B.4. Mean light-saturated photosynthesis rate (A_{\max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), for clones of loblolly pine under well watered and drought treatments. Measurement was taken four days after drought was imposed. Clonal differences were not significant ($P= 0.2405$).

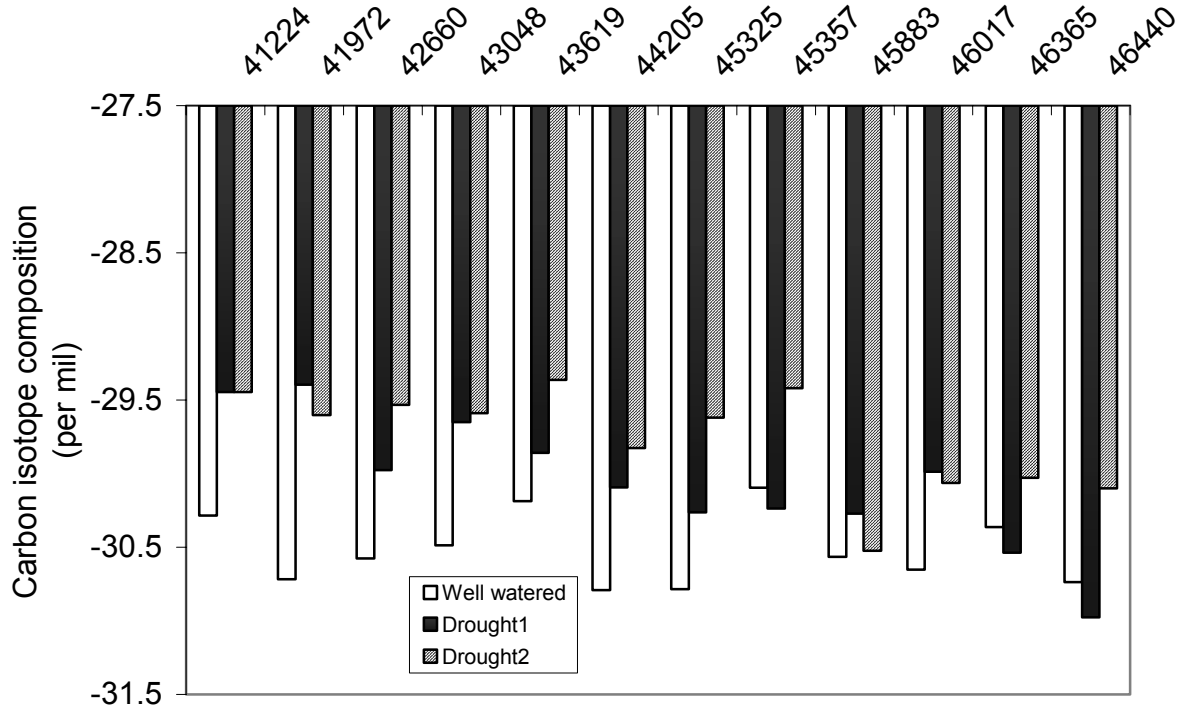


Figure B.5. Mean carbon isotope composition (per mil) in well-watered (open), drought-1 (filled) and drought-2 (hatched) for clones of loblolly pine. Clonal differences were significant ($P = 0.0034$).

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BIOGRAPHICAL SKETCH

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