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# Assessing the Significance of Above- and Belowground Carbon Allocation of Fast- and Slow-Growing Families of Loblolly Pine

**Final Report – 06/15/1997 – 09/14/2000**

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**March 2001**

**Work Performed Under Contract No. DE-FC07-97ID13527**

**For**
**U.S. Department of Energy**
**Assistant Secretary for**
**Environmental Management**
**Washington, DC**

**By**
**Boyce Thompson Institute for Plant Research**
**Ithaca, NY**

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# TECHNICAL REPORT FOR PROJECT NUMBER DE-FC07-97ID13527

**DOE SPONSORING OFFICE:** **Sustainable Forestry Task Group Agenda 2020, Idaho Operations Office**

**AWARD PERIOD:** **6/15/97 through 9/14/00**

**PROJECT TITLE:** Assessing the significance of above- and belowground carbon allocation of fast- and slow-growing families of loblolly pine.

**RESEARCH AREA(S) IN THE RFP TO WHICH THIS WORK IS TARGETED:**
(3) Basic understanding of tree physiological and biochemical processes that control important traits affecting forest productivity.
(4) Develop cost-effective management options for enhancing long-term soil productivity.

**PRIMARY INVESTIGATORS AND COLLABORATORS:**
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## TABLE OF CONTENTS

**Page**

# CHAPTER 1

## SUMMARY

The continued long-term improvement in the performance of any crop, herbaceous or woody, requires a firm physiological understanding prior to genetic alteration. Identifying how whole-plant carbon source/sink relationships may change with age and an edaphic stress, with an emphasis on root system carbon demands, is central to any genetic regulation of photosynthate allocation to harvestable and non-harvestable tissues of trees. We experimentally evaluated belowground biomass and carbon allocation and partitioning of four different fast- and slow-growing families of loblolly pine (*Pinus taeda* L.) located in Scotland County, NC, over the course of three growing seasons. The trees were subjected to two treatments: optimal nutrition and control since planting in 1993. Destructive harvests in 1998 and 2000 were used for whole-plant biomass estimates and to identify possible family differences in whole-tree biomass allocation. Gas exchange characteristics were measured for two years to assess family and treatment differences in carbon acquisition (photosynthesis) and efficiency of water use. At regular intervals throughout each year, we sampled tissues for carbohydrate analyses to assess differences in whole-tree carbon storage. Minirhizotron observation tubes were installed for monitoring root (roots + ectomycorrhizae) system production and turnover using minirhizotron technology. Stable isotope analysis was used to examine possible functional differences in water and nutrient acquisition of root systems between the various families. By linking experimental data with the simulation model TREGRO, we are extending our results across a wide range of Atlantic Coastal Plain sites. A genetic dissection of root ontogenic and architectural traits including biomass partitioning was conducted using molecular markers to better understand the functional implications of these traits on resource acquisition and whole-plant carbon allocation.

### TASK 1: ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

**Section I:** Height growth of families of loblolly pine (*Pinus taeda* L.) from the "Lost Pines" provenance in Texas and the Atlantic Coastal Plains of NC and SC during the first four years has been evaluated as well as volume at age six. Response to fertilizer applications has been large with a 50% increase in height and a 200% increase in stem volume at age six years. The Atlantic Coastal families were significantly taller and had greater stem volume than the LPT families in both the fertilized and control plots. Even given the tendency for low genotype by environment interaction for open-pollinated families of loblolly pine, the adaptability of the Atlantic Coastal families to such extreme environmental conditions was surprising. The long-term performance of the trees will be evaluated to see if this trend continues.

**Section II.** We examined the total biomass allocation in fast- and slow-growing families of two provenances of loblolly pine at 5 years of age in January 1998. Fertilization increased total root, total shoot, and total tree biomass in all families compared to harvested trees in control plots. Although there were treatment and family differences in standing-crop biomass of the total root, total shoot, total tree, and various individual root and shoot components, percent (whole-tree) biomass allocation to these tissues remained similar across treatments. Total nonstructural carbohydrate (TNC) analysis indicated some treatment, family, and provenance differences in TNC concentrations and partitioning to starch and soluble sugars. At the time of harvest, TNC

concentrations of belowground tissues were substantially higher than aboveground tissues. Enhanced partitioning towards starch in root tissues indicates an important carbon storage role for belowground tissues in loblolly pine at this time. More than 90% of the trees starch content was present in root tissue in January. Although constrained by a sample size of three harvested trees per family, the harvest suggests that biomass allocation on a whole-tree level was similar between fast- and slow-growing families of different provenances of juvenile loblolly pine and was not affected by fertilizer treatment.

**Section III.** Source water utilized by four families of loblolly pine (*Pinus taeda* L.) was assessed by comparing the hydrogen isotope composition (δD) of xylem sap and of soil water from four depths (0-20 cm, 20-40 cm, 1.2 m, and 2.1 m) across one year. Soil water δD values varied with soil moisture content in the well-drained, sandy site and at each of the four soil depths. In September and November 1997 and May through November 1998, xylem sap δD values closely matched the soil water δD values of the upper soil horizons (0-20 and 0-40 cm, indicating significant water uptake from upper regions of the soil profile. However, in March 1998, xylem sap δD values closely matched the soil water δD values of the 1.2 m soil depth, indicating that trees were obtaining their water from deep in the soil profile at this time of year, most likely due to low soil temperatures in the upper horizon. Analysis of source water use with a two-ended mixing model in the three months of collection that exhibited a range of soil water δD values across the soil profile confirmed that trees utilized different sources of water depending upon season of the year. In September 1997 and November 1998, source water uptake was primarily from the upper soil profile while in March 1998, source water uptake was from deep in the soil profile. With few exceptions, we did not find striking differences in source water use between drought-hardy families and those that were locally adapted.

**Section IV:** Over a two year period (1998-1999), we conducted a field study using minirhizotron technology to investigate fine root system production and turnover in the four families of loblolly pines used in the physiological studies. A total of 144 minirhizotron tubes were installed to examine potential genetic differences in fertilizer effects on fine root turnover. Data analyses indicated an interaction between these families and fertilizer treatments for total fine root length and total fine root number. Preliminary analyses of data suggest that fertilization increased total root length in slow-growing families, but had no effect or decreased total root length in a faster-growing families. Fertilization decreased total root number in the two fastest-growing families, but increased or decreased root number in the two slowest-growing families. Survivorship analysis indicated that mycorrhizal roots had a shorter life span than brown fine roots. Fertilization prolonged brown root life span but reduced mycorrhizal root life span.

In May and September 1999, soil cores were removed to determine % colonization of loblolly pine roots by ectomycorrhizae, and classify mycorrhizal symbionts by morphotype. Because ectomycorrhizae are significant carbon sinks in pine root systems and more than 90% of short roots in these loblolly pine families were colonized, ectomycorrhizal short roots (clusters) were classified into nine different morphotypes. No treatment nor family interactions were found. Fertilizer treatment decreased the number of mycorrhizal clusters per unit root length on both sampling dates. Dark and brown morphotypes were dominant mycorrhizal morphotypes among all the families in May and September 99. Faster-growing families produced more fine roots than the slower-growing families under ambient soil conditions, but were more responsive

to fertilization, i.e. root number decreased and mycorrhizal colonization decreased, suggesting a larger reduction in root system carbon demands.

**Section V.** We investigated diurnal and seasonal changes in gas exchange and partitioning of recently assimilated carbon in needles of drought-hardy and mesic families of loblolly pine. Although diurnal and seasonal effects on $P_{net}$ and $A_{max}$ were significantly different, few family or treatment differences in gas exchange characteristics were observed. Net photosynthesis peaked at different times during the day over the season, and $A_{max}$ was generally highest in May. Stable isotope analysis and gas exchange measurements did not indicate large differences in water use efficiency (WUE) among the families for most of the sampling period. However, family differences were suggested in July 1999 during a severe drought, with one of the Texas families exhibiting high WUE. Current year foliage exhibited higher rates of $A_{max}$, diurnal $P_{net}$, higher N concentration and higher $\delta^{13}C$ values, suggesting higher water use efficiency.

There were no diurnal effects on foliar starch concentrations or starch/sucrose partitioning among families or treatments. However, strong seasonal effects on foliar concentrations of total nonstructural carbohydrate (TNC) and starch, as well as starch/sucrose partitioning were apparent. For all families, foliar starch concentration peaked in May and decreased to a minimum during winter months. In contrast, reducing sugar concentrations were highest in winter months. High TNC and starch concentrations occurred when photosynthetic rates and spring carbon demands were at their highest. Fertilization reduced TNC concentrations and partitioning of TNC into starch in July and September, possibly reflecting a dilution effect and/or enhanced metabolic demands in fertilized leaves. Lower starch, reducing sugar and sucrose concentration in current year vs. previous year foliage was probably due to different seasonal metabolic demands of these tissues. Overall, our data suggest strong ontogenetic control over gas exchange characteristics and carbon partitioning in loblolly pine that was independent of environment and genetic influences.

**Section VI.** Identifying how whole-plant carbon source/sink relationships may change seasonally and with nutrient stress, with an emphasis on root system carbon demands, is central to any genetic regulation of photosynthate to harvestable and non-harvestable tissues. In the following study, we examined genetic differences and fertilizer effects on whole-tree carbon allocation and partitioning in four families of loblolly pine located in Scotland County, NC. Seasonal differences in TNC and partitioning of TNC (into starch) were quite pronounced in both the control and fertilized treatments, with peak TNC concentrations occurring in March and May for belowground and aboveground tissues, respectively, while the lowest TNC concentrations were found in September. Absolute TNC concentrations were the highest in root tissues (coarse roots = woody roots > fine roots) and lowest in stem tissue. The high partitioning of TNC towards starch in root tissues concomitant with their high TNC concentrations suggest that root tissues serve a primary storage function in loblolly pine. Our data suggest that there were no differences in partitioning to storage carbon between fast- and slow-growing families of loblolly pine; however, fertilizer significantly altered whole-tree carbon source/sink relationships.

**Section VII.** Effects of root and shoot genotypes on productivity and physiology of loblolly pine seedlings were evaluated. The main objective was to elucidate the relative influence of genetic factors in tree roots upon growth in biomass. Twelve-week-old seedlings from contrasting provenances were grafted reciprocally to facilitate distinction of rootstock and scion effects. Five open-pollinated families each from a mesic region (Atlantic Coastal Plain) and from a xeric region (Lost Pines Texas) were used and were planted in a split-plot design on a nutrient-poor site in the Scotland County, NC, field site. Half of the plots were fertilized annually, and after one and two growing seasons, seedlings were harvested for biomass. Total biomass production among families was positively related to proportional biomass allocation to roots. Generally, mesic sources produced more total biomass and allocated proportionally more biomass to roots. Proportional biomass shifts between aboveground parts and belowground parts, depending on root and shoot genotypes, suggested that root system genotype was more influential in determining root:shoot allocation. Rootstock did affect stem growth efficiency, in that the xeric rootstock was associated with increased proportional allocation to stem, regardless of scion type.

Effects of root system genotype on foliar physiology of selected families were evaluated and related to whole-plant growth of genotypes. In four families (two from each provenance), midday light-saturated net photosynthesis ($A_n$) and stomatal conductance to water vapor ($g_s$) were measured monthly during the summer of 1999. Leaf carbon isotope discrimination ($\Delta$) was analyzed for estimation of long-term water use efficiency (WUE) of genotypes. Rootstock affected $g_s$ but not $A_n$ nor $A_n/g_s$ of scions. Rootstocks were associated with lower $g_s$ when paired with scions of the other provenance. Although leaf $\Delta$ did not normally differ significantly between provenances, rootstock did affect $\Delta$. Xeric rootstocks were associated with lower $\Delta$. It was evident that stomatal behavior was pre-conditioned by factors inherent with root genotype. Degree of correlation between $\Delta$ and $WUE_i$ depended on the degree of relatedness between genotypes grafted as scion and rootstock. Our results indicate that root genotype can substantially influence certain aspects of leaf physiology, which can have large repercussions on tree growth.

## TASK 2: COMPUTER MODELING (TREGRO)

Trees at the SETRES II field site are growing under extremes of nutrient conditions (optimum nutrition and nutrient limiting). In order to extrapolate the findings/conclusions of the field study, the TREGRO simulation model, in conjunction with parameter sets from each family/treatment, was used to extend the results from this field study to other Atlantic Coastal Plain sites. To this end, meteorological data files necessary to run the simulations have been constructed for both years of the study (1998 and 1999). In addition, one family/treatment combination has been completely parameterized (Family 81 - fertilized plots). Other parameter sets are under development and will be completed shortly. Once parameter files have been constructed they will be used to predict expected changes in whole-tree growth and carbon allocation for each of the families under a wide range of nutrient conditions.

## TASK 3: GENETICS AND MOLECULAR BIOLOGY

Genetic variation in tree growth and development could play an important role in explaining the patterns and variation in tree productivity. Tree roots are especially important in tree growth and development because a large portion of the photosynthate produced by a tree is

allocated to the roots, especially when soil nutrient levels are low. Roots are the major sink for photosynthate under these conditions. Allocation of carbon to plant parts (foliage, shoots, stem, coarse roots, fine roots) is plastic in response to nutrients, especially nitrogen. Less photosynthate is allocated to roots when nutrients are abundant than when nutrients are scarce. This responsiveness to changes in environmental conditions can be described as phenotypic plasticity. Phenotypic plasticity occurs when organisms alter their phenotypes to better suit different environmental circumstances. Plasticity could be under genetic control, or at least subject to genetic constraints. Plasticity to nutrients could play a role in managing forest productivity because fertilizer is becoming widely used in forest tree plantations.

We carried out three studies to address the role of genetics in controlling responsiveness to nutrients. In the first, we studied seedling biomass changes in response to different nutrient regimes in loblolly pine seedlings. We determined that seedlings from the eastern Texas xeric ecotype (Lost Pines provenance) of loblolly pine differed in root traits and biomass partitioning from the Atlantic Coastal Provenance mesic ecotype. There were significant differences among ecotypes and among nutrient levels. Our results supported the hypothesis that the differences in productivity between the xeric and mesic loblolly pine ecotypes planted at Scotland County are due to belowground differences in root systems. Based on the results of this study, we determined that seedling biomass partitioning could be analyzed as a set of quantitative traits and that the xeric and mesic ecotypes were sufficiently differentiated that we could expect to find meaningful differences in these traits segregating within a family parented by an F1 hybrid between the two ecotypes.

We carried out a genetic dissection of height and diameter growth of loblolly pine OP family 7-1037 at the Scotland County field test. We assayed DNA markers in haploid DNA samples corresponding to 7-1037 trees in the field. This allowed us to detect several quantitative trait loci (QTLs) with average effects in trees at selection age (6 years in the field). Average effects are directly related to breeding value and few studies have defined QTLs this way. To do this, we overcame several challenging problems. We developed DNA marker methods to enable us to efficiently make maps from megagametophyte samples. Megagametophytes from field grown trees have years of field data associated with them. These samples cannot be replaced with equivalent samples and earlier methods had a prohibitively high failure rate. We acquired better software for gel scoring and data analysis. The biggest problem we faced was the small amount of phenotypic variation that is under genetic control in field grown half-sib families. To deal with this, we developed spatial analysis methods to reduce environmental variation and enhance genetic resolution for our study. Originally, we had planned to analyze two families, but we had to scale our effort back to a single family. The QTLs that we detected controlled a small proportion of the phenotypic variation in height and diameter. We had intended to characterize root traits from a sample of trees that had different QTL genotypes. In principle, the above-ground productivity of trees with different QTL genotypes could be due to differences in belowground traits. However, the magnitude of the QTL effects is not large enough to make such an investigation feasible. A large number of root systems would have to be sampled to detect effects that are expected to be small, and the effort required is not feasible.

We initiated a genetic dissection of seedling biomass partitioning in an open pollinated family of loblolly pine selection 5-1065. The seedlings were planted in sand-filled pots and grew

outdoors during the summer and fall of 1999. The seedlings were harvested, measured, and components were separated for biomass analysis. We obtained large root systems in the 10 inch diameter, 20 inch tall pots, but the processing was extremely laborious. DNA samples were made from the megagametophytes and AFLP DNA markers prepared from the DNA. The analysis of this family is in progress and will be completed before spring 2001. We hope to find QTLs for biomass partitioning and address the issue of pleiotropy, i.e., do genes that control root traits affect partitioning in other plant parts as well?

# CHAPTER 2

## GENERAL BACKGROUND

Loblolly pine (*Pinus taeda* L.) is the most widely-planted tree species in the Atlantic Coastal Plain, with over 12.3 million hectares in both natural and planted stands (Allen *et al.* 1990). Yet we have an insufficient understanding of what controls its ability to achieve maximum growth under a wide range of environmental conditions. Because of its wide geographic distribution, it is not uncommon to find significant genotypic variation in growth of various populations (Bongarten and Teskey 1987). On most sites, trees of coastal origins have consistently outgrown trees of continental origin, and trees from southern origins have outgrown those of northern origins (Bongarten and Teskey 1987). Several studies have associated yield variation among genetic families with net assimilation rate, water and nutrient use efficiency, and aspects of aboveground dry matter partitioning (Bongarten and Teskey 1987; Greenwood and Volkaert 1992; Li *et al.* 1991). How genetically-based differences in belowground carbon allocation may influence yield has not been examined in field-grown trees. Since annual fine root production and maintenance costs in trees can be as much as 60-80% of total net primary productivity of forests (Reichle *et al*. 1973; Agren *et al.* 1980), yield variation among families could be associated with belowground carbon demands. Identifying how whole-tree carbon source/sink relationships may change with age and edaphic stress, with an emphasis on root system carbon demands, is central to any genetic regulation of photosynthate to harvestable (e.g., stem or bole) and non-harvestable tissues. In the following study, we examined carbon allocation strategies of fast-and slow-growing populations of loblolly pine using a fast-growing coastal provenance and a slower-growing Texas provenance that may not only be drought-hardy, but exhibit greater root system carbon demands.

Since fine root production represents a substantial carbon sink, more research examining the effects of fertilization on fine root turnover is necessary. Whole-tree destructive harvests and/or soil coring provide root biomass estimates, but little information is available on the dynamic nature of root production and carbon demands. Recent investigations using minirhizotron root observation tubes in the field suggest that the effects of fertilization on root production and turnover may be species and stand-specific (Pregitzer *et al.* 1993; Tingey *et al.* 1996). If fertilizer amendments stimulate fine root production and maintenance, does this occur at the expense of another carbon sink (such as coarse roots or stem), or are whole-plant carbon source/sink relationships maintained because of increased carbon acquisition (due to increased foliage production and photosynthetic rates)? Alternatively, if fertilizer amendments reduce fine root production and maintenance, does extra carbon become available for stem and foliage production, or are whole-plant carbon source/sink relationships maintained? Theoretically, populations that allocate more carbon belowground such as the drought-hardy loblolly pine from Texas could benefit most from soil amendments IF whole-plant biomass partitioning was plastic. However, greenhouse studies with seedlings of the Texas drought-hardy loblolly pine suggest that not only are its root ontogeny and architecture under strong genetic control, regardless of the external soil environment, but overall seedling growth and physiological rates of uptake are not responsive to an enhanced P supply (Topa and McLeod 1986a,b; Topa 1996; Topa and Sisak 1997).

A review of ecological literature suggests that species typical of infertile soils have higher nutrient concentrations in tissues (a lower nutrient-use efficiency), slower growth rates and lower rates of nutrient absorption that are relatively insensitive to a variation in nutrient supply than fast-growing species (Clarkson 1967; Chapin 1980 1988; Chapin and Bieleski 1982; Chapin *et al.* 1982). It is not unreasonable to postulate that certain slow-growing populations of loblolly pine indigenous to soils with limiting resources may also be unresponsive to fertilizer or water amendments. If so, is this non-responsiveness a function of reduced resource acquisition resulting from reduced physiological uptake rates and/or reduced root growth? Are root and shoot growth of these slow-growing populations non-responsive, or is there a shift in shoot/root biomass partitioning (and whole-plant carbon source/sink relationships) under fertilized conditions? The research in this study addresses some critical physiological questions that will be of assistance in genetic tree improvement and silvicultural practice decision-making by examining: (1) population differences in root system production, and shoot/root biomass and carbon allocation in field-grown trees, and how these differences may change with fertilizer amendments; and (2) possible functional differences in water and nutrient acquisition between the various populations.

## OBJECTIVES

The overall objective of this study was to assess possible differences in acquisition, whole-plant allocation, and partitioning of carbon in fast- and slow-growing families of loblolly pine, and how fertilization may alter these differences. We combine aboveground (leaf area and biomass) data provided from our collaborators at North Carolina State University with belowground data to address the following questions:

1) Do fast- and slow-growing loblolly pine families allocate and partition carbon differently to above- and below-ground components? Does fertilization alter these allocation/partitioning patterns?
2) Are root systems of fast-growing families more responsive to fertilization? If so, does this result in increased aboveground growth?
3) Are there genetic differences in root system production, turnover and mycorrhizal associations between fast- and slow-growing families? Do root system production, turnover, and mycorrhizal associations change in response to fertilization?
4) Which carbon allocation and/or growth strategy, singly or in combination, would enable a specific family to flourish under a range of environmental conditions?

Answers to the preceding questions were used to assess the carbon efficiencies of fast- and slow-growing loblolly pine families across a wide range of Atlantic Coastal Plain sites using the simulation model TREGRO. In addition, stable isotope experiments were conducted to examine possible functional differences in resource acquisition of root systems between the various families to address the following questions:

5) Which roots (surface or deep roots) are most active in water uptake during the various seasons? Do drought-hardy Texas families utilize a deeper water source than Coastal Plain families? Are surface roots more active in nutrient uptake rather than water uptake during drier months (functional specialization)?

6) Are there periods of maximum nitrogen uptake in these families? Do they coincide with specific phenophases? Is recently-acquired or stored N used to support new needle or root growth?

A genetic dissection of root ontogenic and architectural traits and biomass partitioning was conducted by Drs. McKeand and O'Malley using molecular markers to better understand possible functional implications of these traits on resource acquisition and whole-plant carbon allocation. They will address the following questions:

7) Are differences in root traits in pine under the control of major genes as has been demonstrated in rice and maize? Can molecular markers be used to select root system traits in breeding as an alternative to destructive phenotypic assays of root traits?
8) In field-grown trees, do major genes (QTLs) for shoot growth and aboveground measures of productivity have physiological correlates in belowground characteristics of root systems?
9) In outbred $F_2$ and backcross seedlings of loblolly pine, are major gene effects on roots and shoots independent or is there a pleiotropic effect on both traits? If root/shoot QTLs are pleiotropic in low nutrient environments, are they independent in high nutrient environments?

**SCOTLAND COUNTY STUDY SITE**

Our research site is located in Scotland County, NC, and was established by the Department of Forestry at North Carolina State University. The study site is adjacent to the U.S. Forest Service/North Carolina State University SETRES study. The soil is Wakulla series – sand to greater than 43 m, sandy, silicaceous, thermic Psammentic Hapludt – very infertile, somewhat excessively drained with a total water holding capacity of 12-14 cm in a 2 m profile. The site receives and annual rainfall of 120 cm. Temperatures average 17°C annually; 26°C summer, and 9°C winter. An existing 10-year-old loblolly pine stand was removed and large block-plots of different family-treatment combinations were established. Five open-pollinated families from the North Carolina and South Carolina Atlantic Coastal Plain (ACP) and five drought-hardy Texas families (LPT) with average or slightly above average breeding values for volume production were selected for this study. Breeding values across the ten families ranged from 8.6 to -9.3 based upon four-year aboveground height growth (breeding value = average family height at 4 years - average height of all families at 4 years) (McKeand *et al*. 1999). Seeds were sown in containers (160 cc RL Super Cells) in the greenhouse in June 1993, and seedlings were field-planted in November 1993.

To facilitate the application of nutrients, a split-split plot design was used with the two nutrient treatments as main plots, provenances as sub-plots, and families within provenances as sub-sub-plots. Each family-plot consists of 100 measurement trees planted in 10 rows of 10 trees at 1.5 m by 2 m spacing. Buffer trees, 12 m around each treatment plot, were planted at the same spacing to eliminate the influence of one nutrient treatment on another. The study was replicated across 10 blocks. The experimental design of the field site is 10 blocks x 2 provenances (ACP, LPT) x 5 families/provenance (fast- and slow-growing) x 2 fertilizer treatments (unfertilized and optimum fertilization) with 100 trees in each family plot for a total of 20,000 trees. It should be noted that the tight spacing was necessary due to space limitations and the required 100 trees/family/treatment necessary for the quantitative trait locus (QTL) objectives in the study.

One-half of each one of the blocks have received annual soil fertilizer additions to maintain optimum foliar nutrient ratios (Hockman and Allen 1990) while control plots have received no nutrient additions. Our goal has been to supply optimal levels of nutrients each year to stimulate rapid growth. Through the first six growing seasons, the total nutrient additions (kg/ha) have been: 425 N, 55 P, 85 K, 3 Ca, 50 Mg, 75 S, 0.5 B, 2 Cu, 5 Fe, 5 Mn, and 2 Zn.

For the physiological and modeling aspects of the current study (Tasks 1 and 2), we selected only the fastest- and slowest-growing families from each provenance based upon evaluation of 4-year aboveground height growth of control trees (McKeand *et al.* 1999). Families ACP-1 (8-118) and ACP-2 (9-1046) were the fast- and slow-growing ACP families, and LPT-1 (BA3L11-1) and LPT-2 (GR1-2) were the fast- and slow-growing LPT families, respectively (four-year heights - ACP-1 183±1.7 cm; ACP-2 165±1.7 cm; LPT-1 165±1.5 cm; LPT-2 157±1.6 cm). We also focused our analysis of this large study on three blocks (6, 8, and 10) because sample size constrained data collection. Average aboveground growth in these three blocks closely approximated the overall study for 4-year aboveground growth (data not shown).

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# CHAPTER 3

## TASK 1: ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

## I. GENETIC VARIATION IN FERTILIZER RESPONSE

**S.E. McKeand[1], J.E. Grissom[2], J.A. Handest[2], D.M. O'Malley[3], and H.L. Allen[1]**

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### INTRODUCTION

Tree improvement has made significant contributions to forestry and plantation management the last 40 years. In the southeastern United States, managers of wood-based manufacturing facilities have realized that the future of their industry depends upon a reliable, ecologically sustainable, and economically affordable supply of wood. Plantations of genetically improved forest trees are critical to maintaining this supply.

Loblolly pine (*Pinus taeda* L.) is by far the most important forest tree species in the South, with over 1 billion seedlings planted annually by forest industry and non-industrial private forest landowners. Genetic gains from tree improvement programs have been large (e.g. Li *et al.* 1999), since geographic and within-provenance variation for growth and adaptive traits in loblolly pine is very large. General trends in productivity variation are that families from southern and eastern coastal sources grow faster than families from northern, western, and interior sources (e.g. McKeand *et al.* 1989; Wells 1983; Wells and Lambeth 1983; Schmidtling 1994). Contrasting the response to nutrient stress of two very different provenances of loblolly pine such as from the "Lost Pines" region of Texas and the Atlantic Coastal Plain may give us insight into the adaptive significance of different ecophysiological traits.

Previous work indicates that the Lost Pines Texas (LPT) sources are generally more stable across environments, while productivity of eastern sources depends more on the environment (van Buijtenen 1978). Eastern sources were very responsive to environmental enhancement, since productivity was high on the better sites, but very low on the droughty sites. In this report, we describe a study designed to assess spatial and temporal variation in response of loblolly pine genotypes to environmental stress. Trees have completed six growing seasons in the field under two different nutrient regimes (severe stress and optimal), and variation in early growth is described.

### MATERIALS AND METHODS

All trees were measured annually for height and starting in year 3 for breast height diameter. Individual tree volumes were calculated, and plot volumes were estimated as the sum of the individual tree volumes and converted to per hectare volumes. Analyses of variance were conducted on a family-plot-mean basis (Table 1). Means and within family-plot standard deviations and coefficients of variation were calculated for height for each 100-tree family plot.

**Table 1.** Significance levels for main effects and interactions tested in the analyses of variance for height over four years and DBH and stem volume at age four.

| | Height | | | | | Vol/ha | |
|---|---|---|---|---|---|---|---|
| Source[1] | Yr1 | Yr2 | Yr3 | Yr4 | Yr5 | Yr6 | Yr6 |
| Treatment | *** | *** | *** | *** | *** | *** | *** |
| Provenance | + | * | * | ** | ** | ** | * |
| Trt x Prov | | * | | | | | |
| Family(Prov) | *** | * | * | ** | ** | ** | + |
| Trt x Fam(Prov) | | | | | | | * |

[1] Treatment and provenance were considered fixed effects. Family within provenance and blocks were considered random effects.
+, *, **, *** Significant at P_≤0.10, 0.05, 0.01, 0.001, respectively.

Error terms for each main effect and interaction listed above are:

Treatment: [Block x Trt] + [Trt x Fam(Prov)] - [Block x Trt x Fam(Prov)]
Provenance: [Block x Prov] + [Fam(Prov)] - [Block x Fam(Prov)]
Trt x Prov: [Block x Trt x Prov] + [Trt x Fam(Prov)] - [Block x Trt x Fam(Prov)]
Family(Prov): [Block x Fam(Prov)] + [Trt x Fam(Prov)] - [Block x Trt x Fam(Prov)]
Trt x Fam(Prov): [Block x Trt x Fam(Prov)]

Within family-plot standard deviations and coefficients of variation were also subjected to analyses of variance to determine if sub-sub-plot uniformity varied.

## RESULTS AND DISCUSSION

Survival and growth of the trees has been excellent in the first six years. Survival averaged 90% after six growing seasons (no treatment or genetic effects), and height averaged 4.7 m. Deer browse and tipmoth caused some problems in the first two growing seasons, and 13.1% of the trees were damaged and not included in the analyses for growth traits.

### *Fertilizer Response*

Growth responses to fertilization were very large and significant each year (Table 1). Height was 21%, 46%, 43%, 43%, 43%, and 50% greater in the fertilized plots for years one to six four respectively (Figure 1). Only volume at age 6 is reported, and differences were even more dramatic (Figure 2), with the fertilized trees having 3.0 times more volume per hectare than

the controls. Although this is a well-drained site, from the results of the nutrition by irrigation study (SETRES) adjacent to this trial, we know that the primary limit to productivity is nutrition (Albaugh *et al.* 1998). The huge increase in productivity in the first six growing seasons is possible since all potential nutrient limitations (i.e. more than just N and P) were ameliorated.

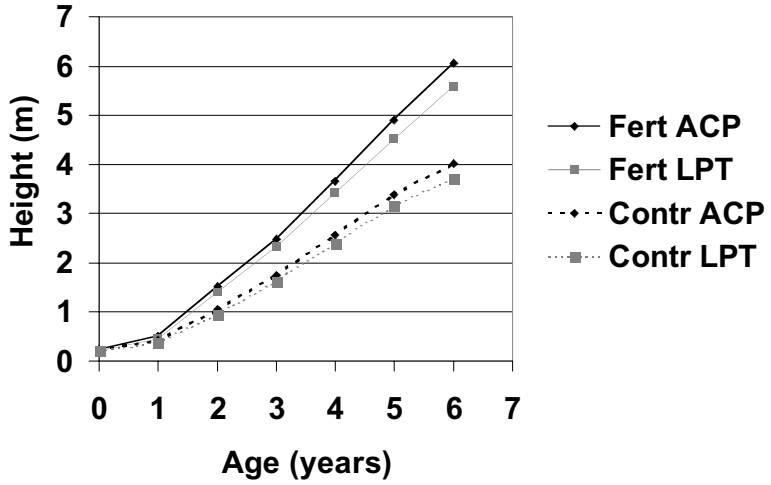


**Figure 1.** Mean tree heights during the first six growing seasons in the field for trees from the Lost Pines Texas (LPT) and Atlantic Coastal Plain (ACP) provenances in the fertilized and control plots. Initial height of seedlings (age 0) at planting was measured in 1994.

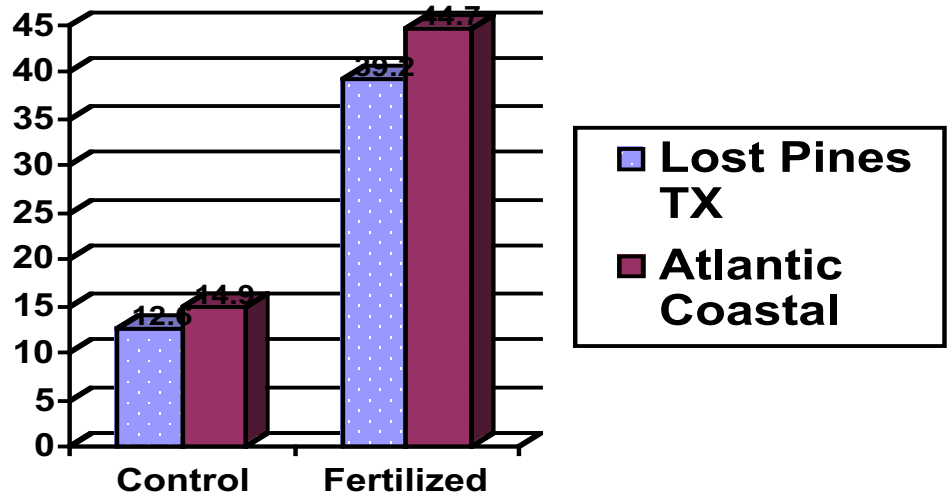


**Figure 2.** Estimates of volume per hectare ($m^3$) for the Lost Pines Texas (LPT) and Atlantic Coastal Plain (ACP) provenances in the control and fertilized plots at age six years.

One of the most dramatic effects of the nutrition amendments has been the increase in uniformity within the 100-tree family plots. The average within-plot coefficient of variation for sixth-year height was 19.7% for the control plots and 9.4% for the fertilized plots. The within plot standard deviations for height were also significantly different and were 0.71 m for the control plots and 0.54 m for the taller fertilized plots. While increased uniformity typically results from nutritional amendments on very poor sites, the dramatic differences in uniformity was surprising.

***Provenance and Family Variation***

As expected, the five families from the Atlantic Coastal Plain grew faster than the five Texas families (Figure 1). We anticipated that under the harsher environmental conditions in the control plots that the Texas families would perform relatively better. However, the ACP families were superior in both environments, and the provenance by treatment interactions for height in all six years were not close to being significant.

The provenance by treatment means for volume per hectare at age six (Table 1) are somewhat indicative of the greater responsiveness to nutritional amendments of the Atlantic Coastal Plain provenance compared to the Lost Pines Texas provenance (e.g. van Buijtenen 1978, McKeand *et al.* 1997). Although there was no provenance rank change in the two environments, the difference in the magnitude of the provenance means (greater in the fertilized plots) is similar to previous trials (van Buijtenen 1978).

The superior above-ground growth of the ACP families could be due in part to differential carbon allocation to above- and below-ground tissues. Our work with one-year-old seedlings of these two provenances indicates that the ACP provenance may have a reduced cost for higher-order lateral root production compared to the LPT provenance especially under high fertility (Wu *et al.* 2000).

Families within provenances also differed for growth traits (Table 1 and Figure 3). The family means at age six for the ACP families varied from 3.72 m to 4.25 m in the control plots and from 5.70 m to 6.37 m in the fertilized plots. The Texas families also differed in the control plots (3.57 m to 3.82 m) and in the fertilized plots (5.49 m to 5.84 m). The marked difference in productivity between the drought-hardy Lost Pines families and the ACP families is illustrated by the almost complete lack of overlap of the family means for height and volume (Figure 3).

The lack of rank change across the treatments both at the provenance and family level was surprising for height and volume. Given the magnitude of the imposed environmental differences and the young age of the trees, differential performance of the families in the two treatments was expected. This result reinforces the tenet of the stability of open-pollinated families of loblolly pine (e.g. Li and McKeand 1989) as well as the better responsiveness of the ACP provenance compared to the Lost Pines provenance.

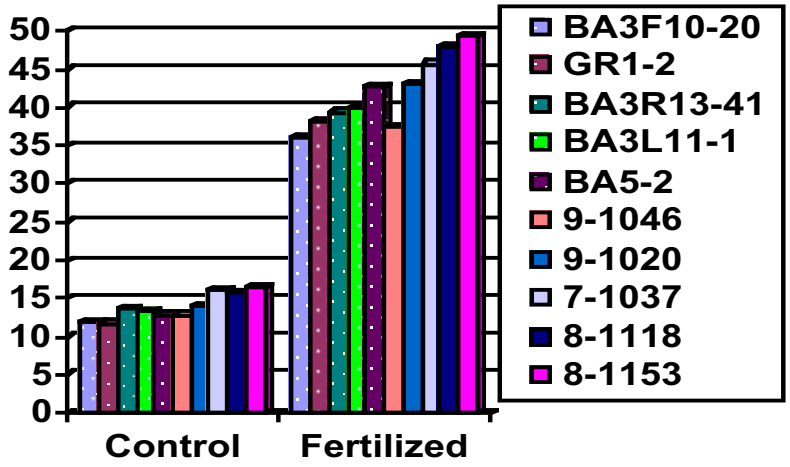


**Figure 3.** Estimates of volume per hectare ($m^3$) for the open-pollinated families from the Lost Pines Texas (LPT) and Atlantic Coastal Plain (ACP) provenances in the control and fertilized plots at age six years. The LPT families start with letters B or G and are the stippled bars. The ACP families are the solid bars and all start with numerals. Families within provenance are ordered by their rank in the fertilized plots.

***Future Work***

This experiment will be a long-term (~20 years) field laboratory for ecologists, physiologists, and geneticists to study the bases for trees' responses to environmental stress. Productivity will continue to be assessed through rotation age to see if the early growth differences are maintained. We suspect that as the stand develops and competition for limited soil resources becomes more intensive, the Lost Pines Texas trees may be superior to the Atlantic Coastal Plain trees in the control plots. Future work will also emphasize both above- and below-ground production and physiological processes and how they interact to affect productivity. Not only will traditional quantitative genetic analyses be conducted to evaluate genetic control for these traits, but genomic mapping to determine the significance of major gene control is also an integral part of the study. Megagametophytes for each of the 19,800 individuals in the trial are in cold storage (-80ºC) and DNA will be extracted and genomic maps developed to determine marker - trait associations. Using the open-pollinated families in such a manner will allow us to determine if major genes with high breeding values (O'Malley and McKeand 1994) are associated with adaptive response to environmental stress.

**CONCLUSIONS**

Height growth of families of loblolly pine from the "Lost Pines" provenance in Texas and the Atlantic Coastal Plains of NC and SC during the first four years has been evaluated as well as volume at age six. Response to fertilizer applications has been large with a 50% increase in height and a 200% increase in stem volume at age six years. The Atlantic Coastal families were significantly taller and had greater stem volume than the LPT families in both the fertilized and control plots. Even given the tendency for low genotype by environment interaction for open-

pollinated families of loblolly pine, the adaptability of the Atlantic Coastal families to such extreme environmental conditions was surprising. The long-term performance of the trees will be evaluated to see if this trend continues.

**Acknowledgments:** In addition to the support received from DOE, financial support for this research has been provided by the Department of Forestry, NCSU; McIntire-Stennis Project NCZ04149; Agricultural Research Service, NCSU; North Carolina Biotechnology Center grant 9413-ARG-0035; USDA Forest Service; Tree Improvement Program, NCSU; Forest Biotechnology Program, NCSU; Forest Nutrition Cooperative, NCSU; Bowater Inc.; Champion International; Georgia-Pacific Corp.; Rayonier; Westvaco Corp.; and Weyerhaeuser Company.

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## TASK 1: ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

## II. GENETIC VARIATION IN WHOLE-TREE BIOMASS ALLOCATION

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### INTRODUCTION

Loblolly pine (*Pinus taeda* L.) is one of the most important tree species in the southern region of the USA, with over 800,000,000 seedlings planted annually (McKeand *et al.* 1999). Great effort has been expended to maximize productivity through genetic selection of trees with traits that maximize capture of carbon and conversion into standing crop biomass. Genetic gains from tree improvement programs have been large (e.g., McKeand and Svensson 1997) because geographic and within-provenance variation for growth and adaptive traits in loblolly pine is large. However, studies examining differences in whole-tree biomass allocation with an emphasis on belowground components in field-grown trees are lacking. Since annual fine root production and maintenance costs in trees can be as much as 60-80% of total net primary productivity of forests (Reichle *et al.* 1973; Ågren *et al.* 1980), yield variation in aboveground production among families could be associated with differences in belowground carbon demands. Strong genetic control of biomass and carbon allocation to above- and belowground components and carbon partitioning to storage reserves may influence a tree's ability to compete for resources (i.e., light, nutrients, and moisture) and to withstand environmental stress. If an environmental stress such as soil fertility or drought decreases the photosynthetic capacity of a plant, then storage carbon may be used to supplement growth demands. Balanced allocation of carbon between immediate use and storage is essential for plant growth and survival during seasonal fluctuations in carbon supply and stress episodes (Geiger and Servaites 1994).

Seedling studies suggest that drought-hardy TX (LPT) families owe their drought hardiness to various avoidance mechanisms including deep root systems and wide ranging laterals (van Buijtenen *et al.* 1976). Other seedling studies also indicate that LPT families allocate more carbon to root growth compared to ACP families, regardless of soil stresses and soil fertility (Topa and Sisak 1997). Whether these differences in whole-tree biomass allocation are maintained in the field as a tree matures is unknown.

Identifying how whole-tree carbon source/sink relationships may change with age and edaphic stress, with an emphasis on root system carbon demands, is central to any genetic regulation of photosynthate to harvestable (e.g., stem or bole) and non-harvestable tissues. In the following study, we examined whole-tree (total standing-crop) biomass and tissue carbohydrate concentrations to compare possible differences in structural and nonstructural carbon allocation

and partitioning between fast- and slow-growing families from two provenances of loblolly pine planted in a droughty, infertile site in NC, USA. More specifically, we examined whether genetically based differences in aboveground yield reflected differences in whole-tree biomass allocation. We hypothesized that drought-hardy trees from Texas preferentially allocated more biomass belowground than trees from the Atlantic Coastal Plain.

## MATERIALS AND METHODS

### *Biomass Harvest*

For the current study, we selected the fastest- and slowest-growing families from each provenance based upon evaluation of four-year aboveground height growth of control trees (McKeand *et al*. 1999). Families ACP-1 (8-118) and ACP-2 (9-1046) were the fast- and slow-growing ACP families and LPT-1 (BA3L11-1) and LPT-2 (GR1-2) were the fast- and slow-growing LPT families, respectively (four-year heights - ACP-1 183±1.7 cm; ACP-2 165±1.7 cm; LPT-1 165±1.5 cm; LPT-2 157±1.6 cm). We also focused our analysis in three blocks (6, 8, and 10) of this large study because sample size constrained data collection. Average aboveground growth in these three blocks closely approximated the overall study for four-year aboveground growth.

Twenty-four trees (1 tree/family plot x 2 families x 2 provenances x 2 treatments x 3 blocks) were harvested in January 1998. Trees were selected for harvest from an outside row of the 100-tree plot. Selected trees represented the average height of all trees in the immediate plot. Trees were cut at the groundline and aboveground biomass was sorted into foliage (age class), branch, and stem components. Only loblolly pine roots emanating from the harvested taproot within a 1x1 m square around the trunk were excavated at two depths (0-20 cm and 20-40 cm). Roots were sifted consecutively through 1.3 cm and 0.64 cm mesh screens. Sifted loblolly roots were sorted by size class: < 2 mm (fine roots), 2-5 mm (coarse roots), > 5 mm (woody roots), respectively. Any remaining lateral roots (below 40 cm soil depth) and the entire taproot were then excavated. All tissues (above- and belowground components) were oven-dried and weighed to obtain standing crop biomass estimates.

### *Carbohydrate Analysis*

Just prior to harvest, tissue samples were collected from two trees per family plot between 1100 and 1700 h (including one tree from total tree harvest) for carbohydrate analysis. Foliar samples of the first two 1997 flushes were collected from three branches from the two trees. Stem cores were removed with an increment borer from the bole of both trees between the 4$^{th}$ and 5$^{th}$ branch whorl (at or just below breast height – approximately 1.3 m). Branch tissue from three branches was collected at the base of the sterile node of 1997 flush one from both selected trees. Taproot cores were removed with an increment borer 10 cm below the whorl of lateral roots nearest the soil surface from both trees. Four soil cores (per tree) were removed within a 30 cm radius of the bole of two trees per family x treatment plot using an AMS (Art's Manufacturing and Supply, American Falls, ID) auger (5.0 cm diameter) in the 0-20 cm soil horizon before the trees were harvested. Live loblolly pine roots from these cores were sifted consecutively through 1.3 cm and 0.6 cm screens, sorted into fine (≤ 1.0 mm) and coarse (> 1.0 mm) roots, and washed in cold water. We have found that total nonstructural carbohydrate

(TNC) concentrations do not vary between size classes > 1 mm (unpublished data). Above- and belowground tissues for carbohydrate analysis were placed immediately on dry ice and stored at -70°C until tissue was freeze-dried.

Freeze-dried tissue was ground with a SPEX® CertiPrep 8500 shatterbox. Ground tissue was extracted with 80% ethanol at 80°C and centrifuged for 10 min at 3000 g (Topa and Cheeseman 1992a). Reducing sugars (glucose + fructose) and sucrose were determined in the ethanol extract using enzymatic analysis (Jones *et al.* 1977). The starch-containing tissue pellet was incubated with amyloglucosidase for 24 h at 55°C and starch concentrations were determined as glucose equivalents using enzymatic analysis. Total nonstructural carbohydrate concentrations represent the sum of starch, reducing sugars, and sucrose concentrations.

### *Statistical Analysis*

The entire experiment is a split-plot design with treatment as the main plot, provenance as sub-plots, and families nested within provenance. Analysis of variance of total and component biomass and carbohydrate content was conducted using SAS® on a plot-mean basis.

## RESULTS AND DISCUSSION

### *Standing Crop Biomass*

As expected, fertilization significantly increased (Figure 1, $P \leq 0.05$) aboveground biomass of all four families corresponding with the previously reported increase in height and volume (McKeand *et al.* 1999). Within a provenance in the fertilized plots, the two harvested faster-growing families (ACP-1 6532±277 g and LPT-1 6327±1637 g) had greater aboveground biomass than their slower-growing counterparts (ACP-2 5468±185 g and LPT-2 3882±483 g). As reported previously for aboveground production (McKeand *et al*. 1999), the two ACP families clearly performed as well or better than the LPT families in terms of total aboveground biomass accumulation in both poor and enriched nutrient environments (Figure 1). It remains to be seen, as the trees shift from the juvenile to the mature life stage and the competition for light and soil resources increases, whether aboveground growth of these families or provenances will remain altered by fertilization.

Although many studies have reported aboveground biomass estimates of loblolly pine (e.g., Ralston 1973; Shelton *et al*. 1984; van Lear *et al*. 1986; Baldwin 1987; van Lear and Kapeluck 1995; Albaugh *et al*. 1998), few have reported belowground biomass estimates for loblolly pine in the field (e.g. Ralston 1973; Harris *et al*. 1977; van Lear and Kapeluck 1995; Albaugh *et al*. 1998) and none from known genetic origin as in the current study. In the present study, fertilization increased the standing crop biomass of belowground tissues of all families (Figure 1, Table 1) and, total tree biomass as well. Similar to the aboveground response, the two faster-growing families (ACP-1 2455±95 g and LPT-1 2278±458 g) had greater belowground biomass than their slow-growing counterparts (ACP-2 2284±75 g and LPT-2 1377±112 g) under fertilized conditions (Figure 1). In fact, under fertilized conditions, the faster-growing LPT family (LPT-1) grew as well as both the ACP families (e.g., above and belowground biomass was similar).

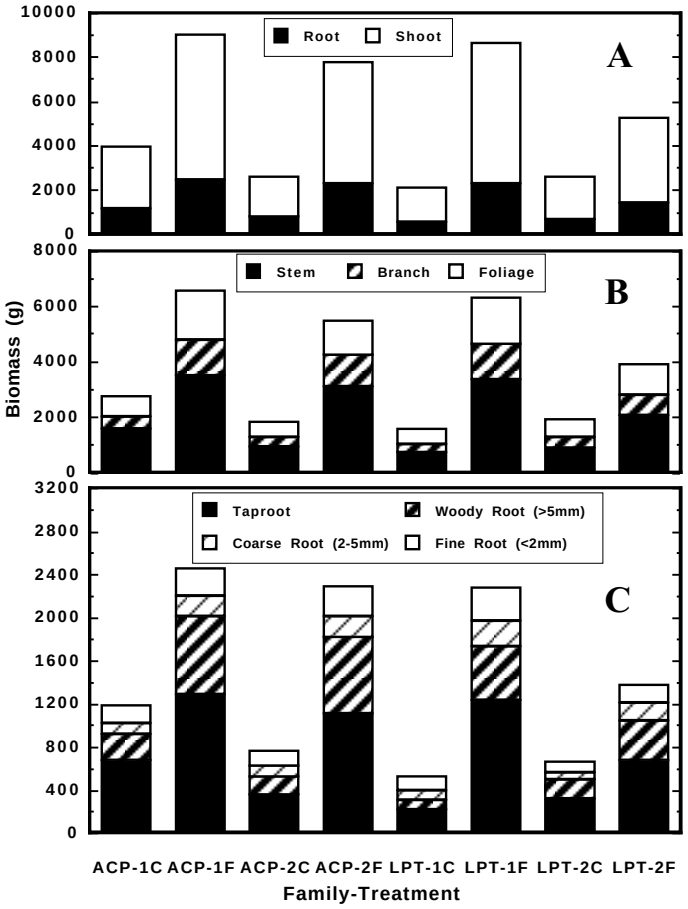



**Figure 1.** Mean biomass data from whole-tree harvest in January 1998 for each of the four loblolly pine families in control (C) and fertilized (F) plots. (a) Total root and shoot biomass; (b) stem, branch, and foliage biomass; and (c) taproot, woody root, coarse root, and fine root biomass. ACP – Atlantic Coastal Plain; LPT – drought-hardy Texas; 1 – fast-growing; 2 – slow-growing; C – control; F – fertilized. n=3.

**Table 1.** Percent of total tree biomass allocated to roots in loblolly pine stands of various ages and site amendments.

| Stand Age (yrs) | % of biomass in roots | Amendments | Method | Reference |
|---|---|---|---|---|
| 5 | <30 | None, Fertilization | 1 | Current study |
| 9-11 | 23-35 | None, Fertilization, Irrigation | 2 | Albaugh *et al*. 1997 |
| 15 | 24 | None | 3 | Harris *et al*. 1977 |
| 48 | 20 | None | 4 | van Lear and Kapeluck 1995 |

1 = Whole-tree excavation of all root classes.
2 = Whole-tree excavation of all root classes > 2 mm; fine roots scaled from cores.
3 = Whole-tree excavation of small number of trees + block excavation and allometry.
4 = Minimal whole-tree excavation of some classes of roots; allometry used to predict lateral root size classes; roots $\leq$ 0.6 cm estimated from cores.

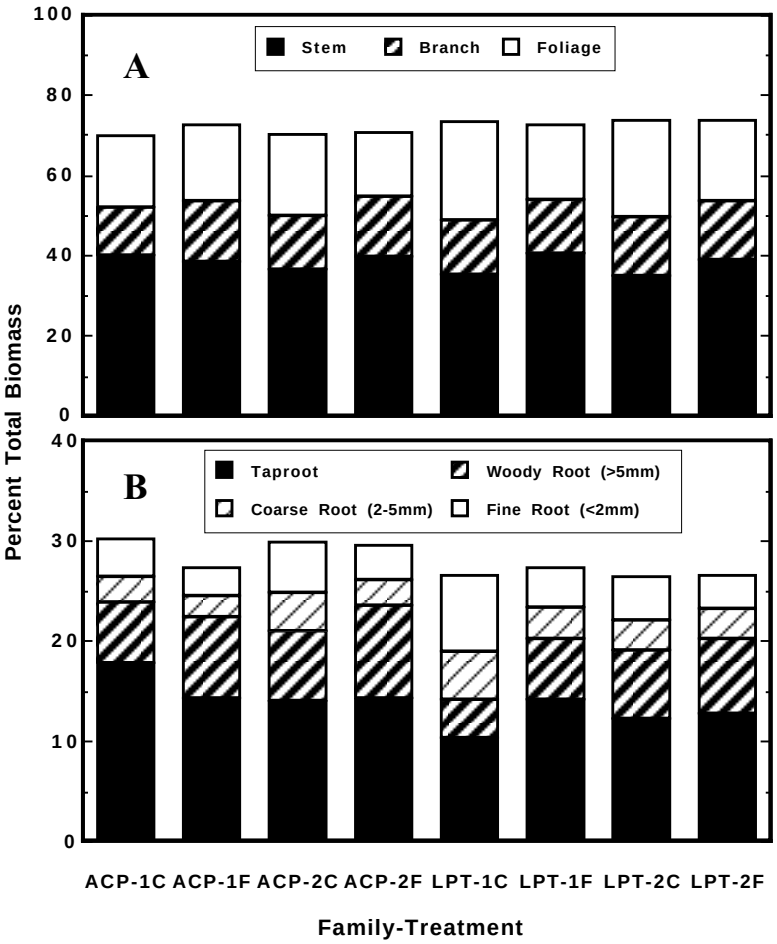



**Figure 2.** Mean percentage of tree biomass allocated to component tissues from whole-tree harvest in January 1998 for each of the four loblolly pine families in control (C) and fertilized (F) plots. (a) Stem, branch, and foliage biomass and (b) taproot, woody root, coarse root, and fine root biomass. Other information as in Figure 1.

Interestingly, although there were treatment and family differences in standing crop biomass of the root, shoot, and various components, percent (whole-tree) allocation to these tissues was the same across treatments (Figure 2, $P > 0.05$). Fertilization increased the standing crop biomass of all tissues except fine roots (Figure 1), but aboveground biomass did not appear to increase at the expense of belowground compartments or vice versa (Figure 2). Our hypothesis that the drought-hardy LPT trees would preferentially allocate more biomass belowground than the ACP trees was not supported by the whole-tree standing crop biomass data. However, our results estimate standing crop biomass at a single time point during the growing season and life span of these trees and also underestimate total root biomass. Further, our constrained sample size limits our conclusions regarding family/provenance differences (n=3 and n=6, respectively), but not treatment differences (n=12).

The percentage of total tree biomass allocated to shoot and root tissues in the current study (>70% shoot and <30% root in all families and treatments) matches very closely with values reported for a number of other biomass harvest studies representing a wide-range of ages and sites within the physiographic region of loblolly pine (Table 1). The cross-study comparison of data would suggest that percent biomass allocation to roots of loblolly pine decreases as trees age. However, caution must be exercised when interpreting these data because of the increase difficulty in extracting the entire root system of trees as they age. Thus, studies are more likely to underestimate standing crop biomass of root systems as trees mature because of a stronger reliance on indirect than direct methods for estimating root biomass than in direct methods (as

**Table 2.** Parameters of the regression log (Y) = a + k log(X) for the entire data set (n=24). Treatment had no significant effect on the regression coefficients ($P \geq 0.05$), nor intercepts ($P > 0.09$). DW = dry weight.

| | a | k | $R^2$ | P |
|---|---|---|---|---|
| Shoot DW on Root DW | -0.2254 | 1.1109 | 0.904 | 0.0001 |
| Root DW on Tree DW | -0.4929 | 0.9844 | 0.971 | 0.0001 |
| Shoot DW on Tree DW | -0.8482 | 1.1143 | 0.9114 | 0.0001 |
| Leaf DW on Tree DW | 0.0294 | 0.8055 | 0.8959 | 0.0001 |
| Stem DW on Tree DW | -0.6471 | 1.0640 | 0.983 | 0.0001 |
| Branch DW on Tree DW | -1.3858 | 1.1459 | 0.9462 | 0.0001 |
| Taproot DW on Tree DW | -1.2923 | 1.1188 | 0.9532 | 0.0001 |
| Coarse + Woody Root DW on Tree DW | -0.9981 | 0.9984 | 0.9282 | 0.0001 |

used in the current study). Of the four studies in Table 1, the current study was the only one that actually harvested all root classes, including taproots and lateral roots < 5 mm diameter, and did not depend upon soil coring and allometric techniques to predict biomass of the various root classes. We did not find soil coring to accurately predict fine root biomass at the tree level.

Standing crop estimates will always underestimate root system carbon demands because they do not include accurate estimates of fine root system (root + mycorrhizae) production and turnover. Estimates of fine root production and turnover in a loblolly pine plantation have been shown to be as high as 9000 kg $ha^{-1}$ $yr^{-1}$ (Harris *et al.* 1977), but more accurate estimates of fine root production and turnover are becoming available with the advent of more sophisticated *in situ* techniques, such as minirhizotron technology. Our preliminary results using such technology is reported in the following section (Section IV).

***Allometric Analysis***

Since S/R ratio and root weight ratios (Figure 2) are subject to genetic, ontogenetic, and environmental influences such as light levels, soil fertility, water availability, growth regime, etc. (Drew and Ledig 1980; Reynolds and Antonio 1996), a more useful measure of assessing treatment effects on biomass allocation during ontogeny is allometric analysis, i.e.,

$$\text{Log } (DW_{shoot}) = a + k \log (DW_{root})$$

where a is a constant, and k is the allometric or regression coefficient of shoot growth on root growth. In the present study, fertilization treatment had no significant effect ($P = 0.9380$) on the

allometric coefficient of shoot dry weight (DW) on root DW (k = 0.997 for control, k = 0.974 for fertilized treatment), nor on the y-intercepts (P = 0.2931). The allometric coefficient for the complete data set of shoot DW on root DW (k = 1.111) is slightly higher or comparable to that reported for southern pine seedlings (e.g. Drew and Ledig 1980; Topa and Cheeseman 1992b).

Although fertilization significantly increased tree biomass or tree size, it had no effect on the instantaneous root weight ratio, nor on the allometric relationships between the dry weight of the various above- and belowground tissues and tree size (Table 2). King *et al*. (1999) reported small shifts in biomass allocation in field-grown loblolly pine under fertilization, with increased partitioning in perennial belowground tissues (taproots + coarse roots) relative to perennial shoots (branches + stems). In the current study, trees in both fertilization treatments allocated more dry weight to perennial (woody) tissues than to foliage, in particular stems, branches and taproots, than to foliage.

***Root Distribution***

Over 70% of lateral roots in all families were found in the 0-20 cm soil horizon (Figure 3). Although taproots of all families extended down more than 0.9 m, we found no proliferation of lateral roots below the 40 cm depth. Existing literature (albeit with seedlings, e.g. van Buijtenen *et al*. 1976) would suggest that drought-hardy families would have deeper taproots and lateral roots when growing on a deep sandy site such as the Scotland County site. It is possible that as our stand matures and stand water demands increase, both taproots and lateral roots will extend and proliferate into deeper soil horizons.

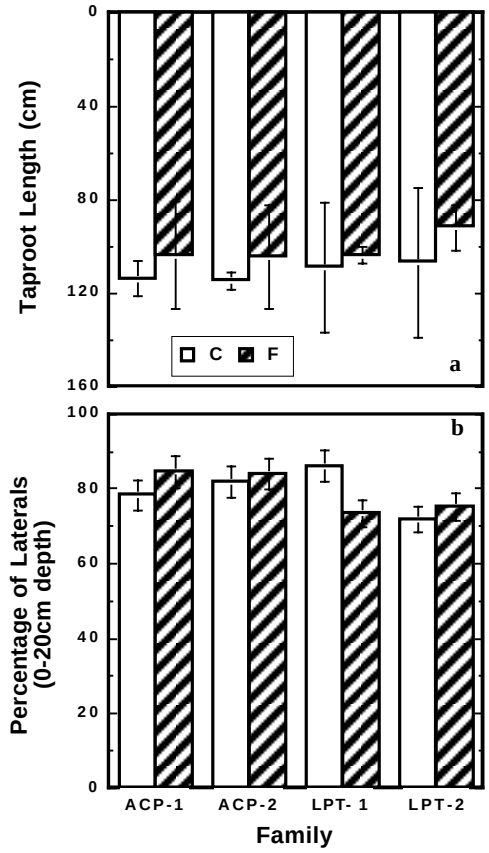



**Figure 3**. (a) Mean taproot length from the January 1998 root excavation. (b) Mean percentage of lateral root biomass in the 0-20 cm soil profile (lateral root biomass in 0-20 cm profile/total lateral root biomass) from the January 1998 root excavation. Bars represent ± SE. n = 3. Other information as in Figure 1.

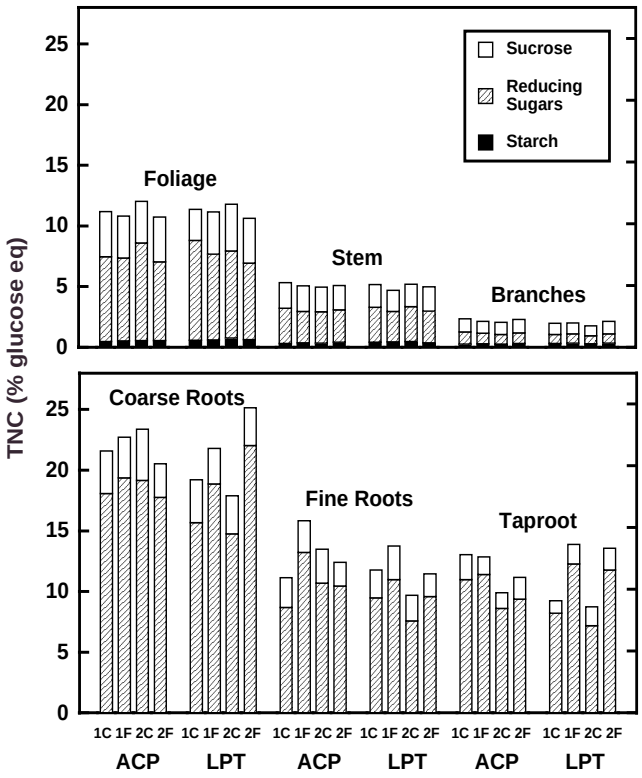
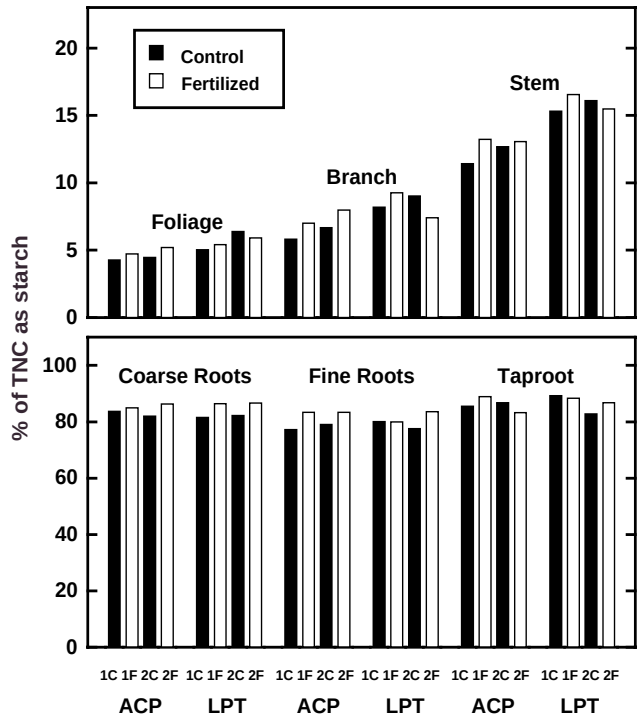



**Figure 4**. Mean total nonstructural carbohydrate (TNC) concentrations in tissue from the whole-tree harvest in January 1998 for each of the four loblolly pine families in control (C) and fertilized (F) plots. Other information as in Figure 1. n = 6.

***Carbohydrate Partitioning***

Fertilization had little effect on TNC concentrations of most tissues, excepting taproots (Figures 4 and 5). Fertilization increased TNC concentrations of taproots by 21%, primarily due to an increase in absolute starch concentrations. Although starch concentrations of all root tissues of fertilized trees were 15-25% higher than control trees, a treatment effect was only significant in taproots. In aboveground tissues, absolute starch concentrations and partitioning of TNC towards starch were most influenced by provenance and family effects, with LPT families having higher starch concentrations in needles, and over 20% higher partitioning of TNC in starch than ACP families.

Field studies with loblolly pine (Adams *et al*. 1986) and slash pine (*P. elliottii* Engelm. var. *elliottii*) (Gholz and Cropper 1991) also found minimal fertilization effects on starch and sugar concentrations in above- and belowground tissues, with seasonal effects more significant than fertilization. The lack of pronounced fertilization (Adams *et al*. 1986; Gholz and Cropper 1991; current study) and family/provenance effects (current study) on tissue carbohydrate storage patterns in southern pines would suggest strong endogenous control over partitioning of assimilated carbon to storage reserves in field trees. A strong seasonal influence on starch concentrations in southern pines is indicated by our study (Sections V and VI), but is not unexpected given the dynamic or seasonal nature of photosynthesis and shoot and root growth. The lowest seasonal concentrations of starch vary with tissue type, but in southern pines, concentrations are often lowest in late fall or early winter (Adams *et al*. 1986; Gholz and Cropper 1991), when photosynthetic rates are at their lowest and the metabolic dependency on carbon stores may be at its highest.




In the present study, the largest differences in absolute TNC concentrations and carbohydrate partitioning were found between above- and belowground tissues. Needles had the greatest TNC concentrations of any aboveground tissue, exhibiting twice as much as branches and five times as much as stems (Figure 4). All root tissue had higher concentrations of TNC than branches or stems (Figure 4), with coarse roots exhibiting the highest TNC concentrations of any tissue. Although fine roots, taproots, and needles had similar TNC concentrations, over 75% of TNC in all root tissues were partitioned towards starch, compared with only 6% starch in needles (Figure 5). In the current study, more than 90% of total tree starch in January was stored in loblolly pine roots (even though roots collectively represented less than 30% of total tree biomass) (Figure 6). Based upon biomass, the highest percentage of tree starch was stored in the larger woody root classes, i.e., taproots (35%) and woody roots (32%) while coarse and fine roots accounted for 14% and 12%, respectively.

Both the absolute TNC and partitioning data in the current study suggest that roots, more than aboveground tissues, function as storage organs for carbohydrate reserves in loblolly pine during this time in the growing season. At this time in the growing season (late winter), any net carbon gain in needles from photosynthesis would be expected to be low (Murthy *et al*. 1997), with recently acquired carbon utilized to support metabolic demands of the closest carbon sinks, i.e. needle and perhaps branch tissues. The carbohydrate partitioning data, in particular the low percentage of TNC present as starch, suggests that needle (and most likely branch and stem) metabolism is probably being supplemented with stored carbon from root tissues. Increased partitioning of soluble sugars to sucrose in stems suggests remobilization of stored carbon for transport to carbon sinks (Table 3).

Our TNC data do not suggest that genetic differences in aboveground growth are the result of differences in carbon partitioning to the various TNC fractions. Overall, our data also suggest (tempered by our constrained sample size) that genetic differences in carbon allocation to belowground biomass in loblolly pine is not a contributing factor towards differences in

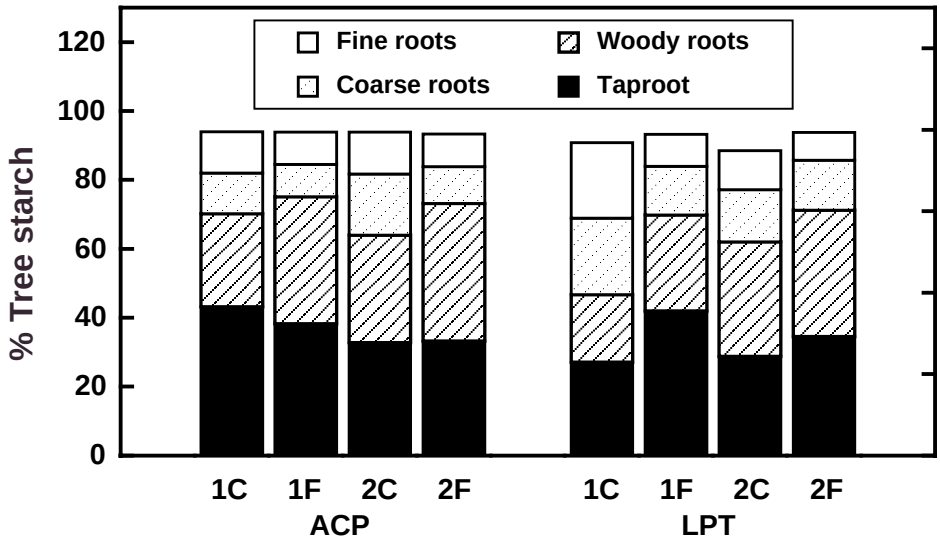



**Figure 6.** Mean percentage of total tree starch in various root tissues from whole-tree harvest in January 1998 for each of the four loblolly pine families in control (C) and fertilized (F) plots. Other information as in Figure 1.

aboveground growth. However, our conclusion is based on standing-crop biomass estimates at one time-point in the juvenile life stage. Slower-growing families could have higher root system carbon demands because of greater root production and turnover and we are currently investigating this possibility. It is also possible that a greater investment in fine root production during the juvenile life stage may ultimately result in faster growth once the canopy closes and root competition for soil resources becomes more intense. One would expect that some identifiable physiological character would be key to define the growth capacity of each genotype.

**Table 3.** Mean percentage of soluble sugars present as sucrose in tissues of loblolly pine from the whole-tree harvest in January 1998. Since there were no significant family (provenance), provenance, and treatment differences ($P > 0.05$), data were pooled. Mean ± S.E. n=48.

| | |
|---|---|
| Flush 1 | 33.00 ± 1.74 |
| Flush 2 | 32.43 ± 1.71 |
| Branch | 41.96 ± 0.47 |
| Stem | 55.23 ± 0.52 |
| Coarse roots | 40.06 ± 1.17 |
| Fine roots | 47.39 ± 1.09 |
| Taproot | 44.71 ± 1.50 |

Genotypes that produce high stem volume may grow fast for many different reasons (e.g. McKeand and Svensson 1997). We will continue to monitor trees in this stand as they progress from the juvenile to mature life stage and the competition for light and soil resources increases to determine whether one or multiple characters is strongly associated with growth capacity.

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## TASK 1: ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

## III. SEASONAL VARIATION IN WATER SOURCE

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### INTRODUCTION

Because of its wide geographic range, it is not unexpected to find significant genotypic variation in growth of loblolly pine (*Pinus taeda* L.) across various environments. The "Lost Pines" Texas (LPT) provenance of loblolly pine is considered to be more drought hardy and more slow growing than its more mesic counterparts from the Atlantic Coastal Plain (ACP). The "Lost Pines" provenance originates more than 160 km west of the continuous range of loblolly, in the Texas counties of Bastrop, Fayette, and Caldwell (van Buijtenen *et al*. 1976). Trees in these isolated pockets receive 25-50 cm less annual and 10-15 cm less July-August precipitation than trees in the pine belt of eastern Texas (Bilan *et al*. 1977). Seedling survival data from both controlled-drought experiments and the field suggest that LPT trees from Bastrop County, TX, the most arid loblolly pine habitat, are more tolerant to drought than trees from eastern Texas (Zobel and Goddard 1955; Goddard and Brown 1959; van Buijtenen 1966; van Buijtenen *et al*. 1976). Seedling studies also suggest that their drought hardiness may be more a function of drought avoidance mechanisms than drought tolerance since (1) rapid transpiration occurs when water is available, but water is conserved during stress, (2) root systems are deep with wide ranging laterals, and (3) needles are small with deep stomatal pits and few stomata per unit leaf surface area (van Buijtenen *et al*. 1976; Bilan *et al*. 1977). Furthermore, greenhouse studies with drought-hardy loblolly pine seedlings suggest that root ontogeny, architecture, and shoot to root biomass partitioning are under strong genetic control (Topa and McLeod 1986a, 1986b).

Strong genetic control of biomass partitioning and carbon allocation to root systems may influence a plant's ability to exploit the various regions of the soil for nutrients and water, particularly when demands are high and resource availability may be low. Very little is known about the patterns of resource utilization by saplings and mature trees in the field because of the difficulty in studying *in situ* processes such as water uptake by roots directly (Ehleringer and Dawson 1992). Recently, stable hydrogen isotope technology has been used to examine source water that a plant is using at the time of analysis (Ehleringer and Dawson 1992).

In the following study, we compared $\delta D$ "signatures" in xylem sap with $\delta D$ "signatures" of soil water from four soil depths to assess possible functional differences in water acquisition between four families of loblolly pine planted at the xeric Scotland County field site in North Carolina. We wanted to examine which roots (surface or deep roots) were most active in water

uptake. We hypothesized that drought-hardy Texas trees from the western portion of the range of loblolly pine would utilize different source water than trees from the Atlantic Coastal Plain because they had evolved deep root systems that would permit access to a deeper, more stable water supply during moisture-limiting periods of the growing season.

## MATERIALS AND METHODS

For the current study, we selected the fastest- and slowest-growing families from each provenance based upon evaluation of four-year aboveground height growth of control trees (McKeand *et al*. 1999). Families ACP-1 (8-1118), ACP-2 (9-1046) were the fast- and slow-growing ACP families, and LPT-1 (BA3L11-1) and LPT-2 (GR1-2) were the fast- and slow-growing drought-hardy Texas families, respectively. Family LPT-1 originates from Bastrop County in the "Lost Pines" region of Texas. We also focused our analysis in three blocks (6, 8, and 10) of this large study because sample size constrained data collection.

### *Tree selection for tissue and soil isotope sampling*

During each sampling period (September and November, 1997, and March, May, July, September, and November, 1998), the order of sample collection from the three blocks, treatment within each block, and row within each family plot was randomly selected. The same row was sampled in all three blocks during each sampling period. Twig and soil samples for $\delta D$ analysis were collected from two trees representing 80% of the population (based upon aboveground height in the selected plot); a third tree was selected using the same criteria for soil sampling only. Soil and twig samples for $\delta D$ analyses were collected between 0800 and 1200 h when the trees were actively transpiring (data not shown). Samples from one block were collected each morning on three concurrent days.

### *Soil and twig sampling*

Soil samples were collected at the dripline of the selected trees from the following depths: 0-20 cm, 21-40 cm, 1.2 m and 2.1 m (Figure 1). Samples in the upper soil horizons (0-40 cm) were collected with an AMS (Art's Manufacturing and Supply, American Falls, ID) 2.54 cm diameter soil probe (three cores per family plot), while deeper samples (1.2 m and 2.1 m) were extracted with an JMC Environmentalist Soil Probe (ESP) Plus (Clements Associates, Inc., Newton, IA) 3 cm diameter soil probe (one core per plot). Both of these soil-coring devices employed a removable plastic liner, which not only allowed intact removal of the soil core, but also minimized evaporation and, therefore, isotopic fractionation (which we discovered could occur in 15-30 seconds and result in significant fractionation) during sample collection.

Soil cores collected with the AMS probe were located at the dripline of the three sample trees. Representative soil samples were collected from the 0-20 cm and 21-40 cm depths by placing a soil-sampling vial into specially constructed forceps and pushing the vial into the soil core. The ESP core(s) were removed from the soil at an additional location in the dripline of the three sample trees. Soil samples were collected from the ESP cores at 1.2 m and 2.1 m.

Two twig samples for isotope analyses were collected from each sample tree (#1 and #2) after all the soil samples had been extracted from the ground. Twigs from branches that were sun exposed (1.2-1.5 m up into the canopy) were severed at the base of the sterile node of the most recent, fully expanded flush and a 4 cm sample was collected. Soil and twig samples collected from each family/treatment plot were sealed immediately after collection and stored at 4°C. Upon return to Ithaca, NY, samples were placed in a -20°C freezer until final processing.

***Precipitation and groundwater sampling***

One bulk precipitation collection gauge was placed in each treatment/block. Each gauge consisted of a 15 cm diameter screened funnel connected to a 4 l Nalgene® bottle with approximately 60 cm of coiled black butyl tubing. Algal growth in the bottles was prevented by painting the bottles black then white and covering the bottle with aluminum foil. Precipitation samples pooled all the precipitation events occurring between each sampling period. Two groundwater samples were also collected from a deep well (40 m) at the adjacent USDA Forest Service site during each sampling period. Collected precipitation and groundwater samples were stored at 4°C. Upon return to Ithaca, NY, samples were placed in a -20°C freezer until final processing.

***Protocol for sampling soils for moisture content***

Soil samples for moisture content analysis were collected at the 0-20 cm, 21-40 cm, 1.2 m and 2.1 m depths from the same cores used for δD sampling. Following collection, soil fresh weights were measured and the samples were oven-dried at 70°C for 7-10 days before dry weights were measured. Soil moisture content was determined by dividing water loss by soil fresh weight.

***Protocol for isotope analysis***

Water from soil and twig samples was cryogenically extracted under vacuum (Dawson and Ehleringer 1991). Extracted water from soil and twig samples and water from precipitation and groundwater samples were reduced to its diatomic form using. All water samples were analyzed by mass spectroscopy at CoBSIL (Cornell University - Boyce Thompson Institute Stable Isotope Laboratory, Boyce Thompson Institute for Plant Research, Ithaca, NY) on a Finnagan® MAT Delta Plus triple collecting isotope ratio mass spectrometer. Deuterium content is expressed in δ-notation at δD (parts per thousand, ‰) relative to Vienna-Standard Mean Oceanic Water (V-SMOW):

$$\delta D = ((D{:}H_{sample}/D{:}H_{standard}) - 1)*1000$$

where $D{:}H_{sample}$ and $D{:}H_{standard}$ represent the molar D/H ratios of samples and the V-SMOW standard, respectively. The overall precision of preparation and analysis was ± 1.5 ‰ and ± 1.0 ‰, respectively. Twig samples from family ACP-2 were not analyzed in all months in order to reduce sample preparation and analysis costs.

### *Statistical analysis*

The entire experiment is a split-plot design with treatment as the main plot, provenance as sub-plots, and families nested within provenance. Analysis of variance of xylem and soil δD were conducted using SAS® on a plot-mean basis.

## RESULTS AND DISCUSSION

### *Bulk precipitation*

Bulk precipitation collected at our Scotland County, NC, field site from July 1997 through November 1998 (pooled precipitation events) ranged from -12.4 to -41.9 ‰ and had an average δD value of -24.6 ‰ (Table 1). For reference, monthly composite precipitation samples collected at Hatteras, NC, by the International Atomic Energy Agency (IAEA) between 1961-81 had a weighted (by precipitation amount) mean δD value of -24.5 ‰ (Yurtsever and Gat 1981). Because of the relatively short time frame (approximately one year) of precipitation collection in our study, it is unclear how great seasonal (over multiple years) differences in isotopically distinct precipitation might be at our Scotland County, NC, study site. However, groundwater isotopic composition reflects a weighted average of annual precipitation inputs (Valentini *et al*. 1994). Since groundwater collected from a 40 m deep well near our field site had an average δD value (-34.3 ‰ over the study period) similar to our precipitation δD values (-24.6 ‰) it is unlikely that extreme seasonal differences (>30 ‰) in isotopically distinct precipitation characterize our Scotland County field site.

Table 1. Bimonthly pooled precipitation δD values collected from July 1997 through November 1998 at SETRES2 in Scotland County, NC.

| Collection Date | δD value (‰) |
|---|---|
| July 1997 | -30.0 |
| September 1997 | -12.4 |
| November 1997 | -41.9 |
| March 1998 | -26.6 |
| May 1998 | -16.5 |
| July 1998 | -19.1 |
| September 1998 | -34.0 |
| November 1998 | -21.3 |
| Average | -24.6 |

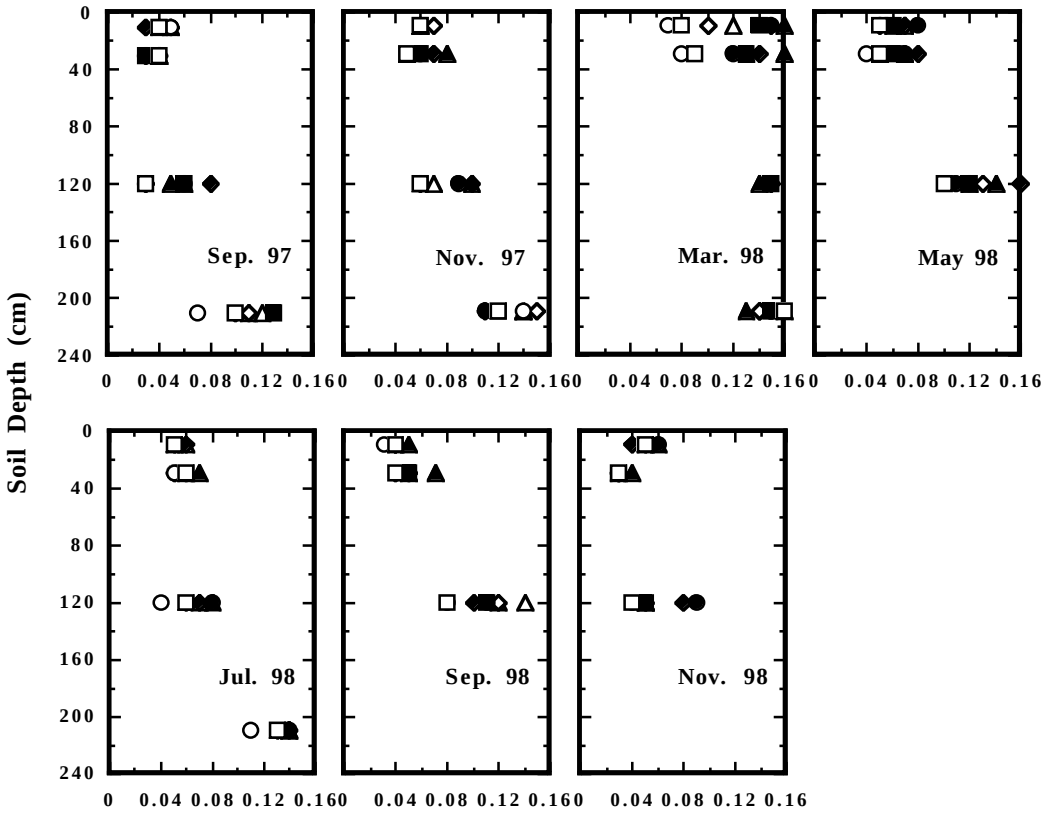


**Figure 1.** Mean soil moisture content values across the soil profile in various family plots bimonthly from September 1997 through November 1998. n=9 in the 0-20 cm and 20-40 cm depth, n=3 in the 1.2 m and 2.1 m depth. Families ACP-1 (□) and ACP-2 (○) are fast- and slow-growing Atlantic Coastal Plain genotypes, while families LPT-1 (◇) and LPT-2 (△) are fast- and slow-growing drought-hardy Texas genotypes, respectively. Open symbols = control plots, closed symbols = fertilized plots.

***Soil water δD***

At the Scotland County study site, soil moisture content increased with increasing soil depth in all months except March 1998 (Figure 1). Therefore, it was considerably drier (soil moisture content < 0.08 vol/vol) in the upper soil layers compared to the deeper soil layers in all months except March 1998. As a consequence of evaporative fractionation and isotopically different precipitation inputs, water samples collected from each soil layer (0-20 cm, 20-40 cm, 1.2 m, and 2.1 m) had varying soil water δD "signatures" in most sampling months (Figure 2). Others have also shown that the soil water δD "signature" varies with soil moisture and soil depth and have used δD "signatures" to identify the source water for different plant species (e.g., Valentini *et al*. 1994; Dawson and Pate 1996; Jackson *et al*. 1999; Meinzer *et al*. 1999).

At our study site, soil water δD values declined in September 1997, and March and November 1998 with increasing soil depth (Figure 2). During dry periods, water in the upper soil layers may become substantially enriched in the heavier isotope of hydrogen (D) through evaporative

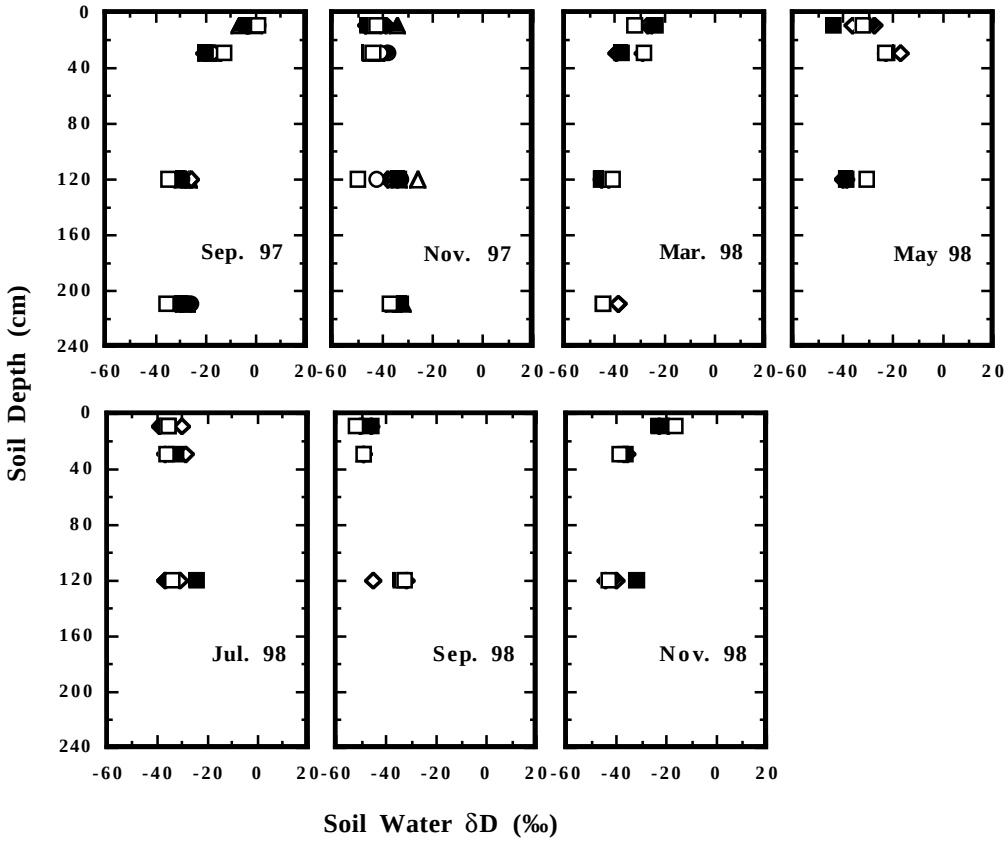


**Figure 2**. Mean soil water δD values across the soil profile in various family plots bimonthly from September 1997 through November 1998. Other information as in Figure 1.

fractionation, resulting in variation in the isotopic composition of soil water with depth (Zimmermann *et al*. 1967; Allison and Hughes 1983). In the current study, δD values in upper soil layer (0-20 cm) during November 1997 and July and September 1998 were less enriched than deeper soil layers (1.2 and 2.1 m) even though soil moisture content increased with depth (Figures 1 and 2). The isotopically lighter soil water in the upper soil profile in these sampling months (compared to δD values in the same soil layers and deeper soil profile δD values in other sampling months) must be a consequence of an individual, isotopically lighter, precipitation event that fell before the data collection periods. Discrepancies between the precipitation δD values and the δD values of the upper soil layers are the result of pooling individual precipitation events. Recorded differences between soil and precipitation δD values in the upper layer indicate that the upper soil profile dries out between rain events and is replenished with isotopically different precipitation.

***Xylem water δD***

Xylem water δD values were not statistically different ($P \leq 0.05$) between families on any of the measurement dates in this study (i.e., all families were obtaining their source water from the same soil depths, Figure 3). Further, within a sampling period and within a soil layer, soil water δD values were not statistically different between treatment plots nor blocks. Xylem water

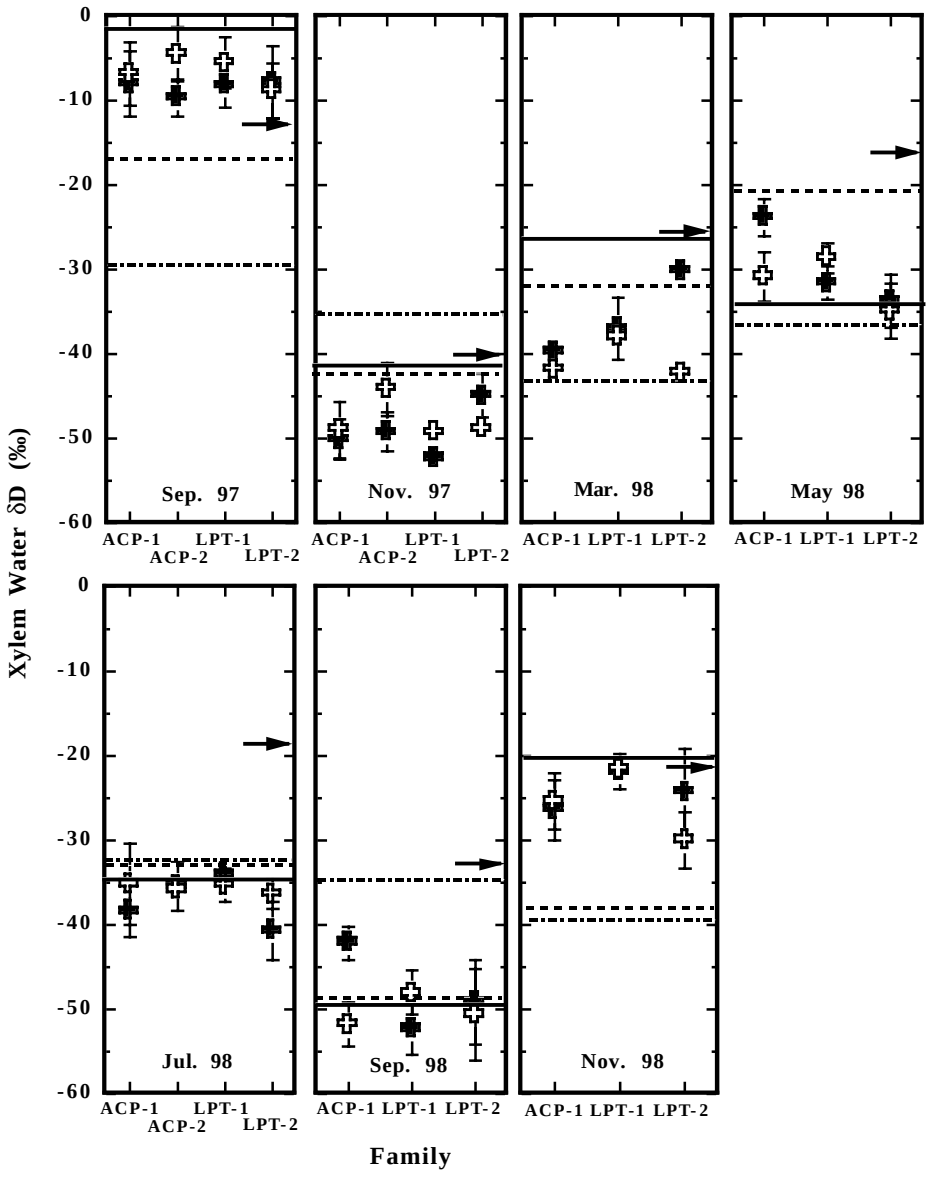


**Figure 3.** Mean xylem water δD values from twigs collected in September and November, 1997. Bars represent ± se and are shown when they are larger than the data symbol. n=6. Control twigs (✚), fertilized twigs (✚). Dashed lines represent the mean soil water δD values from the three different soil depths on each date; 0-20 cm (——), 20-40 cm (·····), and 1.2 m (— · — · ). Arrows represent the δD values of the bulk precipitation collected each month (from Table 1).

δD values from twig samples in all collection months except March 1998 closely matched soil water δD values from the upper two soil depths (0-20 and 20-40 cm). These results indicate that loblolly pine trees at our study site were obtaining the bulk of their source water from the upper soil horizons throughout most of the year (Figure 3). Other *Pinus* species (*P. sylvestris* L. and *P.*

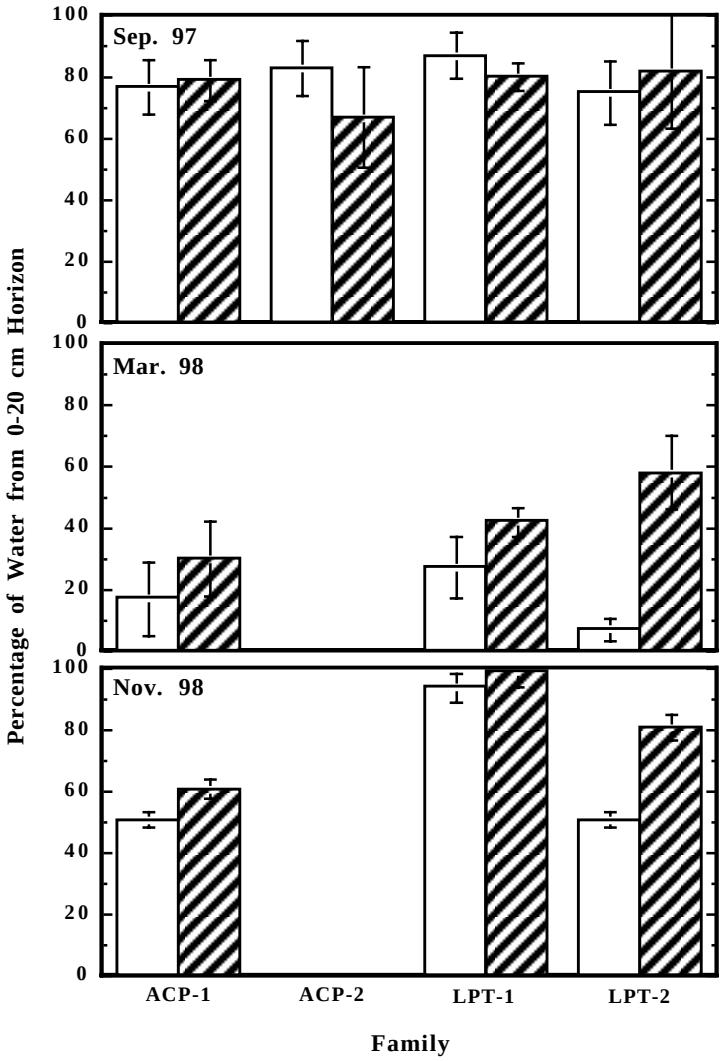


**Figure 4.** Mean percentage source water uptake from 0-20 cm soil horizon in September 1997 and March and November 1998. Values calculated from the following equation: $a = (\delta D_{xylem} - \delta D_{1.2})/(\delta D_{0-20} - \delta D_{1.2})$. Bars represent ± se. n=3. Open bars = control plots, cross-hatched bars = fertilized plots. Other information as in Figure 1.

*cembra* L.) have also been found to utilize water from the upper soil horizons as source water (Valentini *et al*. 1994).

In September 1997, March 1998, and November 1998, δD values of xylem water were between δD values recorded in the 0-20 cm (most isotopically positive) and 1.2 m (most isotopically negative) soil depths indicating that source water may be coming from a range of soil depths. Waters of different isotopic composition in the environment are at some point mixed in various proportions (Dawson 1993). In order to further confirm our hypothesis that drought-hardy families would extract water from deeper in the soil profile, we used the following two-end-member mixing model:

$$\delta D_{xylem} = \delta D_{0-20} a + \delta D_{1.2}(1-a)$$

to calculate the percent source water uptake from the 0-20 cm soil depth ($a$) in September 1997, March 1998, and November 1998:

$$a = (\delta D_{xylem} - \delta D_{1.2})/(\delta D_{0-20} - \delta D_{1.2}).$$

Our calculated values for *a* (percent source water obtained from the 0-20 cm soil depth) suggest that in September 1997 (the month with the lowest mean soil moisture values at 1.2 m), all of the families in the control and fertilized plots obtained between 67-87% of their source water from the upper 20 cm of the soil profile (Figure 4), and at least 13% from deeper in the soil profile. In March 1998, families ACP-1 and LPT-1were obtaining less than 40% of their source water from the upper (0-20 cm) soil profile and more than 60% from deeper horizons. Our minirhizotron data suggest that the high percentage (60%) of water uptake from the upper soil horizon by family LPT-2 in March 1998 is a direct result of increased fine root production by this family in the 0-20 cm soil horizon in the fertilized plots. All other families showed either a reduction or no change in fine root production in response to fertilization and our source water data reflect this. It is interesting to note that deep source water use by loblolly pine in the present study comes at a time when water is not limiting (soil moisture was > 0.08 vol/vol, Figure 1). January through March are typically the wettest months at the Scotland County site (Albaugh, personal communication). Deep source water use during the winter months (November - March) and prior to spring leaf expansion may be the result of cooler soil temperatures, reduced shoot demands, or differing root system function. Wet surface soils are subject to more extreme temperatures than deeper soil horizons and are thus more prone to freezing and a freeze-thaw cycle. Our data suggest that water uptake by fine roots and mycorrhizae in the upper horizons may be at a minimum (as demonstrated by our data) during the cool, wet winter months. Further testing is necessary to see if this is indeed a consistent, winter trend.

It remains to be seen whether families of loblolly pine at this study site would exploit source water from different depths in the soil profile under more extreme moisture limiting (drought < 0.04 vol/vol soil moisture) conditions. All four loblolly pine families in the present study exhibit similar rooting characteristics, i.e. deep penetrating taproots, with over 70% of all lateral root biomass in the upper 0-20 cm soil horizon. Because all families had taproots that extended below 1 m in the soil profile, source water uptake from this soil depth would be possible. The low number/distribution of lateral roots in this deeper soil horizon might suggest minimal contribution to total water uptake. However, our mixing model results demonstrate significant source water uptake from the deeper (below 20 cm) soil depths at certain times of the year. Both the $\delta D$ values and root distribution data indicate that lateral roots of loblolly pine in the upper soil profile are most active in water uptake throughout most of the year despite the intense competition for soil resources in this zone and access to a more reliable water source in deeper soil layers. We hypothesize that the extensive mycorrhizal network (over 90% of short roots are colonized by mycorrhizae at this study site) helps maintain water uptake from the upper soil horizons during the drier summer months. However, taproot extension data suggest that all four families of loblolly pine have the potential to increase water uptake from deeper in the soil profile if necessary (i.e., during extreme drought) as they did in March 1998.

Although field provenance tests indicate that drought-hardy loblolly pine seedlings from the interior regions, such as Texas, consistently suffer less drought-induced mortality than trees from the more mesic, coastal regions (Wells and Wakeley 1966; Wells 1983; Lambeth *et al*. 1984) it is not clear whether similar seedling root traits associated with the drought-hardy families occur in the sapling and mature life stages. Using stable isotope technology, we have shown that Texas drought-hardy loblolly pine families from the western extent of this species natural range do not extensively use deeper sources of water during seasonal dry periods, nor do

they have deeper roots than ACP families, at least at this life stage. Indeed, our mixing model data suggest that both drought-hardy families, LPT-1 and LPT-2, exhibited a great reliance on water sources from the upper soil horizons even though taproots penetrated to deeper soil depths with greater soil moisture (Figure 4). It remains to be seen whether dimorphic patterns of water use by loblolly pine may become more prevalent as trees at our field site mature and it becomes necessary to exploit a larger volume of soil resources to meet demands

A greater reliance in a deeper water source by all families was only observed in late winter. It has been shown in a similar study in Scotland County, NC, that loblolly pine growth appears to be limited primarily by nutrients and secondarily by water (Albaugh *et al*. 1998). It may be that source water uptake from the upper soil profile is a consequence of root system function related to nutrient uptake in this rooting zone. Utilizing the same roots for water and nutrient uptake in an area of the soil where mycorrhizal associations are most prevalent would be the most carbon efficient growth strategy – if the fine root system could meet the tree's water demands. Whether drought would induce further proliferation of roots in the deeper soil horizon, particularly in the drought-hardy families of loblolly pine, is unknown at this time. The ability to switch rapidly among different water sources could put a plant at an advantage if competition for water occurs within the ecosystem (Ehleringer and Dawson 1992) or water becomes extremely limited. However, maintenance of two root systems in different soil horizons (one for nutrient and one for water uptake) would come at an additional carbon cost.

**Acknowledgments:** This research was conducted by the Boyce Thompson Institute for Plant Research and the North Carolina State University as part of cooperative agreement U.S. DOE Agenda 2020 Sustainable Forestry Research Initiative #DE-FC07-97ID13527. Additional funds were provided by the endowment of the Boyce Thompson Institute for Plant Research, the North Carolina State University Tree Improvement Program, the North Carolina State University Forest Nutrition Cooperative, Bowater Inc., Champion International, Georgia-Pacific Corp., Rayonier, Westvaco, Weyerhauser Company, and Southern Illinois University Edwardsville.

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## TASK 1: ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

## IV. FINE ROOT SYSTEM TURNOVER IN LOBLOLLY PINE: INFLUENCE OF GENETICS AND ENVIRONMENT

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Root system carbon demands, particularly fine root turnover, may represent one of the largest carbon sinks in loblolly pine (*Pinus taeda* L.) Yet, we have a poor understanding of not only what controls root lifespan, but how much annual carbon is used to support fine root system (roots + associated mycorrhizae and microbes) growth, respiration, maintenance, nutrient uptake and carbon exudation. Estimates of fine root production and turnover are noticeably lacking for southern pine forests. Other coniferous forest studies suggest that fine root system production may account for 60-77% of net primary productivity (Harris *et al*. 1977; Agren *et al*. 1980; Fogel 1983). A study with slash pine (*Pinus elliottii*) suggests that fine root production may represent 30-50% depending upon stand age (Gholz *et al*. 1986). Quantifying *in situ* fine root system carbon demands associated with construction (structural carbon + respiratory losses), ion uptake and maintenance is key to understanding whole-tree carbon allocation and partitioning patterns in southern pine forests, and the contribution of fine root system turnover to stand and nutrient budgets.

Since fine root production and maintenance costs in conifers may be as much as 60-80% of total net primary productivity, yield variation among loblolly pine families could be associated with genetic differences in belowground carbon demands. Identifying how whole-tree carbon source/sink relationships may change with age and edaphic stress, with an emphasis on root system carbon demands, is central to any genetic regulation of photosynthate to harvestable (e.g. stem or bole) and non-harvestable tisses. Because fine root systems of trees are dynamic, undergoing aging, death and regeneration, standing crop estimates of fine root biomass (at any singular point in time) will underestimate annual carbon demands associated with fine root system production. Accurate estimates of fine root system production and mortality (turnover) are necessary to quantify annual belowground net primary productivity in southern pine and other coniferous forests. Unfortunately, accurately quantifying root system turnover has been one of the most intractable problems in the study of forest ecosystems (Eissenstat and Yanai 1997) because of the technological difficulty in directly measuring fine root system demography. The paucity of fine root production and mortality data is further confounded by the variety of methods that have been used to obtain such data, often making cross-study comparisons difficult.

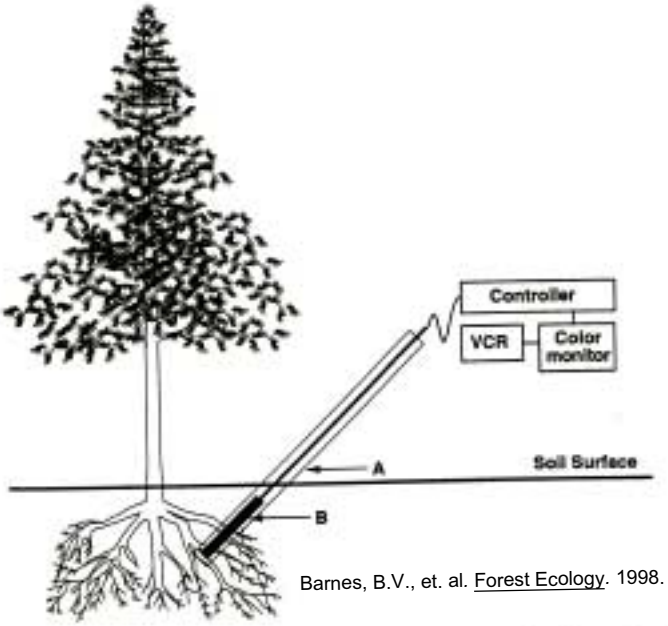


**Figure 1.** With minirhizotron technology, a clear plastic tube is placed permanently in the soil at a 45° angle (A), and (B) a micro-video agricultural camera can then be inserted into the tube to record root images at various locations along the tube. Thus, the same cohort of roots can be observed over time.

Until recently, the most popular method for studying fine root mortality and production has been sequential soil coring (usually on a monthly basis, Vogt and Persson 1990). However, minirhizotron technology has emerged as perhaps the best direct method for examining fine root system turnover (Figure 1), but estimates for root production are best obtained using a combination of the two methods. Clear plastic tubes are inserted in soils of established forests, and using miniature agricultural cameras, root images can then be recorded on video tape (or directly on computers) on a regular basis. The births and deaths of the same cohort of roots can then be followed over time. In the following study, we used a combination of soil coring and minirhizotron techniques to examine whether there are genetic and treatment differences in fine root system production and turnover in selected families at the Scotland County field site. We hypothesized that faster-growing families would be more responsive to fertilization than the slower-growing families (i.e. exhibit a more plastic response), showing larger reductions in fine root system production. We also hypothesized that mycorrhizal colonization would be affected by the host loblolly pine family and by the fertilization treatment. We used gross morphological traits to categorize mycorrhizal root tips into ectomycorrhizal morphotypes.

## MATERIALS AND METHODS

### *Root image collection*

One hundred forty four clear acrylic minirhizotron tubes (6 feet in length) were installed from September 97 through January 98 in blocks 6, 8 and 10 at the Scotland County, NC, field site. Two tubes were installed at a 45° angle per tree, and three contiguous trees were selected from each family plot; thus, there were three trees per experiment unit. The disturbance associated with tube insertion, i.e. removal of soil and associated roots, necessitates a lag period of at least 6-12 months before roots recolonize the soil volume and turnover data reflect growth and mortality in undisturbed soil (Hendrick and Pregitzer 1993; Joslin and Wolfe 1998; Steele *et al*. 1997). Consequently, we did not start collecting root data until September 1998, and recorded images until January 2000. Using a Bartz Technology BTC-2 color video minirhizotron camera system, we recorded root images from the upper 0-40 cm soil horizons approximately every 4 weeks (Figure 2). The view field for this camera is 11 mm x 16 mm. A

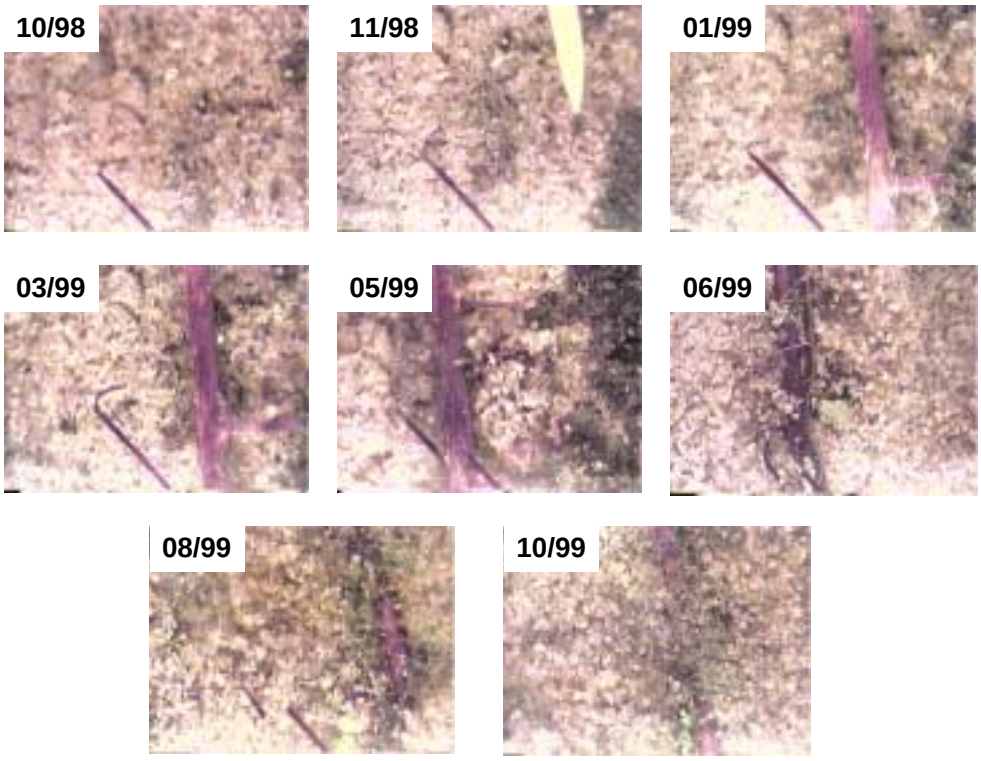


**Figure 2**. Example of root images and demographic data that can be collected over time on the same cohort or roots using minirhizotron technology. A white root tip appears in 11/98 (i.e. is born), turns brown and begins to disappear or die by 10/99.

mechanical advancing handle attached to the camera was used to assure precise camera registration within each minirhizotron tube during each image collection event. Images were recorded on VHS videotapes.

Using Adobe Premier software, root image movies were converted to individual frames and then digitized using MAC Graphics Converter software. Roots in the individual frame were then traced using RooTracker software; the condition of the root (color, live or dead, etc.) and root diameter class and category (root tip, feeder root, mycorrhizal root, etc.) were identified. Root length and number were also recorded. Raw data from Root Tracker were then manipulated in Microsoft Excel by writing Visual Basic code to generate the data format suitable for statistical analysis of the following root demographic data: birth, death, longevity, total root length and total root number of the various root classes.

Root length and root number are reported per frame basis. For survivorship analysis, the roots present at the second sampling date but absent at the first sampling date were classified as new root cohort. Only brown and mycorrhizal root cohorts had sufficient new roots for survivalship analysis. Survivorship curves were generated by plotting percent of remaining live roots over time.

### *Root tip morphotyping*

Four soil cores (5 cm x 20 cm) were taken from each tree using a soil auger in May and September 99. There were 192 soil cores collected during each sampling period. Four soil cores from each tree were pooled, and fine roots (< 2 mm) and mycorrhizal root clusters were carefully collected using a 2-mm mesh sieve. Root samples were stored in 40 ml of 50% ethanol solution containing 0.5% sodium hexametophosphate to help disperse the soils from the roots with gentle shaking. Fine root samples were then microscopically examined to determine number of mycorrhizal clusters. A mycorrhizal cluster was defined as one or more mycorrhizal root tip originating from a short root. Mycorrhizal clusters were further classified into three categories based on the number of mycorrhizal tips as cluster type I (1-2 tips), cluster type II (2-4 tips), and cluster type III ( $\geq$ 5 tips). Each of these clusters was further divided into three groups based on cluster color (dark, brown, and golden-yellow), thus resulting in nine mycorrhizal morphotypes. The length of each individual root with or without mycorrhizal clusters were measured and total root length was the sum of all the roots. The intensity of mycorrhizal colonization is reported as number of mycorrhizal clusters per unit root length (number of clusters $mm^{-1}$), which was calculated by dividing cluster numbers by total root length in each morphotype.

### *Data analysis*

The data for both minirhizotron and morphotyping were analyzed as a split-plot design with treatment as main plot, provenance as sub-plot, and families nested within provenance. Trees in each family were treated as sub-samples for both minirhizotron and morphotyping data analyses. Tubes and frames within each experiment unit were also treated as sub-samples. Fisher's protected LSD was used for mean comparisons when treatment and provenance interactions were not significant; or the comparison among least square means were used where appropriate. All analyses were performed using GLM SAS procedure (SAS Institute, Gary, NC) and the differences were considered significant at $P \leq 0.05$.

## RESULTS AND DISCUSSION

Figure 2 is an example of how root images collected using minirhizotron technology are used to collect demographic data. A new root appearing for the first time in a frame is classified as a new root. The root in Figure 2 on 11/98 is thus described as a white tip born that month turning into a brown root on 1/99. Color is an arbitrary classification suggesting that the root is undergoing secondary growth, i.e. developing a cork or periderm. The brown root begins to disappear or die by 10/99. By summing all the root data over the 17-month collection period, we found that brown roots (with diameter < 2.0 mm) were observed more than any other diameter root class, representing over 76% of all roots (Figure 3). Indeed, small diameter fine roots (less than 1 mm) represented the largest diameter class root. From a functional standpoint, there is probably no difference between lateral roots > 1 mm, since these roots usually serve both as transport conduits for nutrients, water and carbon, and as storage tissue. Fine roots < 1 mm diameter are morphologically and physiologically distinct from larger lateral roots, since lateral roots, short roots and mycorrhizal roots are included in this diameter class.

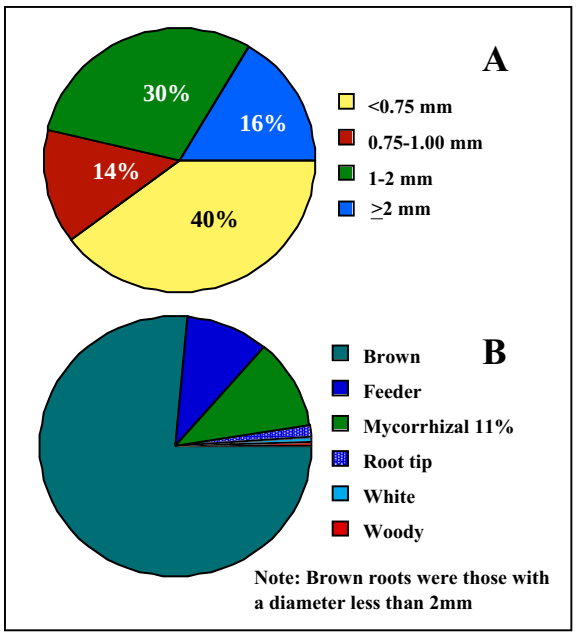


**Figure 3.** % of fine roots in loblolly pine in the various diameter classes (A) and root categories (B). Data are pooled from the entire 17-month observation period.

Because of the small size and fragile nature of short roots (both nonmycorrhizal and mycorrhizal), they are easily lost during soil sieving and are probably not included in most standing crop or production estimates. Unfortunately, these roots are probably the largest carbon sinks in root systems of southern pines in terms of production and maintenance costs, and probably exhibit the highest turnover. Their small size and lack of secondary tissues make them very difficult to detect in methods estimating both live and dead standing crop biomass, particularly if their turnover and decay rates are high. Hand-sorting and dry sieving can result in a 30-60 % loss of roots < 1 mm in diameter (McClaugherty *et al*. 1982; Fogel 1983; Hendrick and Pregitzer 1993). Consequently, minirhizotron technology is a better method than coil coring in estimating turnover of these smaller root classes, particularly since they tend to have higher turnover than larger woody root classes (Vogt and Bloomfield 1991; Hendrick and Pregitzer 1993; Eissenstat and Yanai 1997). Because of the large input of fine roots and mycorrhizae to decomposition processes, it is critical that we get better estimates of these small-diameter fine lateral roots (< 1 mm), short roots and mycorrhizal roots + extramatrical hyphae. Unfortunately, most root standing crop estimates do not include mycorrhizal roots + extramatrical hyphae. Mycorrhizae may account for an additional 5-15% in standing crop biomass in trees (Fogel and Hunt 1979; Vogt *et al*. 1982).

In the current study, virtually all root tips observed through the minirhizotron tubes were mycorrhizal or pre-mycorrhizal (i.e. were feeder roots); these roots represented 11% and 10% of total roots, respectively. Feeder roots are short, stubby lateral root tips less than 0.5 mm in length which appear to exist solely for mycorrhizal colonization; if feeder roots weren't colonized with 2 weeks of appearance, they usually disappeared. Under fertilized conditions, there was little to no feeder root production (Figure 4). For much of the sampling period, the two faster-growing families from each provenance (ACP-1 and TX-1) had greater feeder root production than their slow-growing counterparts. The slow-growing ACP-1 family consistently exhibited the lowest production of feeder roots during the 17-month observation period. High

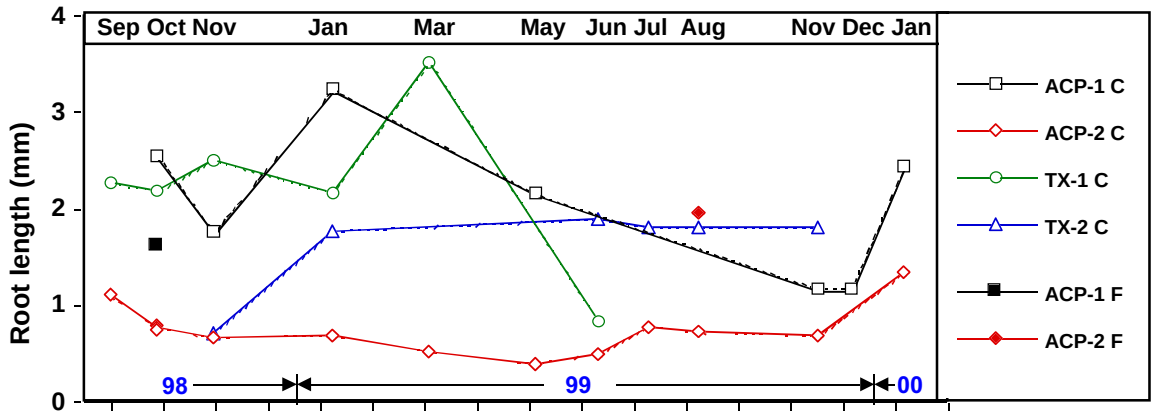


**Figure 4.** Live feeder root length (mm per 2.4 $cm^2$) in the four loblolly pine families during the 1998-2000 observation period. Note the absence of feeder roots in the fertilized treatment.

feeder root production in the faster-growing families could suggest higher mycorrhizal colonization rates or simply more plastic root ontogeny.

Treatment effects on fine root production was quite pronounced (Figure 5). Fertilization decreased both fine root number and length. Family x treatment interactions on fine root length are suggested by differences in how the fast- and slow-growing responded to fertilization (Figure 6). Fertilization decreased total root length and number (data not shown) in the faster-growing families from each provenance (ACP-1 and TX-1). In contrast, fertilization increased root length and number of the slowest-growing of all four families (TX-2), and either increased or decreased fine root production in ACP-2, depending upon the sampling date. Family differences between the fast-and slow-growing families within each provenance were most pronounced under control (ambient) conditions, with the two faster-growing families (ACP-1 and TX-1) generally exhibiting greater fine root production (both length and number) than the slow-growing families (ACP-2 and TX-2) during most of the observation period (Figure 7). The two faster-growing families also exhibited a much higher seasonal variation under ambient soil conditions, while root length of the slower-growing families remained fairly constant throughout the observation period. Under fertilized conditions, there were no consistent differences between the families.

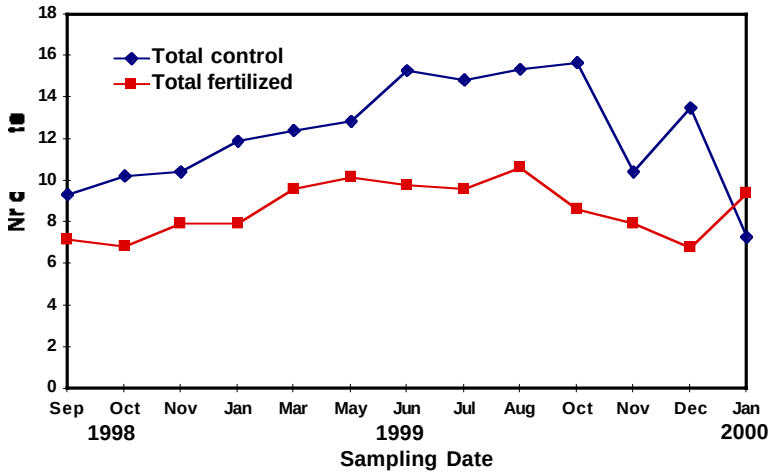


**Figure 5**. Fertilizer effects on the number of live roots per 2.4 $cm^2$ during the 1998-2000 observation period. Data from all loblolly pine families are pooled.

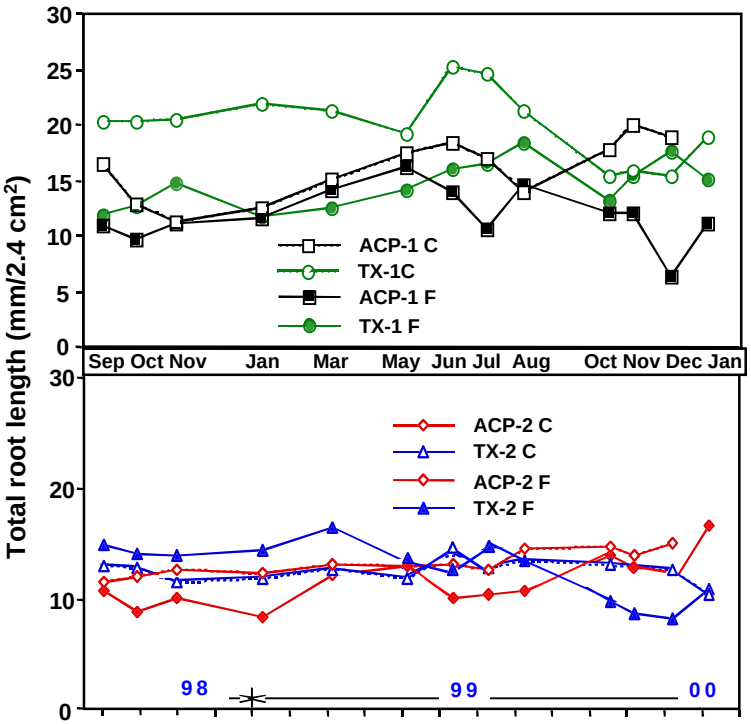


**Figure 6.** Treatment effects on total live root lengths of the individual loblolly pine families during the 1998-2000 observation period. C = control, F = fertilized

Annual production often exceeds standing crop biomass because fine roots undergo multiple cycles of growth, death and replacement during the year. These cycles may occur in microsites, and may not be in phase with one another. Seasonal patterns in growth of fine roots may be most pronounced in upper soil horizons (Gholz *et al.* 1986; Hendrick and Pregitzer 1993; Steele *et al*. 1997).

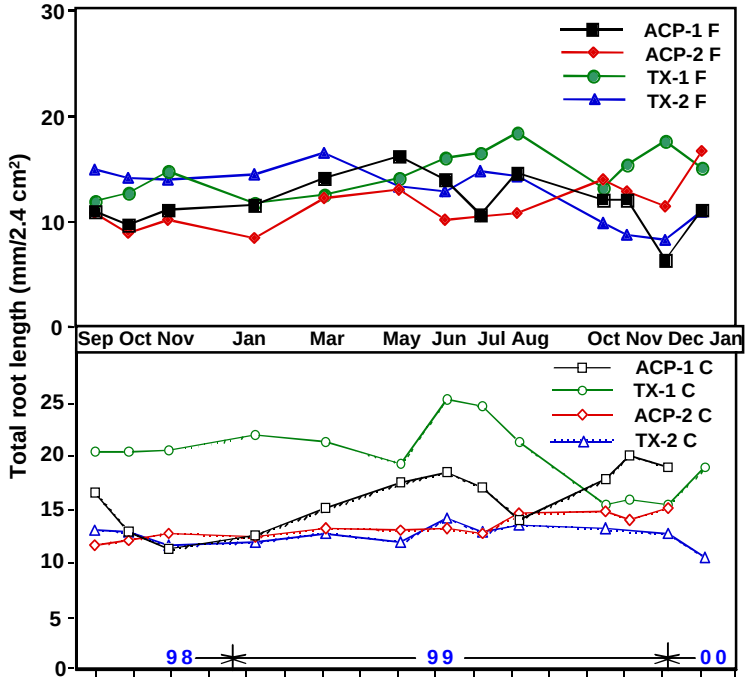


**Figure 7.** Family differences in total live root length of loblolly pine during the 1998-2000 observation period.
C= Control, F = Fertilized.

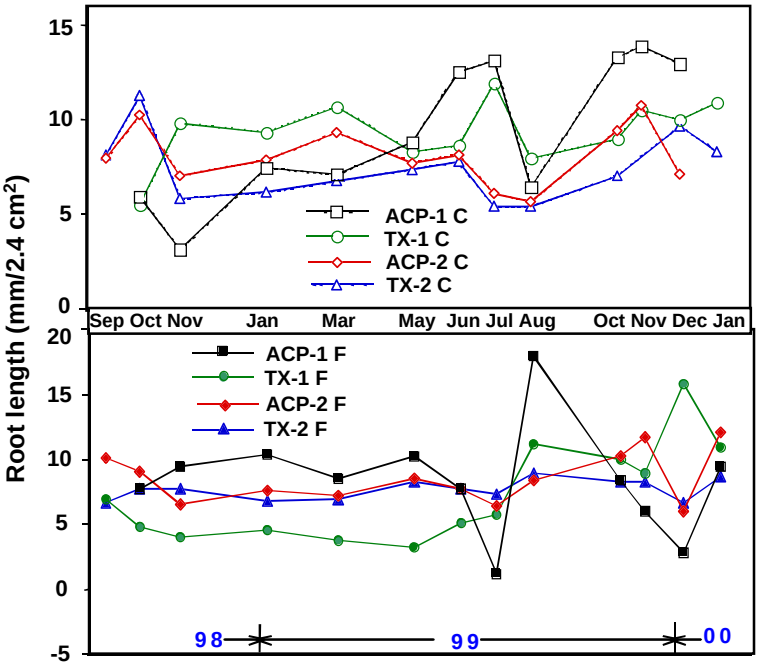


**Figure 8.** Dead root lengths of the four loblolly pine families under control (C) and fertilized (F) conditions during the 1998-2000 observation period.

Fertilization had a greater effect on dead root length in the two faster-growing families (ACP-1 and TX-1), but had little effect on the slower-growing ACP-2 and TX-2 families (Figure 8). The reduced plasticity in live root production in the slower-growing families was thus mirrored in dead root production. Under control conditions, family TX-1 generally had the highest numbers and lengths of dead roots of any family (a pattern mirrored in live root length and number), while TX-2 exhibited the lowest. Survivorship analysis of 0-2 mm brown roots indicates that there was higher root death in control trees than in fertilized trees (Figure 9). Roots born in October through November lived longer under fertilized conditions. At the end of the sampling period, 92% and 74% of brown roots born in October-November were still alive in fertilized and control trees, respectively.

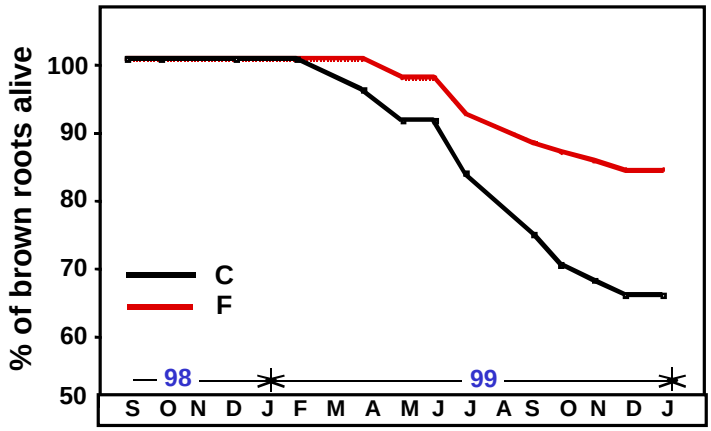


**Figure 9.** Survivorship curve for 0-60-day brown root cohorts born in October – November 98 under control (C) and fertilized (F) conditions.

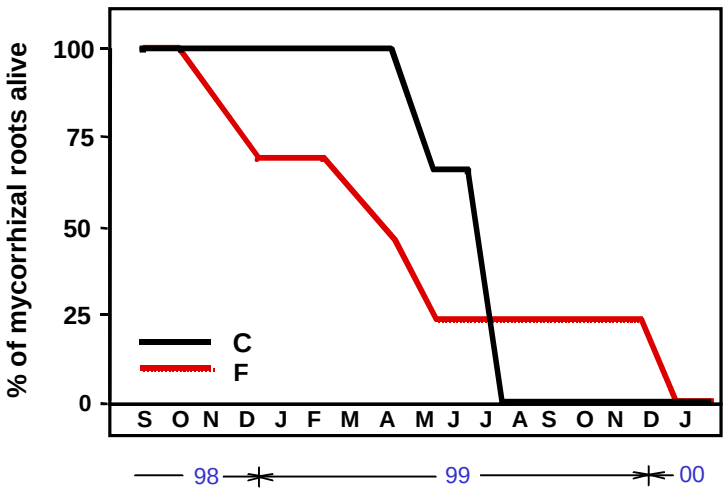


**Figure 10.** Survivorship curve for 0-60-day mycorrhizal root cohorts born in October – November 98 under control (C) and fertilized (F) conditions.

Interestingly, fertilization had the opposite effect on mycorrhizal root tips, i.e. it increased root mortality during the first 320 days after birth (Figure 10). The rapid increase in mycorrhizal root tip mortality in early to mid summer in the control treatment is most likely a function of the severe drought in 1999. All mycorrhizal roots from the October-November cohort under control conditions were dead in late July, while less than 25% of the fertilized cohorts survived through early winter. The median root lifespans (MRL) for mycorrhizal roots in the fertilized and control treatments were 230 and 315 days, respectively. Although minirhizotron technology may be the best direct method for estimating fine root turnover, assessing root death by visual approaches can underestimate the number of dead roots by 12-15% compared with more direct methods such examination of root tissues with tetrazolium (Wang *et al*. 1995).

The mechanism(s) controlling root longevity or life-span are poorly understood (see Vogt and Bloomfield 1991; Eissenstat and Yanai 1997 for review). Root longevity generally increases with increasing root diameter (Vogt and Bloomfield 1991; Eissenstat and Yanai 1997) and seems to be the case with southern pines (Gholz *et a.l* 1986). Fine root system longevity is a function of stand characteristics, e.g. climate or latitude (Hendrick and Pregitzer 1993), species composition (Frederickson and Ledaker 1995), tree or stand age (Gholz *et al*. 1986; Black *et al*. 1998; Johnson *et al*. 2000), species (Vogt and Bloomfield 1991; Shoettle and Fahey 1994; Steele *et al.* 1997; Black *et a.l* 1998), soil environmental variables such as fertility, water availability and temperature at both stand and microsite levels, e.g. at various soil depths (Gholz *et al*. 1986; Hendrick and Pregitzer 1993; Sword *et al*. 1996; Rytter and Rytter 1998), carbon status of the tree (Eissenstat and Duncan 1992; Pregitzer *et al*. 1995), physiological status of the tree and mycorrhizal infection (Harley 1969; Harley and Smith 1983; Fogel 1983; Vogt and Bloomfield 1991). Consequently, it is not surprising that several minirhizotron studies show opposite effects of N fertilization on root demography (e.g. Pregitzer *et al*. 19931995; Majdi and Nyland 1996; Majdi and Kangas 1997; Tingey *et al*. 1997; Johnson *et al*. 2000).

What is noticeably lacking are studies examining genetic variability in fine root system production and life-span within a species. Most production and longevity studies are based on a limited number of trees or sites over a relatively short time period, and conclusions are often drawn at the population, stand or ecosystem level. Conclusions suggesting site to site variation or a stand's response to fertility could easily be a function of genetic variability within a population**.** Indeed, our study using only four families of loblolly pine suggests significant

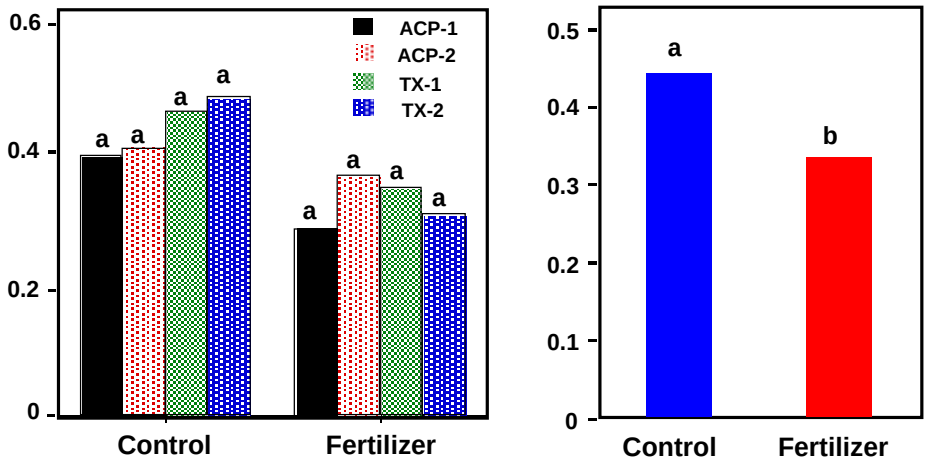


**Figure 11.** Total number of mycorrhizal clusters per unit root length (mm) in the four loblolly pine families under control (C) and fertilized (F) conditions. Roots were collected in September 1999 from the Scotland County, NC, field site.

genotype x environment variation in fine root length density and number within these families, as well as in their response to fertilization. With the exception of a couple of recent studies (Majdi and Nylund 1996; Rygiewicz *et al.* 1997), information on life-span of mycorrhizal root tips under field conditions is also lacking. Given the high carbon costs of mycorrhizal fungi to its host and its potential to be a substantial carbon pool in the soil, more information on mycorrhizal turnover is necessary to assess their role in belowground carbon sequestration and carbon/nutrient cycling.

More than 90% of short roots in these loblolly pine families were colonized by ectomycorrhizal fungi. Fertilization decreased the number of colonized short roots or mycorrhizal clusters in both May (data not shown) and September 1999 (Figure 11), but had no family effect. Although there was no difference in total mycorrhizal colonization among fast and slow growing families, there were differences in the number of mycorrhizal clusters of the dominant morphotypes (Figure 12). As a result, nine ectomycorrhizal short roots (clusters) were classified as different morphotypes. There were no interactive family and treatment effects on the total or different morphotypes per unit of root length. Among all families, dark and brown morphotypes were dominant mycorrhizal morphotypes in May and September 99.

Seedling studies suggest that mycorrhizal development and colonization by specific mycorrhizal fungi vary substantially among plant families, genera and even among closely-related genotypes (Lundeberg 1968; Marx and Bryan 1971; Krishna *et al.* 1985; Hatchell and Marx 1987; Manske 1989; Graham *et al.* 1991; Burgess *et al.* 1993; Graham and Eissenstat 1993; Johnson *et al.* 1997). Similarly, mycorrhizal fungal genotypes significantly affect host growth, with some more beneficial than others, i.e. host response varies along a positive-neutral-negative continuum (Marx and Bryan 1971; Marx *et al.* 1977; Hatchell and Marx 1987; Bougher *et al.* 1990; Dosskey *et al.* 1991; Colpaert *et al.* 1992; Burgess *et al.* 1993; Graham *et al.* 1996;

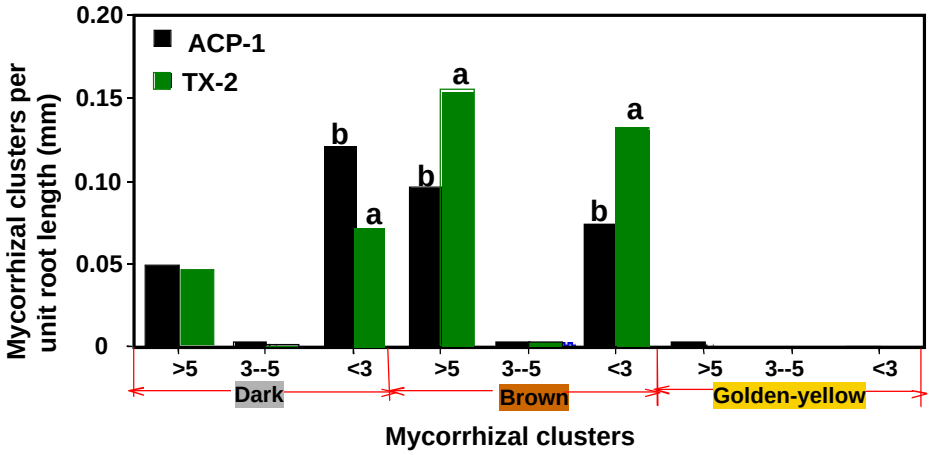


**Figure 12.** Mycorrhizal morphotypes categorized from gross morphological traits on roots of loblolly pine families at the Scotland County, NC, field site.

Smith and Smith 1996; Johnson *et al*. 1997). But whether similar growth promotion or depressions occur in older trees has not been examined in part because of the dynamic nature of the host/fungal genotype interaction with its environment, and the technical difficulty in characterizing the mycorrhizal phenotype.

In the current study, fertilization decreased fine root length density and root numbers in the two faster-growing families, but either increased or had little effect on fine root production in the slower-growing families. In addition, the two slower-growing families exhibited little seasonal variation in root number and root length density. These results suggest a potential reduction in root system carbon demands in faster-growing trees in the fertilized plots, a more plastic response to soil fertility, and greater seasonal variability in production. Theoretically, a population that allocates more carbon to fine root system production would be at a competitive advantage for exploiting soil resources under infertile conditions. In infertile soils, increasing the root surface area available for absorption of immobile nutrients such as phosphate and ammonium, is more critical than increasing physiological rates of uptake. Mycorrhizal associations are beneficial in infertile soils because they increase the exploitation potential of the root system, but they come at a considerable carbon cost. In our study, over 90% of short roots in unfertilized soil conditions were colonized by a minimum of 10 different morphotypes. Whether differences in aboveground growth in field-grown trees are correlated with differences in colonization rates by the various genera/species is unknown. All families exhibited a reduction in degree of mycorrhizal colonization in the fertilized plots.

In fertilized soils, the cost of maintaining mycorrhizal fungi may outweigh their benefits, with negative growth responses possible because the fungi may no longer behave as mutualists (Graham and Eissenstat 1998). Presumably extra carbon becomes available for aboveground growth under fertilized conditions because root system carbon demands resulting from fine root production and mycorrhizal colonization decreases. Indeed, fertilization decreased the degree of colonization by mycorrhizal fungi in all loblolly pine families examined at the Scotland County field site. Fertilization decreased fine root production in the faster-growing families, but either increased or had no effect on fine root production in the slowest-growing family. Both results

suggest a reduction in root system carbon demands in the fertilized plots, and a more plastic root response in the faster-growing families.

## Conclusions

Loblolly pine fine root production, turnover, and survival were strongly influenced by season and the interactions between family and fertilization treatments. Greater fine root production and turnover were observed in faster-growing families. Fine root production and turnover in slow-growing families showed less seasonal variability than in fast-growing families. Faster-growing families exhibited a greater reduction in fine root production under fertilization than slower-growing families, suggesting a greater plasticity in fast-growing families. In general, fertilization decreased total fine root production, particularly feeder root production. The reduction in feeder root production under fertilized conditions may account for the reduction in mycorrhizal colonization in the fertilizer treatment. Fertilization also prolonged brown root life span, but reduced mycorrhizal root life span. Although there were no differences in the survivorship of brown and mycorrhizal roots among all four families, greater fine root production and turnover were observed in fast-growing families.

Survivorship analysis indicated that mycorrhizal roots had a shorter life span than brown fine roots. Brown fine roots accounted for 76% of all fine roots and more than 80% of them were still alive after 500 days, suggesting long-term minirhizotron studies are needed to determine the median root life span of brown roots (<2.0 mm) in loblolly pines. More than 90% of short roots in these loblolly pine families were colonized by ectomycorrhizal fungi. As a result, nine ectomycorrhizal short roots (clusters) were classified as different morphotypes. There were no interactive family and treatment effects on the total or different morphotypes per unit of root length. However, fertilization reduced total mycorrhizal colonization but family had no effect. Among all families, dark and brown morphotypes were dominant mycorrhizal morphotypes in May and September 99. Our results suggest possible genetic differences and treatment effects on root system carbon demands of loblolly pine.

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## TASK 1: ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

# V. DIURNAL AND SEASONAL CHANGES IN GAS EXCHANGE, WATER USE EFFICIENCY AND FOLIAR CARBON RESERVES

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## INTRODUCTION

The "Lost Pines" provenance of loblolly pine (*Pinus taeda* L.) is a disjunct population originating more than 160 km west of the continuous range of loblolly pine in the Texas counties of Bastrop, Fayette and Caldwell (van Buijtenen *et al.* 1976), where the annual rainfall is 25-50 cm less than in eastern Texas. The "Lost Pines" Texas provenance is known for its drought hardiness and resistance to natural pests/pathogens, but is generally described as a slower-growing pine than its more mesic Atlantic Coastal Plain counterparts. Seedling experiments suggest that their drought hardiness is more a function of avoidance than drought tolerance since seedlings rapidly transpire when water is available, but conserve water under stress (Bilan *et al.* 1977; van Buijtenen *et al.* 1976). Their drought avoidance may be a function of deeper taproots and wide-ranging laterals, small needles with deep stomatal pits and thicker cuticles with fewer stomata per unit leaf surface area. In contrast, eastern populations from North and South Carolina are more mesic, generally faster-growing, but less resistant to drought and natural pathogens such as fusiform rust and tipmoth.

But whether saplings and mature trees of the drought-hardy Texas loblolly pine population have higher water use efficiencies under droughted field conditions has not been thoroughly examined. Population differences in gas exchange characteristics and water use efficiency may be most pronounced in the seedling stage before roots tap into a reliable water source (Teskey *et al.* 1987), or in mature trees exposed to a severe and lengthy drought. Since drought is probably the greatest limitation to photosynthesis in southern pines (Ellsworth 2000), a closer examination of potential water conservation and gas exchange characteristics of these drought-hardy populations and differences in net carbon gain is warranted.

Although net photosynthesis is often considered a determinant of growth, genetic differences between fast- and slow-growing trees may be more a function of light interception (i.e. leaf area, leaf orientation and morphology) or the relationship between net photosynthesis and leaf area (Linder 1986; Bongarten *et al.* 1987). Net carbon gain of a tree is a function of rate of photosynthesis per leaf area, respiration rate and metabolic demands of leaf tissue, leaf area and respiration rate of nonphotosynthetic tissue (Teskey *et al.* 1987), and is determined by the tree's genetic potential and its environment. Seasonal (Boltz *et al*. 1986) and diurnal differences in photosynthesis may also account for population differences in growth or productivity of

loblolly pine. Faster-growing families may maintain higher net photosynthesis in the fall and spring or for longer periods of time during the day. Indeed, carbon gain in the fall may be important in restoring carbohydrates depleted during late summer (Murthy *et al.* 1997).

Understanding how carbon assimilation and partitioning may change diurnally or seasonally is central to any genetic regulation of whole-tree source/sink relationships. In the following study, we examined genetic, seasonal, diurnal, and fertilizer effects on gas exchange characteristics and partitioning of recently assimilated carbon in needles of four families of loblolly pine from a 5-year-old plantation in Scotland County, North Carolina, USA. We hypothesized that the faster-growing, mesic population from the Atlantic Coastal Plain would have higher photosynthetic rates and lower water use efficiencies than the drought-hardy families from Texas. Gas exchange characteristics were monitored during a wet and dry year.

## MATERIALS AND METHODS

Because the amount of field data collection precluded sampling all families and blocks, only the fastest and slowest growing families from each provenance in three blocks (6, 8 and 10) were chosen based on the evaluation of aboveground height growth of 4 year-old control trees (Mckeand *et al.* 1999). The families selected in this study were the faster-growing ACP-1 (ACP 8-118) and TX-1 (TX BA3L11-1), and the slower-growing ACP-2 (ACP 9-1046) and TX-2 (TX GR1-2) from their respective provenances. Studies presented here consist of 3 blocks x 2 treatments x 4 families (two from each provenance). **Please note change in the acronym for the drought-hardy Texas families in this section from the previous sections: TX-1 = LPT-1; TX-2 = LPT-2.**

During each sampling period, the orders of sample collection from the three blocks, treatment within each block, and row within each family plot were randomly selected. The same row was sampled in all 3 blocks.

### *Gas exchange and water use efficiency*

During the growing seasons in 1998 and 1999, diurnal changes in gas exchange parameters were monitored bimonthly; however, in May and September of 1999, only light saturated maximum photosynthesis ($A_{max}$) was measured. Gas exchange data for each block were collected on 3 consecutive days. All gas exchange measurements were made on needles removed from the tree's upper canopy using a portable photosynthesis system (Li-Cor 6400, Li-Cor, NE, USA). Once removed, needle fascicles were immediately placed upright in a glass vial containing distilled water, and gas-exchange rates were determined. Needles from the current, fully expanded flush (usually flush 1 in the current year) were removed, and needles from the previous year (flush 1 or the healthiest flush if multiple flushes exist) were also removed if present.

During measurements, reference $CO_2$ concentration (400 ppm for diurnal and 385 ppm for $A_{max}$) was maintained in the cuvette by using an external $CO_2$ source; light intensity and relative humidity were adjusted to approximate average light and humidity levels for that interval period. For diurnal measurements, two trees from each family plot were selected and four to six

measurement intervals were conducted depending on day length. $A_{max}$ was determined using four to six trees from each family plot between 0900 and 1300 h using predicted daily maximum light level in May and September 1999. $A_{max}$ data for months other than May and September 1999 were the composite of diurnal $P_{net}$ data from all intervals prior to 1300 h exhibiting maximum daily light levels. Instantaneous water use efficiency (WUE) was calculated by dividing $P_{net}$ by transpiration rate.

Long-term measurement of WUE was assessed using stable carbon isotope technology. Foliar tissue $\delta^{13}C$ in September 1998 and 1999 was obtained using a Europa Scientific ANCA-SL Stable Isotope Analysis System (Europa Scientific Ltd, Crewe, UK) at the Cornell Boyce Thompson Institute Stable Isotope Laboratory. The $^{13}C/^{12}C$ ratios in foliage were calculated against the PDB standard, and the precision of the $^{13}C$ analysis was ±0.2‰. It should be noted that precipitation differed significantly during 1998 and 1999. In 1999, precipitation through July was 40% of 1998.

***Diurnal carbohydrate analysis***

Needles representing current and previous year foliage were removed for carbohydrate analysis from the same trees used for gas exchange measurements. Fully-expanded needles were selected from a branch in the upper third of the canopy from two trees per family plot. Four fascicles from the selected branch were removed over a 24-hr period at dawn, dusk, and the following dawn. Preliminary experiments indicated that between branch variability in total needle nonstructural carbohydrates and partitioning was within 10%, and that maximum starch concentration in needles were generally reached by 1300 h and remained constant until sunset (data not shown). Foliage tissue was placed on dry ice immediately after removal, and then stored at -70°C until freeze-dried. Tissues were freeze dried at -40°C for two days and then at ambient temperature in a Lab Conco freeze dryer. Tissues were then ground with a SPEX® CertiPrep 6750 freezer mill. Starch and soluble sugar concentrations in needles were determined as described in Section I. Total nonstructural carbohydrate (TNC) concentrations are the sum of reducing sugars (glucose and fructose), sucrose, and starch concentrations. Starch, sugar and TNC concentrations are reported as glucose equivalents $g_{DW}^{-1}$ tissue.

***Data analysis***

The experiment was analyzed as a split-plot design with treatment as main plot, provenance as sub-plot, and families nested within provenance. Trees in each family were treated as sub-samples for both photosynthesis and carbohydrate data analyses. Fisher's protected LSD was used in mean comparisons when treatment and provenance interactions were not significant; or least square means were presented with Bonferroni adjustment where appropriate. Linear regression techniques were used to determine the relationships between photosynthesis and stomatal conductance, and between WUE and $^{13}C$ isotope discrimination. All analyses were performed using GLM SAS procedure (SAS Institute, Gary, NC) and the differences were considered significant at $P \leq 0.05$.

## RESULTS

### *Gas exchange characteristics: Diurnal $P_{net}$*

Overall, seasonal ($P \leq 0.001$) and diurnal ($P \leq 0.001$) differences in diurnal rates of net photosynthesis ($P_{net}$) were more pronounced than treatment or family effect. Fertilization increased $P_{net}$ only during the first two intervals in July 1998, and the fourth and fifth intervals in November 1999 (Figures 1 and 2). On most sampling dates, family had no significant effect on $P_{net}$. Family differences in $P_{net}$ of current year foliage were observed only during the first three intervals in July 1999 (Figure 1). The fastest growing family (ACP-1) had the highest $P_{net}$ during the first two intervals, while the slowest growing family (TX-2) had the lowest $P_{net}$ at the second interval but the highest $P_{net}$ at the third interval. Similarly, in previous year foliage, there were significant treatment and family interactions in diurnal $P_{net}$ only during the first few intervals in July 1999 (Figure 2), with ACP families exhibiting higher $P_{net}$ rates than the Texas families during the first two intervals.

Net photosynthesis peaked during the first interval in July 1998 and 1999 and steadily declined during the remainder of the measurement period. In September through March, peak $P_{net}$ rates were maintained for a longer period of time, and often did not occur until mid to late afternoon (e.g. Nov, 98 and 99) (Figures 1 and 2). Diurnal variation in $P_{net}$ was most pronounced in July 98 and 99, with needles exhibiting a 50-70% reduction from peak to minimum, compared to a 25-45% reduction during other months. Current year foliage had higher $P_{net}$ rates than previous year foliage (Table 1), with diurnal differences between age classes most pronounced in July 99.

### *$A_{max}$ and WUE*

There was no difference in $A_{max}$ between fertilizer and control treatments except in July 1998, when fertilized trees had over 35% higher $A_{max}$ than control trees (Figure 3). Family differences in $A_{max}$ were only apparent in July 1999, with family ACP-1 exhibiting a higher $A_{max}$ than the other families. Seasonal trends for $A_{max}$ were similar between families and treatments. $A_{max}$ was highest in July 1998 and May 1999, and lowest in July 1999. Rates during late fall through spring were 40-50% lower than peak rates in July 98 and May 99. $A_{max}$ was 21-37% higher in current than in previous year foliage in July and September 98, and July 99 (Table 1).

In general, stomatal control of photosynthesis was strongest in July 98 and 99 (Table 2). The relationship between $A_{max}$ and $g_s$ was its weakest in previous year foliage in September 98, with $g_s$ accounting for less than 10% of variation in $A_{max}$ in three families (Table 2). Although treatment had no effect on the relationship between $A_{max}$ and $g_s$, families differences were generally significant ($P \leq 0.001$) in current and previous year foliage (Figure 4). Within the same foliage class, regression coefficients were generally much higher in July 99 than in July 98 for families ACP-1, ACP-2, and TX-1, and stomatal control accounted for a higher percentage of variation in $A_{max}$. In 1999, family TX-2 had lowest regression coefficients and values of any family. It is worth noting that July 1999 had the lowest stomatal conductances of any month (Figure 4), most likely due to the drought that occurred in 1999. Annual precipitation for 1998 and 1999 through September was 1115 and 772 mm, respectively.

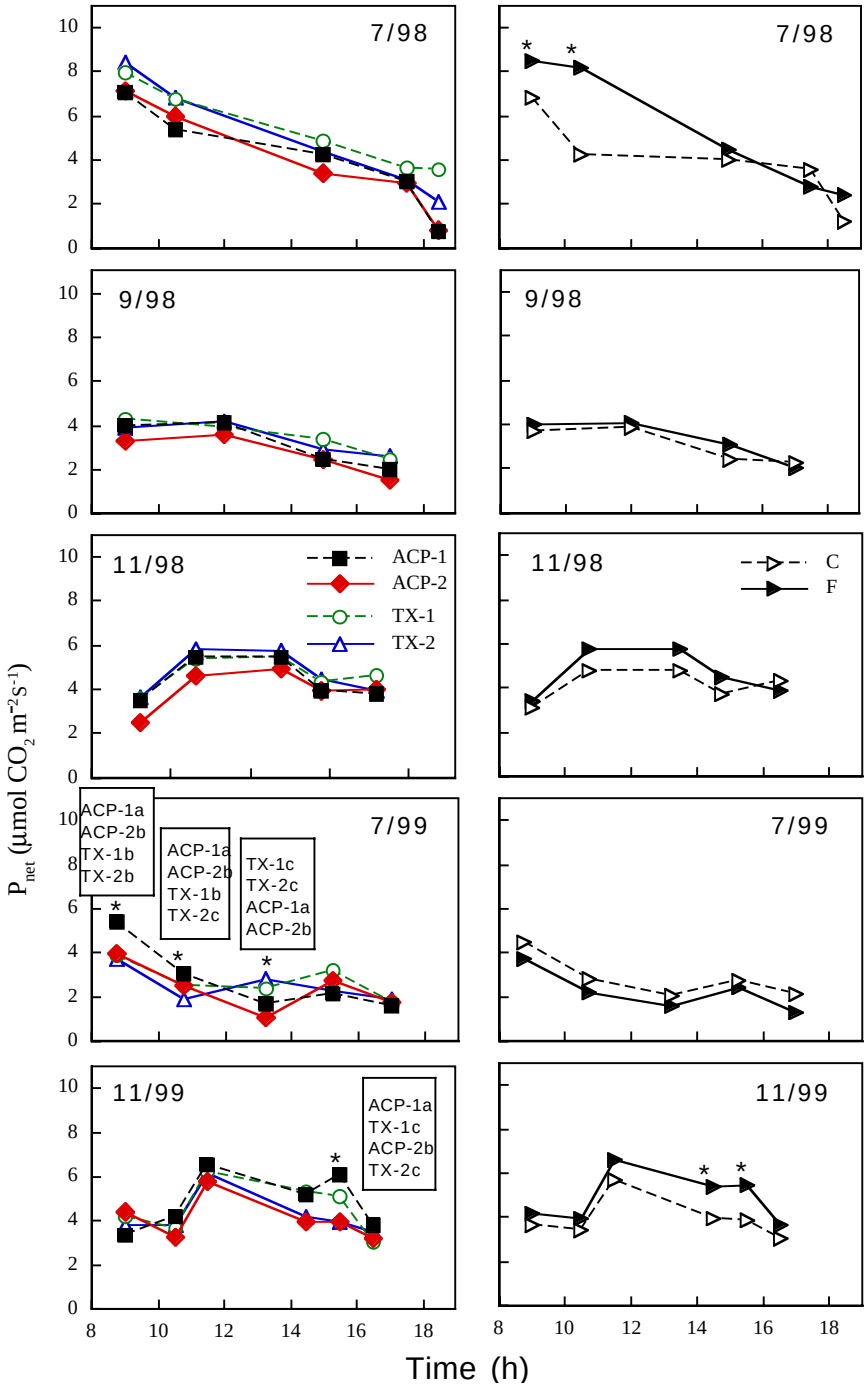


**Figure 1.** Effect of family and fertilization on rates of diurnal net photosynthesis ($P_{net}$) of current year foliage. Treatments differ significantly as determined by *lsd* test ($P \leq 0.05$, *). Family means followed by the same lower case letters are not significantly different ($P > 0.05$).

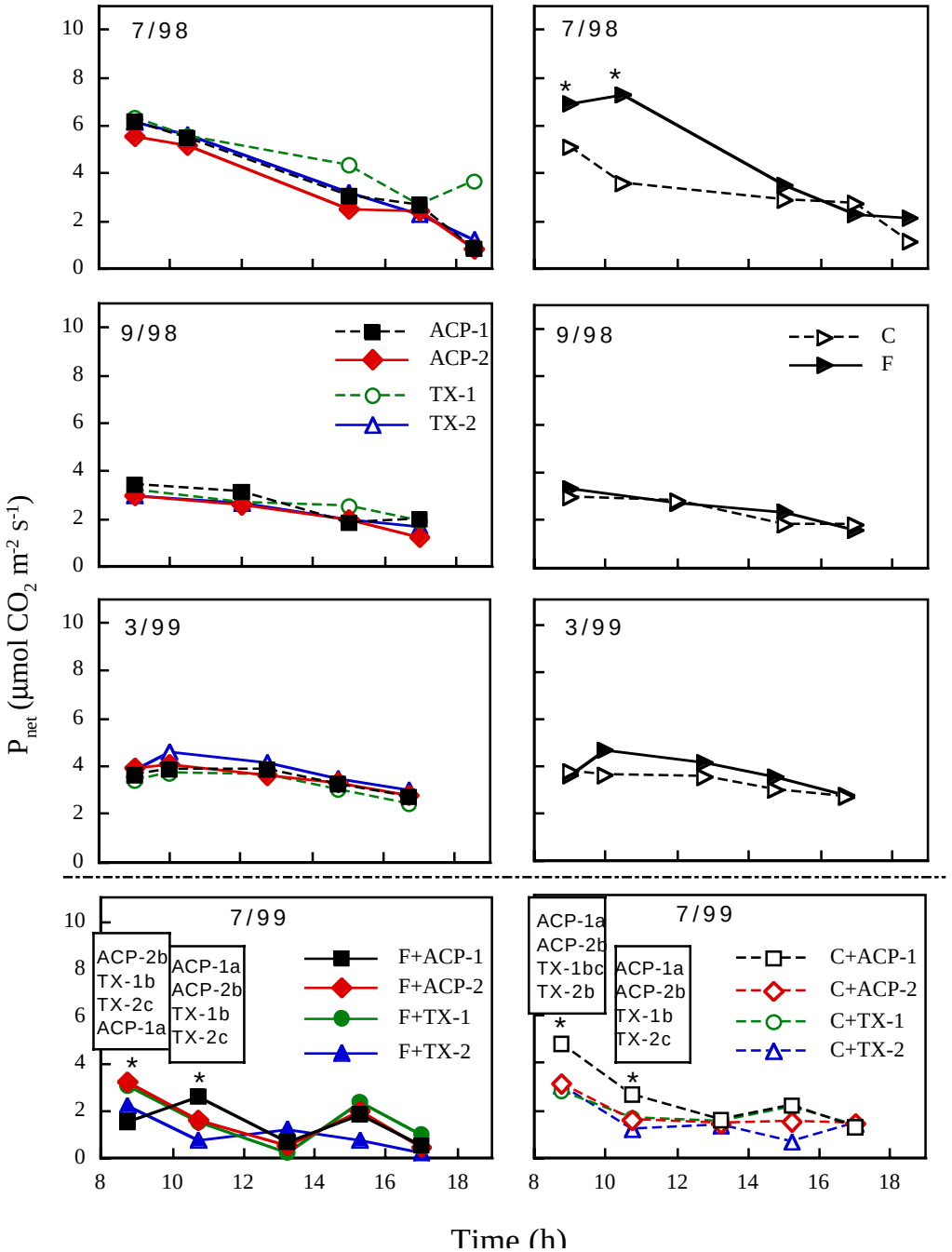


**Figure 2.** Effect of family and fertilization on rates of diurnal net photosynthesis ($P_{net}$) of previous year foliage. Treatments differ significantly as determined by *lsd* test ($P \leq 0.05$, *). Family means followed by the same lower case letters are not significantly different ($P > 0.05$).

**Table 1**. Mean diurnal rates (µmole $m^{-2}$ $s^{-1}$) of photosynthesis and $A_{max}$ in needles of different age classes. [z] P values indicate significance level between current and previous year foliage.

| | Amax | Diurnal intervals | | | | | Interactions | |
|---|---|---|---|---|---|---|---|---|
| 7/98 | | 1 | 2 | 3 | 4 | 5 | age x trt | age x family |
| Current year foliage | 6.91 | 7.62 | 6.21 | 4.22 | 3.17 | 1.78 | | |
| Previous year foliage | 5.72 | 6.02 | 5.43 | 3.24 | 2.50 | 1.76 | | |
| P (F) [z] | 0.0975 | 0.130 | 0.054 | 0.079 | 0.048 | 0.196 | 0.708 | 0.886 |
| 9/98 | | | | | | | | |
| Current year foliage | 3.92 | 3.87 | 3.95 | 2.78 | 2.14 | -- | | |
| Previous year foliage | 2.90 | 3.13 | 2.76 | 2.05 | 1.69 | -- | | |
| P (F) | 0.001 | 0.087 | 0.015 | 0.001 | 0.019 | -- | 0.775 | 0.622 |
| 7/99 | | | | | | | | |
| Current year foliage | 3.28 | 4.17 | 2.48 | 1.85 | 2.59 | 1.73 | | |
| Previous year foliage | 2.39 | 3.03 | 1.71 | 1.09 | 1.69 | 1.00 | | |
| P (F) | 0.023 | 0.018 | 0.066 | 0.119 | 0.014 | 0.293 | 0.877 | 0.799 |

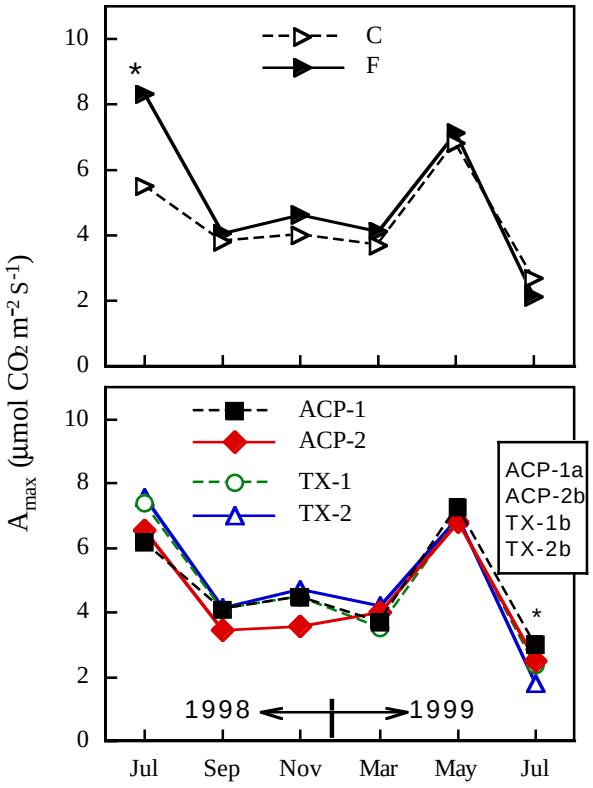


**Figure 3**. Effect of family and fertilization on maximum rates of photosynthesis ($A_{max}$) of needles as they age. Treatments differ significantly as determined by *lsd* test ($P \leq 0.05$, *). Family means followed by the same lower case letters are not significantly different ($P > 0.05$).

**Table 2.** Linear regression equations between maximum photosynthesis ($Y = A_{max}$) and stomatal conductance ($X = g_s$) in current and previous year foliage among different loblolly pine families. Level of significance for linear regression is indicated by $^{ns}$ $P > 0.05$, $^{*}$ $P \leq 0.05$, $^{**}$ $P \leq 0.01$, and $^{***}$ $P \leq 0.001$ respectively. The coefficient of determination ($r^2$) for each equation is given in parenthesis. n=24.

| | MONTH/YEAR | |
|---|---|---|
| | 7/98 | 9/98 |
| Current year foliage | | |
| ACP-1 | $Y=40.9X+3.1^{*}$ (0.254) | $Y=24.0X+2.5^{**}$ (0.418) |
| ACP-2 | $Y=49.0X+2.5^{**}$ (0.355) | $Y=19.5X+2.1^{*}$ (0.256) |
| TX-1 | $Y=39.8X+2.7^{***}$ (0.773) | $Y=25.3X+2.0^{***}$ (0.621) |
| TX-2 | $Y=43.7X+2.1^{***}$ (0.728) | $Y=16.7X+2.5^{*}$ (0.262) |
| Previous year foliage | | |
| ACP-1 | $Y=47.0X+2.4^{*}$ (0.220) | $Y=5.2X+2.9^{ns}$ (0.035) |
| ACP-2 | $Y=71.0X+1.1^{*}$ (0.279 ) | $Y=18.8X+1.7^{ns}$ (0.041) |
| TX-1 | $Y=44.6X+1.8^{***}$ (0.671) | $Y=17.6X+1.7^{*}$ (0.258) |
| TX-2 | $Y=42.8X+1.7^{***}$ (0.758) | $Y=12.1X+1.9^{ns}$ (0.100) |
| | 7/99 | 9/99 |
| Current year foliage | | |
| ACP-1 | $Y=72.0X+1.5^{***}$ (0.847) | $Y=37.2X+2.4^{*}$ (0.244) |
| ACP-2 | $Y=60.4X+1.5^{***}$ (0.824) | $Y=59.5X+1.1^{**}$ (0.584) |
| TX-1 | $Y=61.9X+1.4^{***}$ (0.715) | $Y=16.8X+1.8^{ns}$ (0.178) |
| TX-2 | $Y=40.7X+1.7^{**}$ (0.464) | $Y=16.1X+1.7^{ns}$ (0.135) |
| Previous year foliage | | |
| ACP-1 | $Y=95.9X+0.7^{***}$ (0.634) | -- |
| ACP-2 | $Y=69.0X+0.9^{***}$ (0.753) | -- |
| TX-1 | $Y=84.1X+0.4^{***}$ (0.728) | -- |
| TX-2 | $Y=30.4X+1.4^{ns}$ (0.052) | -- |

There were no significant differences in instantaneous water use efficiency (WUE) between treatments or among families for all the diurnal and $A_{max}$ measurements. Only instantaneous WUE during $A_{max}$ measurements in July and September of 98 and 99 were reported for direct comparison with $^{13}C$ WUE data (Table 3). Previous year foliage had more negative $^{13}C$ values than current year foliage in 98 and 99, suggesting higher WUE in current year foliage. In 1999, $^{13}C$ values of expanding foliage were similar to those of current year foliage. Fertilization treatment had no effect on $^{13}C$ values nor on instantaneous WUE in September in both years. Significant family effects on $^{13}C$ values were observed in September 1998 and 1999, with family TX-2 exhibiting the most negative $^{13}C$ values.

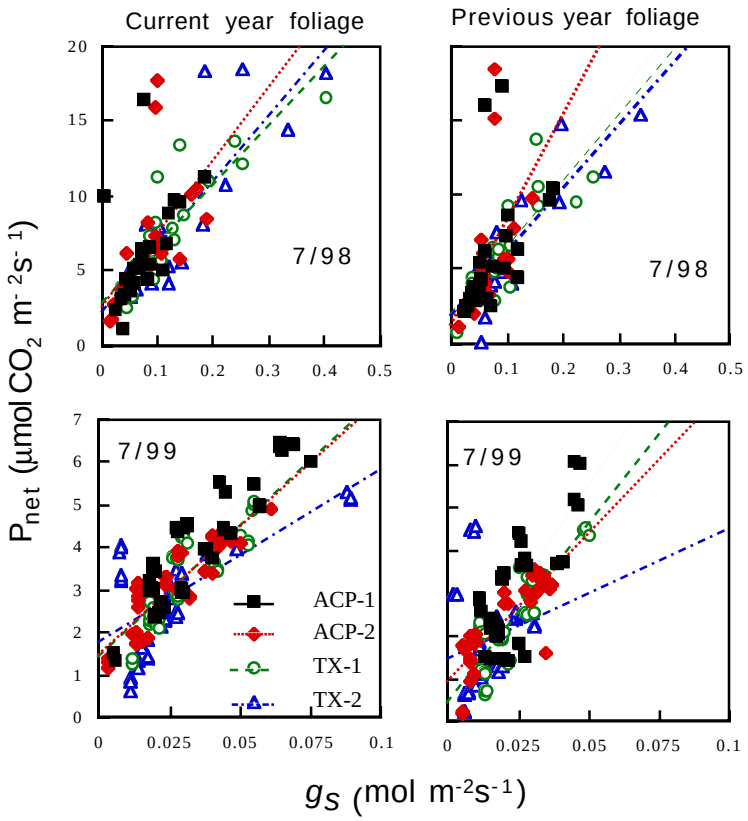


**Figure 4.** Linear relationship between stomatal conductance and net photosynthesis in current and previous year foliage. Regression equations and related statistics are presented in Table 2.

As expected, current year foliage had 20% higher leaf N concentration over previous year foliage. Fertilization increased leaf N concentration about 40-50% (Table 4). There were no differences in leaf N concentration among the families.

***Carbon partitioning in needles***

We found no strong significant diurnal differences (P = 0.106) in total nonstructural carbohydrates (TNC) concentrations and partitioning towards starch in needles sampled at dawn and dusk during all sampling dates. Consequently, only data for the dawn sampling period are shown. However, seasonal differences ($P \leq 0.001$) in needle concentrations of TNC, starch, reducing sugars, and sucrose were apparent, as were the partitioning of TNC into starch and soluble sugars ($P < 0.001$) (Figures 5 and 6). The highest concentrations of TNC, starch, and percent of TNC as starch were observed in May, while reducing sugar concentrations were the highest in winter months (November-March). Percent of TNC present as reducing sugars peaked in late fall, and was at its lowest in May. Reducing sugars (sucrose and fructose) were the dominant soluble sugars in needles of loblolly pine, representing over 70% of soluble sugars. Fertilization generally decreased needle concentrations of TNC and starch, and the partitioning of TNC towards starch during most of the year except for winter months (November to March), and increased the partitioning of TNC towards reducing sugar from summer to early fall. Family differences in carbohydrate concentrations were significant during some sampling dates, but these differences were not as pronounced as treatment effects. In general, the two Texas families exhibited higher concentrations of starch, TNC, and partitioning towards starch in May, July and September, which was accompanied by a decrease in partitioning towards reducing sugar.

**Table 3**. Effect of age, treatment, and family on mean foliage $\delta^{13}C$ values (n=3) and water use efficiency (WUE) (n=16). Means followed by the same lower case letters are not significant different ($P \leq 0.05$).

| | $\delta^{13}C$ (per mil) (n=3) | | WUE (μmole $CO_2$/mmole $H_2O$) | | | |
|---|---|---|---|---|---|---|
| | 9/98 | 9/99 | 7/98 | 9/98 | 7/99 | 9/99 |
| Tissue | | | | | | |
| Current year foliage | -28.3561 a | -27.9314 a | 2.79 a | 3.27 a | 2.63 a | 3.47 |
| Previous year foliage | -28.9671 b | -28.7182 b | 2.54 a | 3.01 a | 2.78 a | -- |
| Treatment | | | | | | |
| Control | -28.8673 a | -28.2459 a | 2.22 a | 2.84 a | 2.98 a | 3.64 a |
| Fertilizer | -28.5312 a | -28.1381 a | 3.12 a | 3.44 a | 2.41 a | 3.31 a |
| Family | | | | | | |
| ACP-1 | -28.5925 a | -27.9040 a | 3.23 a | 4.61 a | 2.71 a | 3.79 a |
| ACP-2 | -28.5450 a | -27.8467 a | 2.68 a | 2.74 a | 2.48 a | 3.71 a |
| TX-1 | -28.6081 a | -28.2825 ab | 2.41 a | 2.73 a | 2.17 a | 3.35 a |
| TX-2 | -29.0401 b | -28.5940 b | 2.36 a | 2.47 a | 3.43 a | 3.07 a |

**Table 4**. Effect of needle age, treatment and family on mean nitrogen concentration (%) in loblolly pine. Means (n=3) followed by the same lower case letters are not significant different ($P \leq 0.05$).

| | Month | | | |
|---|---|---|---|---|
| | 7/98 | 9/98 | 7/99 | 9/99 |
| Tissue | | | | |
| current year foliage | 1.182 a | 1.188 a | 1.115 a | 1.152 a |
| previous year foliage | 0.948 b | 0.923 b | 0.928 b | 0.898 b |
| Treatment | | | | |
| Control | 0.842 b | 0.854 b | 0.887 b | 0.928 b |
| Fertilizer | 1.258 a | 1.218 a | 1.155 a | 1.198 a |
| Family | | | | |
| ACP-1 | 1.088 a | 1.062 a | 1.017 a | 1.011 a |
| ACP-2 | 1.077 a | 1.088 a | 0.977 a | 1.173 a |
| TX-1 | 1.045 a | 0.991 a | 1.052 a | 1.139 a |
| TX-2 | 1.043 a | 1.028 a | 1.036 a | 0.996 a |

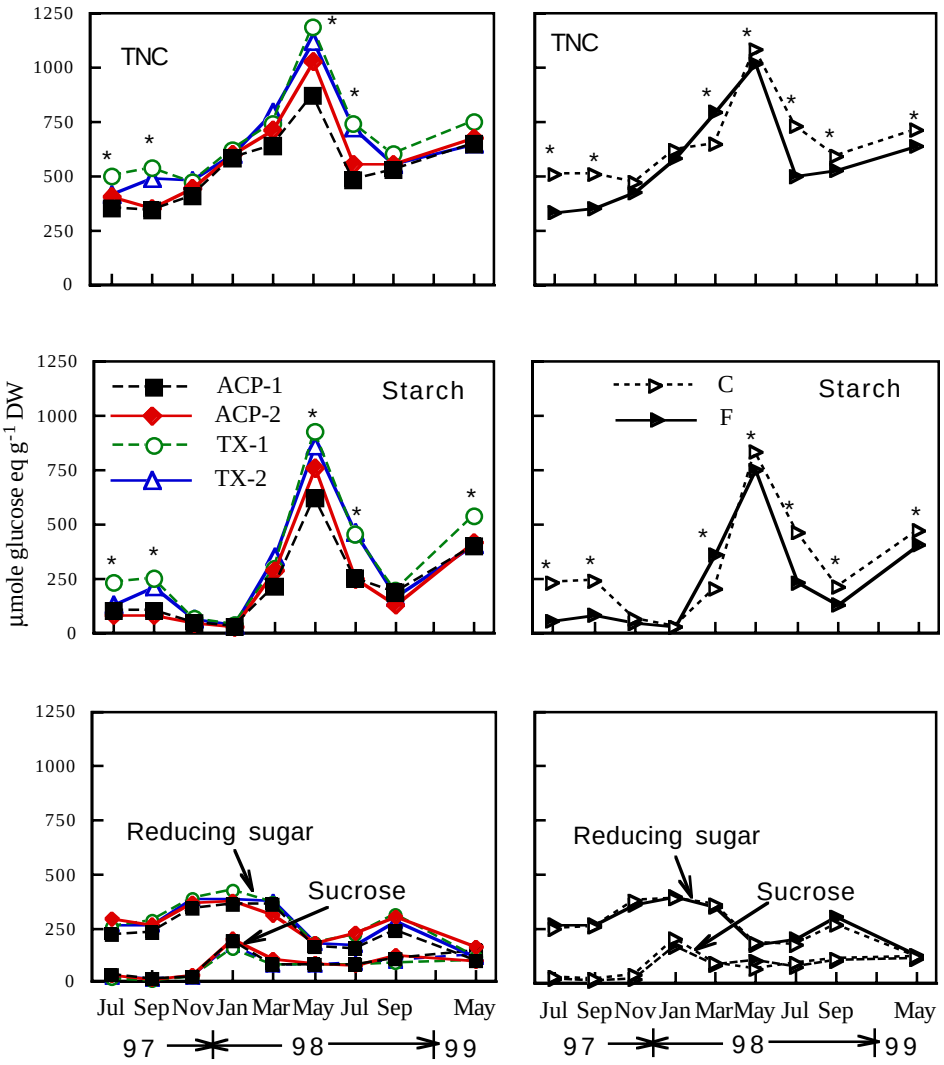


**Figure 5.** Effect of family and fertilization on carbohydate concentrations of needles as they age. Since there was no significant diurnal effect on carbohydrate concentration ($P > 0.05$), only data for needles sampled at dawn are presented. May 1999 data are for current year foliage. Treatments differ significantly as determined by *lsd* test ($P \leq 0.05$, *).

Foliage age had significant effects on carbohydrate concentrations (Table 5). Previous year foliage had higher needle concentrations of TNC, starch, sucrose, and reducing sugars than current year foliage except in September 98. However, partitioning of TNC towards starch was not different between the two foliage classes except in September 98. There was no difference in partitioning of soluble sugars towards reducing sugars between two foliage classes in 97.

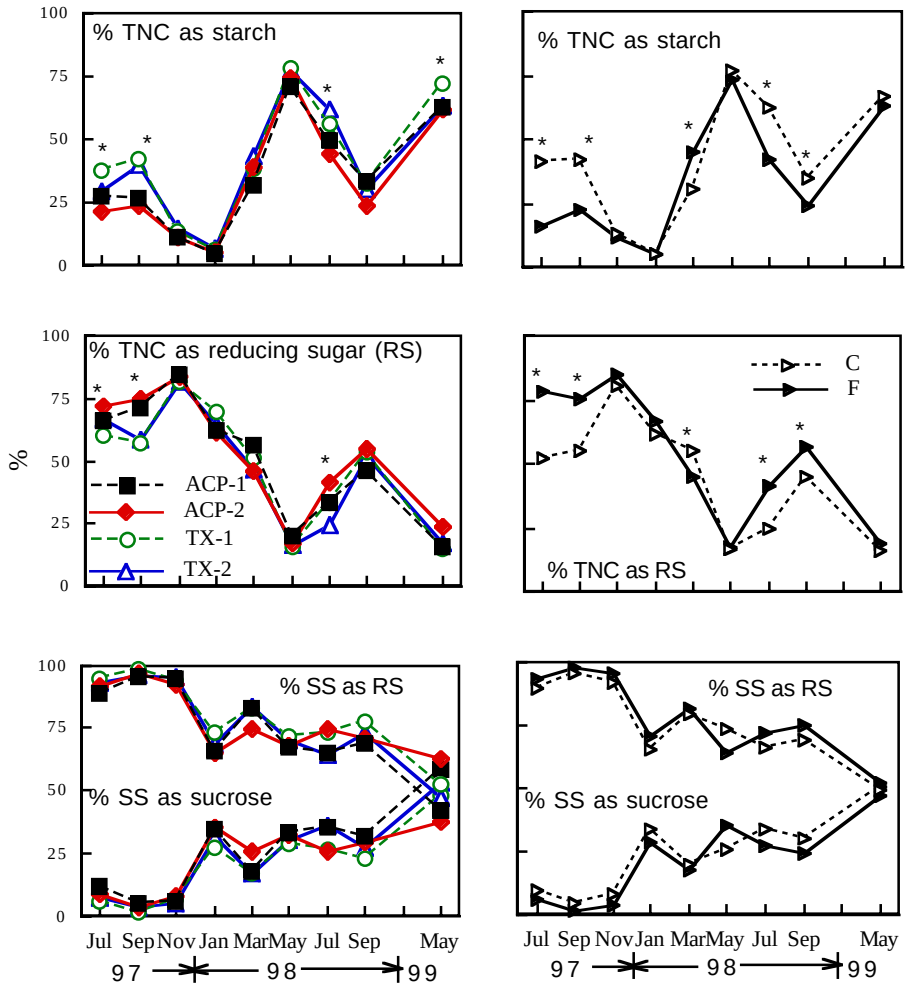


**Figure 6.** Effect of family and fertilization on carbohydate partitioning of needles as they age. Since there was no significant diurnal effect on carbohydrate concentration ($P > 0.05$), only data for needles sampled at dawn are presented. May 1999 data are for current year foliage. Treatments differ significantly as determined by *lsd* test ($P \leq 0.05$, *).

## DISCUSSION

### *Genetic differences in gas exchange*

Net photosynthesis of a forest canopy is one of the principle processes determining net forest carbon gain, storage and productivity. Despite considerable efforts in these areas, the degree of genetic control over $P_{net}$ and an associated variation in growth or productivity are still poorly understood and needs to be further examined in field-grown trees (Bongarten and Teskey 1987; Major and Johnsen 1996; Svensson 1996; Johnson *et al*. 1999). Some recent field studies attempting to determine the physiological basis for observed genetic differences in growth of trees and genetic x environment interactions using photosynthetic gas exchange, water relations and leaf carbon isotope discrimination have met with limited success (Major and Johnsen 1996; Svensson 1996; Johnsen and Major 1999; Major and Johnsen 1999). When photosynthesis has

**Table 5**. Mean carbohydrates concentrations (n=6) (μmoles glucose eq/g DW) in needles of different age classes. Means are significantly different ($P \leq 0.05$) between current and previous year foliage except those followed by ns.

| | July 97 | Sept 97 | July 98 | Sept 98 |
|---|---|---|---|---|
| Total nonstructural carbohydrates (TNC) | | | | |
| Current year foliage | 419.66 | 433.23 | 493.6 | 499.52 |
| Previous year foliage | 534.96 | 523.69 | 624.24 | 559.56 |
| Starch | | | | |
| Current year foliage | 140.31 | 164.46 | 255.38 | 227.07 |
| Previous year foliage | 205.05 | 207.74 | 351.89 | 171.66 |
| Reducing sugars (RS) | | | | |
| Current year foliage | 23.03 | 8.43 | 41.89 | 123.53 |
| Previous year foliage | 34.01 | 17.68 | 80.96 | 104.86 |
| Reducing sugars (RS) | | | | |
| Current year foliage | 279.34 | 268.75 | 238.22 | 272.44 |
| Previous year foliage | 329.90 | 315.94 | 269.33 | 387.29 |
| % TNC as starch | | | | |
| Current year foliage | 28.6 ns | 32.5 ns | 51.0 ns | 40.7 |
| Previous year foliage | 32.9 ns | 35.6 ns | 52.8 ns | 29.6 |
| % soluble sugar as RS | | | | |
| Current year foliage | 92.0 ns | 96.7 ns | 82.3 | 54.3 |
| Previous year foliage | 89.1 ns | 94.3 ns | 69.0 | 72.4 |

been related to growth, it is usually because estimates of leaf area have been incorporated for scaling purposes (Bongarten and Teskey 1987; Michael *et al.* 1990; Wang *et al.* 1995; Major and Johnsen 1996). Strong correlations between genetic variation in $P_{net}$ and growth may be most pronounced under environmental stress, e.g. drought (Major and Johnsen 1996), or if seasonal (Boltz *et al.* 1986) and diurnal patterns are examined.

In the current study, we found little genetic variation in seasonal $A_{max}$ and diurnal rates of photosynthesis in four families of loblolly pine during both a wet (1998) and very dry (1999) year. Family differences in both foliage classes were only evident in July 99 during a long drought, but family variation in diurnal $P_{net}$ in July was not consistent. Precipitation through July 1999 was 40% of the previous year. Photosynthetic data for that month would suggest that $A_{max}$ was positively correlated with growth of the four families, i.e. ACP1>ACP2>TX1>TX2, since the average tree height in 1999 for these families followed the same order (ACP-1= 499 cm, ACP-2 = 467 cm, TX-1 = 461 cm, TX-2 = 428 cm, from McKeand, unpublished data).

Although the current study used a small sampling size (four families), most of our photosynthetic data would indicate that photosynthetic rate may not be a good predictor of genetic variation in yield, at least in loblolly pine. In loblolly pine, leaf area index, rather than photosynthesis may be more positively correlated with tree growth (Bongarten and Teskey 1987; Vose and Allen 1988; Albaugh *et al.* 1998). Historically, in agronomic crops, the largest increases in yield have generally occurred via genetic manipulation of light interception and increased allocation of photosynthate to the harvested organ (Gifford and Evans 1981).

Diurnal patterns of photosynthesis are similar to those reported in earlier loblolly pine studies (Green *et al.* 1991; Ellsworth 2000). In the current study, fertilization similarly had little effect on diurnal rates of photosynthesis or $A_{max}$; when significant, rates were slightly higher under fertilized conditions. However, $A_{max}$ was positively correlated with foliar N concentration ($r=0.892$, $P<0.001$) as has been reported in previous studies (Green and Mitchell 1992; Murthy *et al.* 1996). Fertilization increased foliar N concentrations by 45% and 30% in 1998 and 1999, yet the effect on $A_{max}$ was much less pronounced. Interestingly, the severe drought in 1999 reduced foliar N concentrations by 33% most likely due to reductions in fine root system growth.

In the current study, needle age had a significant effect on diurnal rates of photosynthesis and $A_{max}$, with current year needles exhibiting higher photosynthetic rates than previous year needles (Table 1). Other studies with field-grown loblolly pine have shown a similar depression in photosynthetic rates as needles age (Murthy *et al.* 1997; Ellsworth 2000). Ellsworth (2000) has estimated that current year needles may account for 51% of annual net carbon gain in loblolly pine and play a critical role in supporting foliage and twig expansion during budbreak the following spring. In the current study, higher N concentrations in current vs. previous year foliage may account for higher photosynthetic rates; at the very least, it suggests that younger foliage is a stronger N sink. But higher photosynthetic rates in current year foliage may also be a function of the higher metabolic demands of this tissue as well as close non-photosynthesizing carbon sinks (Chung and Barnes 1980; Myers *et al.* 1999a,b). Indeed, the lower TNC and starch concentrations in current year foliage would indicate that carbon demands are high. Starch accumulation in leaves generally occurs when photosynthate production exceeds immediate metabolic demands and exports out of source leaves.

Differences in $\delta^{13}C$ but not instantaneous WUE among families may partially be a function of strong seasonal changes in $A_{max}$, gs, transpiration rate and WUE. However, family differences in $\delta^{13}C$ values are so small (range is less than 0.6 per mil) that although statistically significant, their biological significance is questionable. Carbon isotope discrimination integrates $c_i/c_a$ throughout the year; like height or biomass, it will reflect the integration of these instantaneous measures with environmental conditions. Previous studies with several tree species have observed a stronger correlation between water use efficiency (as measured by carbon isotope discrimination) and growth, than between $P_{net}$ and growth (e.g. Zhang *et al.* 1994; Major and Johnsen 1996; Johnsen *et al.* 1999; but see Li 2000).

A strong stomatal control over $A_{max}$ is indicated by a positive correlation between photosynthesis and stomatal conductance in plants (Farquhar and Sharkey 1982). Water stress in summer imposes a strong stomatal limitation on $A_{max}$ (Ellsworth 2000; Green and Mitchell 1992; Day *et al.* 1991). In the current study, the higher regression coefficient and $r^2$ in July vs.

September of 98 and 99 in both foliage classes demonstrate that summer drought resulted in a strong stomatal limitation to $A_{max}$ in most families. In July 99, family TX-2 exhibited the weakest correlation between $A_{max}$ and $g_s$, indicating a non-stomatal limitation to $A_{max}$. Uncoupling between needle $P_{net}$ and $g_s$ during a severe drought is suggestive of superior drought tolerance of this family.

Because current year needles tend to be more sensitive to drought than previous year needles (Ellsworth 2000), the presence of older foliage classes during the summer may supplement carbon demands of younger foliage during drought. Although stomatal control of photosynthesis in July 1999 was higher in previous year than current year foliage, younger foliage exhibited 37% higher $A_{max}$, suggesting higher water use efficiency in this foliage. Although foliage $\delta^{13}C$ data indicated little difference in WUE between current and previous year foliage during 1998, $\delta^{13}C$ data for September 99 suggest a higher WUE in current vs. previous foliage (Table 3). In the present study, we detected statistical differences in needle $\delta^{13}C$ among the four loblolly pine families. Family TX-2, a drought-hardy and slowest growing of the four families, exhibited the lowest $\delta^{13}C$ values during 1998 and 1999, and lowest instantaneous WUE of any of the families except in July 99. In July 99, TX-2 had the highest instantaneous WUE of any family, and also exhibited the least amount of stomatal control over photosynthesis, with uncoupling occurring in previous year foliage. In contrast, the faster growing (mesic) ACP families had higher WUE during both years.

Loblolly pine from the "Lost Pines" region in Texas are known for their drought hardiness. In the current study, we hypothesized that Texas families would exhibit higher water use efficiencies than the ACP families, particularly during the 1999 drought. Both WUE estimates obtained from gas exchange measurements and $\delta^{13}C$ data do not indicate that Texas pines used water more efficiently than the more mesic ACP families except during a severe drought (as in July 1999). However, family differences might be more pronounced under the more consistent drought conditions that are experienced by the Texas loblolly pine in their natural habitat.

***Carbon partitioning in needles***

Genetic differences in net carbon gain in trees could not only be a function of difference in rate of photosynthesis and total leaf area available for carbon fixation, but in respiration rate and immediate metabolic demands of leaf tissue. The supply available for export out of source leaves to nonphotosynthesizing carbon sinks is more a function of diurnal net carbon gain and seasonal change in net carbon gain. Needles of loblolly pine exhibited pronounced seasonal changes in absolute concentrations of TNC, starch, sugars, and in partitioning of TNC towards starch and sugars. A strong seasonal influence on starch concentrations in southern pines is not unexpected given the dynamic or seasonal natures of photosynthesis and shoot and root growth. Seasonal peaks in needle starch in the spring near budbreak have been reported for loblolly and slash (*P. elliottii*) pines (Adams *et al.* 1986; Cranswick *et al.* 1987; Gholz and Cropper 1991). In the current study, concentrations of TNC and starch in previous year foliage were highest in May during spring foliage expansion, and peak photosynthesis, whereas starch concentrations were lowest in winter.

Starch accumulation in foliage occurs when carbon supply exceeds leaf metabolic demands and carbon export out of source leaves. Although foliage and branch expansion in the spring are strong carbon sinks (i.e. spring foliage has not yet become a carbon source), high starch concentrations in previous year foliage are most likely a function of high photosynthetic rates and remobilized starch reserves from woody tissues (Yang and Topa, unpublished data). However, it is also likely that carbon demands of expanding foliage were beginning to decline in May because the photosynthetic apparatus is becoming functional. Thus, if expanding needles are beginning to transition from being carbon sinks to sources, carbon demands on older foliage would decrease and starch accumulation would occur (Turgeon 1989). In southern pines, foliar starch concentrations are often the lowest in late fall or early winter (current study, Adams *et al.* 1986; Gholz and Cropper 1991), when photosynthetic rates and metabolic demands are low and the dependency on stored carbon may be at its highest.

Gholz and Cropper (1991) found little seasonal variation in sugar concentrations in needles of slash pine. In the current study, seasonal differences in foliar soluble sugar concentrations (sucrose and reducing sugars) were significant, but the seasonal differences were not as pronounced as those for starch. Unlike seedling studies with loblolly and pond pines (*P. serotina*) (Topa and Cheeseman 1992; Kuehny and Topa 1998), sucrose was not the predominant sugar in needles, and reducing sugars represented over 70% of soluble sugars in needles of loblolly pine. The highest concentrations of reducing sugars occurred in the fall/winter, and may be, in part, osmotic adjustment for the prevention of freezing injury.

In the current study, seasonal peaks in TNC and starch concentrations generally followed those of photosynthesis. Such a pattern would argue against feedback inhibition of photosynthesis by chloroplast starch. In addition, the absence of diurnal changes in leaf starch concomitant with pronounced diurnal patterns in net photosynthesis would indicate that diurnal changes in $P_{net}$ are independent of chloroplastic starch concentrations. The lack of diurnal effects on foliar starch concentrations or starch/sucrose partitioning (data not shown) is surprising given the strong diurnal pattern in $P_{net}$ in loblolly pine, and evidence for coordinated diurnal partitioning of newly fixed carbon into starch and sucrose in both herbaceous (e.g. Fondy and Geiger 1985; Fondy *et al.* 1989; Servaites *et al.* 1989a,b; Li *et al.* 1992) and some woody species (seedling studies only, Topa and Cheeseman 1992; Wullschleger *et al.* 1992; Topa *et al.* 2000). Coordinated diurnal partitioning of newly-fixed carbon to starch and sucrose ensures that a balanced supply of carbon is available for export out of source leaves during the day and night (Fondy *et al.* 1989; Li *et al.* 1992; Topa *et al.* 2000), when photosynthetic rates fall below a minimum. The evergreen characteristic of loblolly pine foliage, combined with a long growing season and continued (albeit low) rates of photosynthesis in winter (Murthy *et al.* 1997) may result in a lower dependence upon foliage starch for maintenance of leaf metabolism at night, and perhaps greater dependence upon twig and stem reserves. In trees, storage carbon in all plant tissues play a critical role in buffering temporary fluctuations in supply and demand.

In the current study, although fertilization had little effect on $P_{net}$, it reduced TNC concentrations and partitioning of TNC into starch in July and September. The lower TNC concentrations in fertilized trees may be a dilution effect and/or reflect enhanced metabolic demands of fertilized leaves. Field studies with *P. taeda* (Adams *et al.* 1986) and *P. elliottii* (Gholz and Cropper 1991) found minimal fertilization effects on starch and sugar concentrations

in foliage. The lack of pronounced fertilization (Adams *et al.* 1986; Gholz and Cropper 1991; current study) and family/provenance effects (current study) on foliar carbohydrate storage patterns in southern pines would suggest strong endogenous control over partitioning of fixed carbon to storage in field trees that is independent of environments. Needle age appears to have more of an effect on TNC, starch and soluble sugar concentrations in loblolly (Myers *et al.* 1999a,b) and slash pine than fertilizer (Adams *et al.* 1986; Gholz and Cropper 1991; current study) or family effects (current study). In the current study, previous year foliage consistently exhibited higher concentrations of TNC, starch and soluble sugars than current year foliage. Although photosynthetic rates in July and September were higher in current year foliage, metabolic demands of this tissue at this time were probably also higher than older foliage.

### *Conclusion*

In the current study with field-grown loblolly pine, diurnal and seasonal effects on photosynthesis were more pronounced than family or treatment effects. Despite diurnal changes in photosynthesis, there were no diurnal effects on foliar starch concentrations or starch partitioning among families or treatments. However, strong seasonal effects on foliar starch concentrations and starch partitioning were apparent. Fertilization reduced TNC concentrations and partitioning of TNC into starch in July and September, possibly refecting a dilution effect or enhanced metabolic demands of fertilized leaves. A phenological effect by different foliage cohorts on carbon source/sink relationships and whole tree carbon gain was suggested by gas exchange and foliar carbohydrate data. Overall, our data suggest strong ontogenetic control over gas exchange characteristics and carbon partitioning in loblolly pine that was independent of environment and genetic influences.

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## TASK 1: ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

# VI. SEASONAL AND TREATMENT EFFECTS ON WHOLE-TREE CARBON STORAGE

**W.Q. Yang[1], R.P. Phillips[2], G.K. Blaisdell[3] and and M.A. Topa[4]**

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## INTRODUCTION

Loblolly pine (Pinus taeda L.) is the most widely planted tree species in the Atlantic Coastal Plain, with over 12.3 million hectares in both natural and planted stands. Because of its wide geographic distribution, it is not uncommon to find significant genotypic variation in growth of various populations. Populations of loblolly pine from the western extent of its physiographic range, i.e. Texas, are often described as slow-growing, but known for their drought and resistance to many natural pests/pathogens. Their drought hardiness is more a function of avoidance, e.g. deeper taproots and wide-ranging laterals, than drought tolerance. Eastern populations from North and South Carolina are generally faster-growing, but less resistant to drought and natural pathogens such as fusiform rust and tipmoth. Whether differences in aboveground growth between the various populations is a function of differences in whole-tree carbon source/sink relationships, particularly root system carbon demands, has not been examined. In the following study, we examined whole-tree carbon allocation and partitioning strategies of some fast- and slow-growing families of loblolly pine to assess genetic x environment differences in whole-tree carbon source/sink relationships.

## MATERIALS AND METHODS

Approximately every two months, tissue samples were collected for carbohydrate analysis from two trees/family plot in each of the three blocks. Current-year foliage, previous-year foliage, branch and stem cores (at breast height) were removed from each tree between 11:00 AM and 6:00 PM. Soil cores were removed within the dripline of each tree using a 15-cm diameter stovepipe. Roots from these cores were sifted through 1.3 cm and 0.6 cm screens, sorted into fine (≤ 2.0 mm diameter), coarse (2-5 mm) and woody roots (> 5 mm), and washed in water. Tissues were placed upon dry ice immediately and stored at –70 °C until freeze-dried.

Tissue was ground and extracted with 80% ethanol at 80°C (Topa and Cheeseman 1992). Reducing sugars (glucose + fructose) and sucrose were determined in the ethanol extract using enzymatic analysis and measured as glucose equivalents at 340 nm on a Beckman 640 spectrometer. The starch-containing pellet was incubated with amyloglucosidase for 24 h at 55°C. Starch concentration was determined enzymatically as glucose equivalents. Total nonstructural carbohydrates (TNC) represent ∑ starch + reducing sugars + sucrose.

## RESULTS

There were seasonal effects on TNC concentrations and partitioning towards starch in previous year foliage with peak levels occurring in May (Figure 1). Family and treatment effects were significant in late summer. Peak TNC concentrations and partitioning towards starch in current year foliage occurred in July and declined through November (Figure 2). Family and treatment effects were most significant in late summer. TNC concentrations and partitioning towards starch in branches peaked in May, but remained low for most the year (Figure 3). Control branches had higher TNC concentrations in late summer/early fall. Stem tissue showed significant seasonal trends, with peak TNC concentrations and partitioning towards starch highest in May, July and November (Figure 4).

Although peak TNC concentrations in coarse roots occurred in March, partitioning towards starch remained high from January through May (Figure 5). Family effects were not significant while fertilization reduced partitioning towards starch in late summer/early fall. Woody roots exhibited similar TNC concentrations and starch partitioning as coarse roots. Fine roots followed similar trends as coarse roots, but fine roots exhibited lower TNC concentrations in spring (Figure 6).

Absolute TNC concentrations were the highest in root tissues; i.e. coarse roots = woody roots > fine roots = previous year needles > current year needles > branch > stem. The high partitioning of TNC towards starch in root tissues concomitant with their high TNC concentrations suggest that root tissues serve as the primary storage organ in loblolly pine. In May, peak TNC concentrations and partitioning towards starch in old foliage and branches are probably a function of remobilized carbon reserves from roots and high $P_{net}$ rates. The increased partitioning of TNC towards starch in November in root, branch, and stem tissues suggest a reduction in shoot carbon demands and switch to carbon storage. Our data suggest that there were no differences in carbon partitioning to storage carbon between fast- and slow-growing families of loblolly pine; however, fertilizer altered whole-tree carbon source/sink relationships.

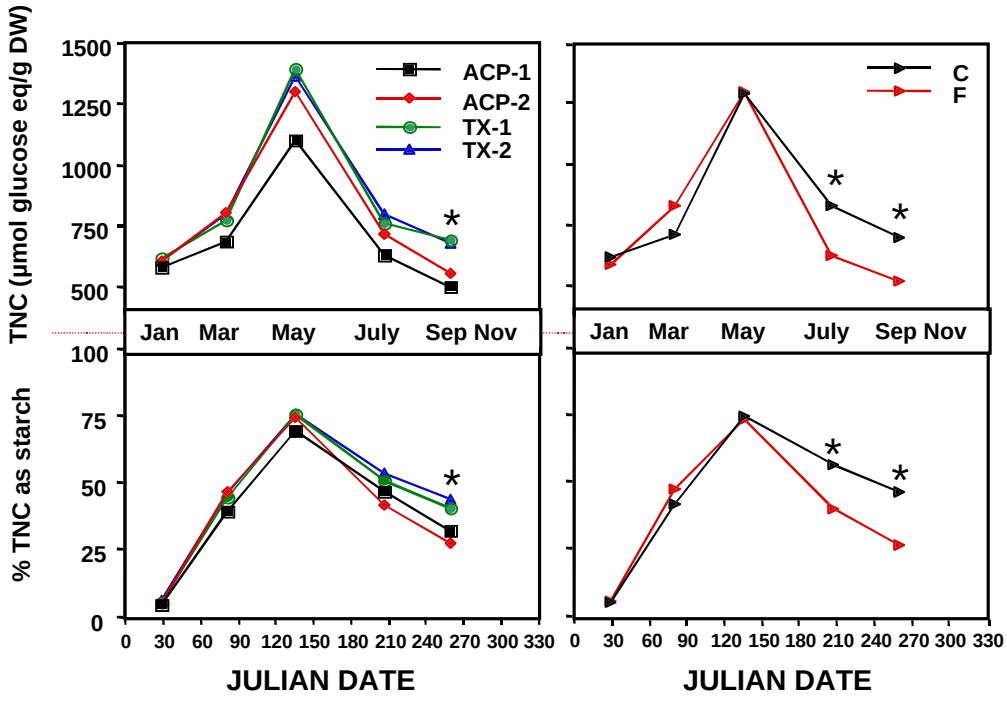


**Figure 1.** TNC concentrations and partitioning of TNC into starch in previous year foliage of loblolly pine in control (C) and fertilized (F) treatments. Family and treatment effects are shown.

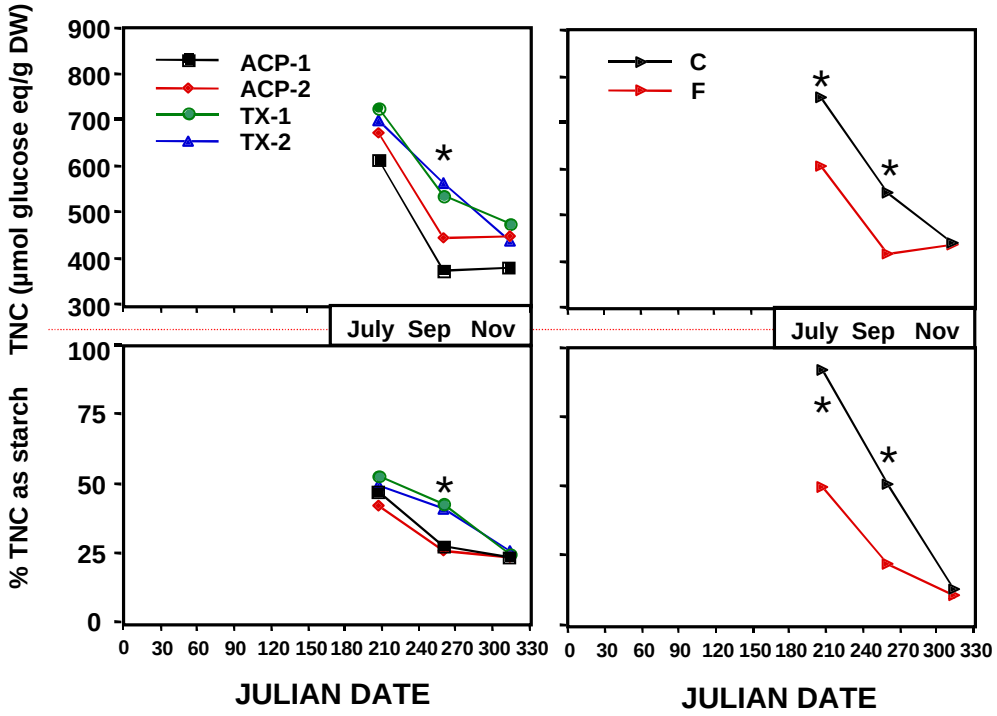


**Figure 2.** TNC concentrations and partitioning of TNC into starch in current year foliage of loblolly pine in control (C) and fertilized (F) treatments. Family and treatment effects are shown.

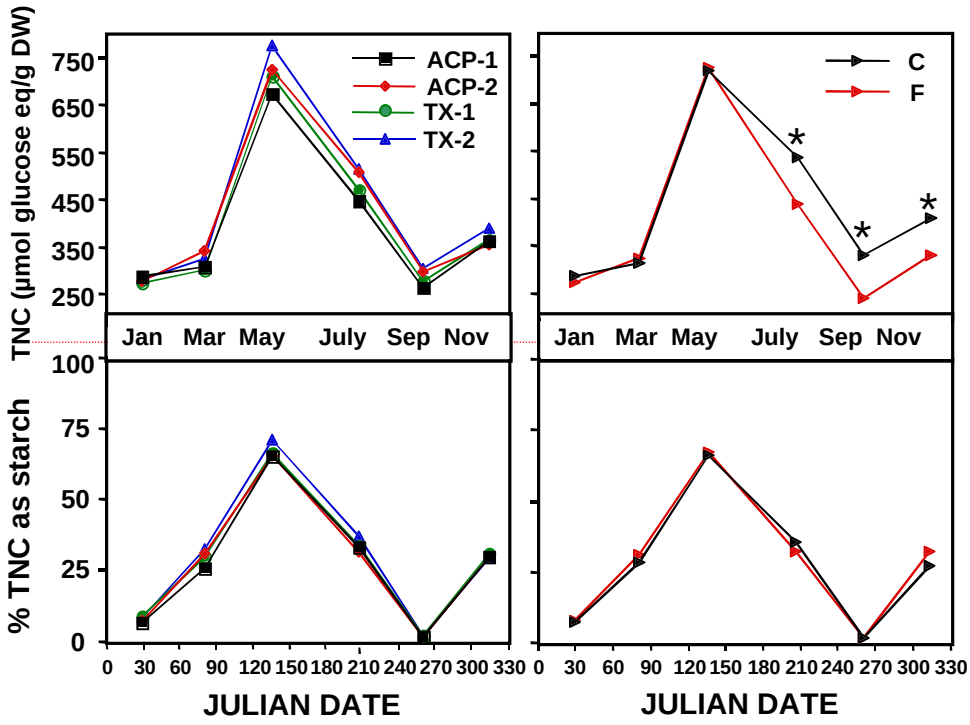


**Figure 3.** TNC concentrations and partitioning of TNC into starch in branches of loblolly pine in control (C) and fertilized (F) treatments. Family and treatment effects are shown.

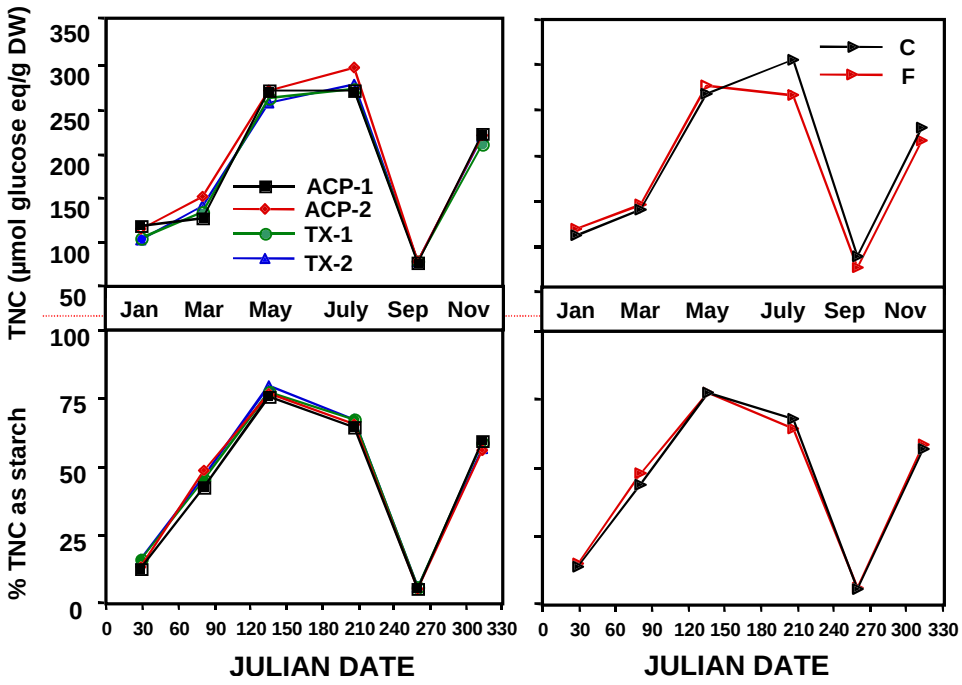


**Figure 4.** TNC concentrations and partitioning of TNC into starch in stems of loblolly pine in control (C) and fertilized (F) treatments. Family and treatment effects are shown.

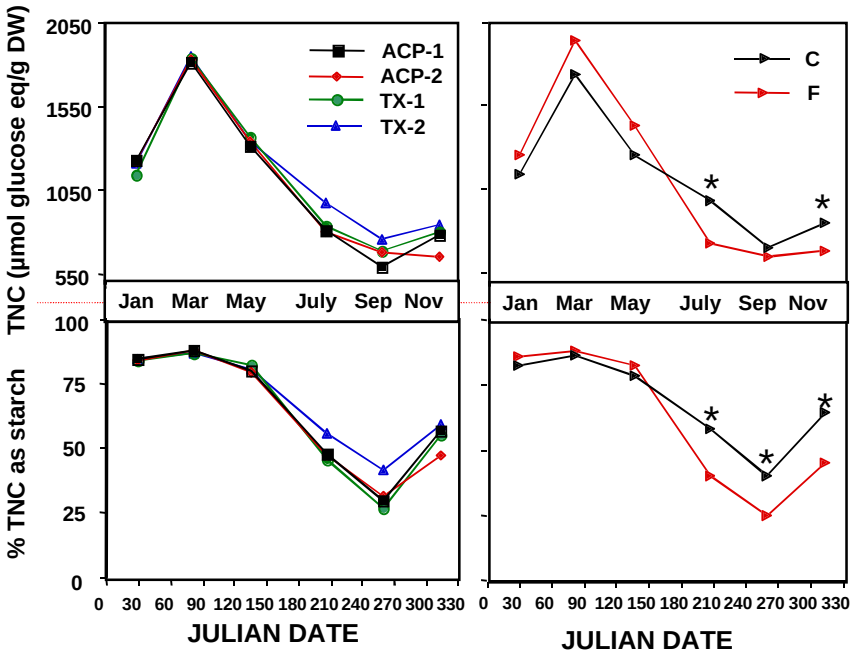


**Figure 5.** TNC concentrations and partitioning of TNC into starch in coarse roots of loblolly pine in control (C) and fertilized (F) treatments. Family and treatment effects are shown.

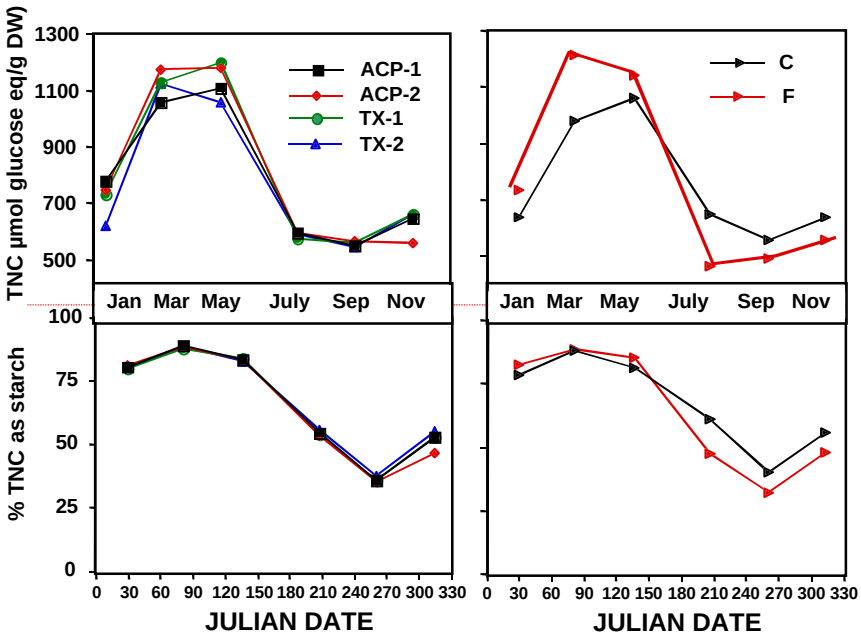


**Figure 6.** TNC concentrations and partitioning of TNC into starch in fine roots of loblolly pine in control (C) and fertilized (F) treatments. Family and treatment effects are shown.

## TASK 1. ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

## VII. GROWTH AND PHYSIOLOGY OF LOBLOLLY PINE SEEDLINGS AS AFFECTED BY GENETICS OF THE ROOT SYSTEM

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### OBJECTIVES

Effects of root and shoot genotypes on productivity and physiology of loblolly pine (*Pinus taeda* L.) seedlings were evaluated. The basic purpose was to elucidate the relative influence of genetic factors in tree roots upon growth in biomass.

Twelve-week-old seedlings from contrasting provenances were grafted reciprocally to facilitate distinction of rootstock and scion effects. Five open-pollinated families each from a mesic region (Atlantic Coastal Plain) and from a xeric region (Lost Pines Texas) were used and were planted in a split-plot design on a nutrient-poor site in the Sandhills region of central North Carolina. A total of 1800 seedlings were used, including ungrafted trees as controls. Half of the plots were fertilized annually, and after one and two growing seasons, seedlings were harvested for component biomass determinations.

### RESULTS

#### *Biomass allocation as affected by root system genotype*

Total biomass production among families was positively related to proportional biomass allocation to roots. Generally, mesic sources produced more total biomass and allocated proportionally more biomass to roots. This finding may reflect that soil resources of water or nutrients were limiting tree growth on the site. No substantial family differences were found in biomass distribution among aboveground parts (foliage, branches, stem).

When fertilized, mesic rootstocks were always associated with greater mass in aboveground components, regardless of scion genotype. Proportional biomass shifts between aboveground parts and belowground parts, depending on root and shoot genotypes, suggested that root system genotype was more influential in determining root:shoot allocation. This effect may also have been accentuated by the growth-limiting edaphic conditions of the site.

Rootstock did affect stem growth efficiency, in that the xeric rootstock was associated with increased proportional allocation to stem, regardless of scion type. The main trade-off in that case, as in most others, was between stem versus root allocation. It was concluded that different root genotypes are associated with subtle changes in biomass allocation which may accrue over time to substantial productivity differences.

***Foliar physiology as affected by root system genotype***

Effects of root system genotype on foliar physiology of selected families were evaluated and related to whole-plant growth of genotypes. In four families (two from each provenance), midday light-saturated net photosynthesis ($A_n$) and stomatal conductance to water vapor ($g_s$) were measured monthly during the summer of 1999. Leaf carbon isotope discrimination ($\Delta$) was analyzed for estimation of long-term water use efficiency (WUE) of genotypes.

Provenances differed in $g_s$ but not in $A_n$. The mesic sources had lower $g_s$ and higher intrinsic WUE ($WUE_i$; $A_n/g_s$). Rootstock affected $g_s$ but not $A_n$ nor $A_n/g_s$ of scions. Rootstocks were associated with lower $g_s$ when paired with scions of the other provenance. Although leaf $\Delta$ did not normally differ significantly between provenances, rootstock did affect $\Delta$. Xeric rootstocks were associated with lower $\Delta$. Photosynthesis and WUE were limited more by stomatal factors than by nonstomatal factors during the measurement periods, which varied from mild to severely droughty. It was evident that stomatal behavior was pre-conditioned by factors inherent with root genotype.

Degree of correlation between $\Delta$ and $WUE_i$ depended on the degree of relatedness between genotypes grafted as scion and rootstock. Within-provenance grafts showed the expected negative correlation between $\Delta$ and $WUE_i$, but between-provenance grafts showed no such trend. This finding bolstered the claim that root genotype can substantially influence physiological performance in leaves. Leaf tissue $\Delta$ was related to stem growth efficiency (GE) and to root mass among families. The opposite-signed correlations suggest a trade-off between allocation to stem and roots, the balance of which depends heavily on root genotype. In this study, low leaf $\Delta$ was associated with high GE and low root mass allocation, though not strongly related to total biomass production. The results show that root system genotype can substantially affect certain aspects of leaf physiology, which can have large repercussions on tree growth.

## TASK 1: ROOT ONTOGENETIC AND PHYSIOLOGICAL STUDIES

## VIII. SEASONAL AND GENETIC VARIATION IN NITROGEN UPTAKE

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In 1999, $^{15}N$ experiments were conducted to determine whether maximum uptake occurs during peak growth periods. $^{15}N$ was applied as $NH_4^+$ three times during the year (in June, July and September) to assess when maximum uptake may be occurring. Ten to fifteen injection sites were located within the drip canopy of selected trees (in a 1 $m^2$ area around the bole), extending 20 cm deep into the soil. We assumed that with subsequent rain events, some of the $^{15}N$ would diffuse into the 20-40 cm horizon. Our whole-tree harvest data suggest that over 90% of lateral roots are in the 0-40 cm horizon. Above- and belowground tissues were removed from enriched trees 1.5-2 months after application; soil cores from the 0-40 soil horizons were also removed in the 1 $m^2$ application area. Soil and plant tissue is being analyzed for total N and $^{15}N$ by the Cornell and Boyce Thompson Institute Stable Isotope Laboratory, and is nearly completed. Whole-tree and soil budgets will be constructed to help answer the following questions:

1) Are there periods of maximum nitrogen uptake in the four loblolly pine families? Do they coincide with specific phenophases?
2) Is recently-acquired or stored N used to support new needle or root growth?
3) Are some families more efficient at acquiring soil nitrogen?

# CHAPTER 4

## TASK 2: COMPUTER (TREGRO) MODELING

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## OBJECTIVE

We used previously published and experimentally-collected data from this study in conjunction with the TREGRO (Figure 1) simulation model to develop accurate carbon budgets for each of the four loblolly pine families under nutrient limiting (CONTROL) and non-limiting conditions (FERTILIZED) (8 total parameter sets). TREGRO parameter sets could then be used to predict expected changes in whole-tree growth and carbon allocation for each of the families under a wide range of nutrient conditions.

## TREGRO DESCRIPTION

TREGRO is a physiological simulation model of the carbon, water, and nutrient fluxes of an individual tree (Weinstein *et al*. 1991). TREGRO was developed to analyze the response of trees to multiple environmental conditions, such as temperature, drought, nutrient deficiency, and exposure to pollutants. In the model, the tree is divided into the following compartments: a canopy of leaves grouped by age class, branches, stem, and coarse and fine roots in three soil horizons. In each compartment, the model keeps track of three carbon pools: structure (living, respiring tissue); wood (the non-respiring tissue); and total non-structural carbohydrate (TNC). The model calculates the carbon assimilation of the entire tree each hour as a function of ambient environmental conditions and the availability of light in the canopy, water, and nutrients using the Farquhar equations (Farquhar *et al*. 1980). Carbon is redistributed daily within the plant for respiration, growth, storage, and replacement of senescent tissues. Priority for the carbon varies inversely with the distance between source and sink and varies directly with the relative sink strength.

The interaction between tree growth and the environment in TREGRO is achieved through the linkage of separate data files. The PARAMETER file defines species-specific characteristics including (but not limited to): maximum photosynthetic rate, rates of maintenance and growth respiration, specifics of nutrient uptake kinetics, phenological patterns of growth, growth rates of individual tree compartments, and carbon partitioning within compartments between living structure, dead wood, and carbon reserves (total non-structural carbohydrates - TNC). The METEOROLOGICAL file defines the site-specific hourly environmental conditions including air temperature (°C), relative humidity (%), rainfall (mm), photosynthetic photon flux density ($\mu mol\ m^{-2}\ s^{-1}$), and $O_3$ concentration (ppb).

TREGRO parameters are set by employing an interactive interface that is separate from the model, but is programmed to read and write model input text files. The interface makes it convenient for a user to browse and change inputs to the model parameter file while working in an environment that helps the user choose logical and consistent parameter values for model inputs.

Parameterization requires knowledge of quantities and forms of carbon in each of the tree compartments (foliage, branch, stem, and coarse and fine root in three soil horizons). Once the user has an initial and final estimate of each compartments' biomass, carbon is allocated within each compartment to the three different forms described above. The amount of wood in the stem, branch, and coarse root compartments is usually set to equal a measured or published amount of heartwood (non-respiring tissue) in the stem. In addition, the amount of TNC in the foliage, branch, stem, and coarse root compartments is also set to equal to a measured or published amount from each of these compartments.

Calendar (and preferably degree-days) dates were necessary to initiate and end growth activities in each of the parameterized growth periods. Further, year-long meteorological files that match the site of parameterization were collected or constructed for each simulation year. All TREGRO simulations in the present study were two years in length and use meteorological data files collected on-site at SETRES II, NC, in each year of the study.

The biomass and carbon allocation of each of the loblolly pine trees was set in each of the parameter files. The simulation target was to grow a loblolly pine tree from each of the four families (under control or fertilized conditions) with two annual biomass increments (from year 1998 to year 2000).

***Initial tree biomass***

Biomass of the initial loblolly pine tree (parameterized on a family/treatment basis) was based on the measured biomass increment (Tables 1-8) calculated from the whole-tree biomass harvests in January 1998 and January 2000. Tree biomass in each component was further partitioned into TNC, structure, and wood (in woody tissue) (Tables 9-23). The proportion of wood in the initial tree's stem, branches, and coarse (woody) roots was set to be zero (0) since these were juvenile trees. The remainder of the initial biomass was divided between TNC and structure based upon measurements made during the whole-tree harvest. Values generated in these biomass and allocation tables will be used in the appropriate parameter files as they are developed.

***Soil parameters***

The soil rooting area in this study was set to be equal the area defined by the within- and between-row spacing (1.5 m x 2 m = 3.0 $m^2$). Depths of the A, $B_1$, and $B_2$ soil horizons were set to 0.2, 0.2, and 0.6 m, respectively, based on measured depths of the root systems at harvest. Soil water conditions were set to be non-limiting in all of the simulations. Nutrient conditions for each of the trees were specifically match the field treatment to the biomass, carbon allocation, and carbon acquisition data collected at the field site.

### *Seasonal phenology*

Seasonal phenology was monitored over the two years (1998 and 1999) of this study and parameters in the model were set to reflect the observed conditions. Observations indicated that bud break and foliage growth occurred on approximately day 60 in each year for all family/treatment combinations and that all tissue growth ceased on day 336. It was assumed that stem, branch, and root growth could occur between day-of-year (DOY) 15 to 335 when conditions are favorable in all simulations. Trees in this study were set to be "dormant" in the model (no growth ocurring) from DOY 336 until DOY 15 in the following year. Loblolly pine trees at this study site senesced all 2-year-old foliage, retaining only current-year foliage over winter.

### *Carbon assimilation*

Carbon assimilation of all families was measured in May 1999 under environmental conditions that were assumed to produce maximum photosynthetic rates. These values (for each family/treatment combination) were converted to maximum grams of carbon assimilated per gram leaf carbon per hour for entry into the parameter sets (Tables 25-32).

### *Final Parameter Criteria*

After entering all the parameter values in the appropriate parameter sets, each TREGRO simulated tree was calibrated by adjusting tissue growth rates and senescence rates in fine roots until two conditions are met: (1) when the simulated carbon gain of each of the tree components (foliage, branch, stem, and coarse and fine roots) and the total tree carbon gain is within 10 percent of the value for projected carbon gain from the field-site measurements, and (2) when the proportion of TNC and the ratio of structure to wood in each of the tree components at the end of a simulation matches that parameterized for the tree at the beginning of the simulation. Fine root senescence was set to approximate one complete root turnover per year for all trees in this study based on field measurements with a minirhizotron camera system.

### *Progress*

Meteorological data files have been constructed for both years of the study (1998 and 1999). One family/treatment combination has been completely parameterized (Family 81 - fertilized plots) in TREGRO (Table 33). The simulated values for this tree meet criteria 1 and 2 described above. Other parameter sets are under development and are expected to be completed shortly. Once parameter files have been constructed they will be used to predict expected changes in whole-tree growth and carbon allocation for each of the families under a wide range of nutrient conditions.

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**Table 1**. Biomass of family 81 in the fertilized plots in January 1998 and January 2000. Values represent grams carbon (2 grams biomass = 1 gram carbon). Measured 2-year growth = 2000 biomass - 1998 biomass.

| | | | | | |
|---|---|---|---|---|---|
| | Family | Treatment | | YEAR | |
| | 81 | F | | 1998-2000 | |
| | | | | | |
| | | | | | |
| | | | | MEASURED | |
| | COMPONENT | 1998 Biomass | 2000 Biomass | 2yr growth | |
| | | | | | |
| | stem | 1735.67 | 4942.17 | 3206.50 | |
| | | | | | |
| | branch | 662.65 | 1414.22 | 751.57 | |
| | | | | | |
| | foliage | 758.77 | 789.40 | 30.63 | |
| | | | | | |
| | root (+stump) | 1227.81 | 2118.35 | 890.54 | |
| | | | | | |
| | total tree | 4384.90 | 9264.14 | 4879.24 | |
| | | | | | |
| | total above ground | 3157.09 | 7145.79 | 3988.70 | |
| | | | | | |
| | Foliage Class | | | | |
| | 0 | 344.72 | 297.65 | -47.07 | |
| | 1 | 414.05 | 491.75 | 77.70 | |
| | | | | | |
| | | | | | |
| | Crs Root A | 765.04 | 1367.66 | 602.62 | |
| | Crs Root B | 333.30 | 696.32 | 363.02 | |
| | | | | | |
| | Fine Root A | 114.95 | 45.62 | -69.33 | |
| | Fine Root B1 | 11.54 | 5.27 | -6.27 | |
| | Fine Root B2 | 2.98 | 3.48 | 0.50 | |
| | | | | | |
| | | | | | |
| | Values are in grams carbon! | | | | |
| | | | | | |
| | Boxes shaded in gray indicate values for entry from field data. | | | | |

**Table 2**. Biomass of family 81 in the control plots in January 1998 and January 2000. Other information as in Table 1.

| | | | | | |
|---|---|---|---|---|---|
| | Family | Treatment | | YEAR | |
| | 81 | C | | 1998-2000 | |
| | | | | | |
| | | | | | |
| | | | | MEASURED | |
| | COMPONENT | 1998 Biomass | 2000 Biomass | 2yr growth | |
| | | | | | |
| | stem | 775.17 | 1611.67 | 836.50 | |
| | | | | | |
| | branch | 240.82 | 462.17 | 221.35 | |
| | | | | | |
| | foliage | 342.80 | 353.09 | 10.29 | |
| | | | | | |
| | root (+stump) | 591.02 | 879.85 | 288.83 | |
| | | | | | |
| | total tree | 1949.81 | 3306.78 | 1356.97 | |
| | | | | | |
| | total above ground | 1358.79 | 2426.93 | 1068.14 | |
| | | | | | |
| | Foliage Class | | | | |
| | 0 | 131.20 | 100.77 | -30.43 | |
| | 1 | 211.60 | 252.32 | 40.72 | |
| | | | | | |
| | Crs Root A | 326.94 | 554.77 | 227.83 | |
| | Crs Root B | 181.96 | 277.35 | 95.39 | |
| | | | | | |
| | Fine Root A | 76.14 | 41.93 | -34.21 | |
| | Fine Root B1 | 4.66 | 2.17 | -2.49 | |
| | Fine Root B2 | 1.32 | 3.63 | 2.31 | |
| | | | | | |
| | | | | | |
| | Values are in grams carbon! | | | | |
| | | | | | |
| | Boxes shaded in gray indicate values for entry from field data. | | | | |

**Table 3**. Biomass of family 91 in the fertilized plots in January 1998 and January 2000. Other information as in Table 1.

| | | | | | |
|---|---|---|---|---|---|
| | Family | Treatment | | YEAR | |
| | 91 | F | | 1998-2000 | |
| | | | | | |
| | | | | | |
| | | | | MEASURED | |
| | COMPONENT | 1998 Biomass | 2000 Biomass | 2yr growth | |
| | | | | | |
| | stem | 1539.00 | 3883.33 | 2344.33 | |
| | | | | | |
| | branch | 576.68 | 1127.55 | 550.87 | |
| | | | | | |
| | foliage | 568.22 | 711.98 | 143.76 | |
| | | | | | |
| | root (+stump) | 1142.07 | 1757.45 | 615.38 | |
| | | | | | |
| | total tree | 3825.97 | 7480.31 | 3654.34 | |
| | | | | | |
| | total above ground | 2683.90 | 5722.86 | 3038.96 | |
| | | | | | |
| | | | | | |
| | Foliage Class | | | | |
| | 0 | 243.25 | 240.88 | -2.37 | |
| | 1 | 324.97 | 471.10 | 146.13 | |
| | | | | | |
| | Crs Root A | 702.58 | 1197.00 | 494.42 | |
| | Crs Root B | 303.05 | 498.13 | 195.08 | |
| | | | | | |
| | Fine Root A | 123.73 | 56.02 | -67.71 | |
| | Fine Root B1 | 9.92 | 5.75 | -4.17 | |
| | Fine Root B2 | 2.79 | 0.55 | -2.24 | |
| | | | | | |
| | | | | | |
| | Values are in grams carbon! | | | | |
| | | | | | |
| | Boxes shaded in gray indicate values for entry from field data. | | | | |
| | | | | | |

**Table 4**. Biomass of family 91 in the control plots in January 1998 and January 2000. Other information as in Table 1.

| Family | Treatment | | YEAR |
|---|---|---|---|
| 91 | C | | 1998-2000 |
| | | | |
| | | | |
| | | | MEASURED |
| COMPONENT | 1998 Biomass | 2000 Biomass | 2yr growth |
| stem | 466.83 | 1299.83 | 833.00 |
| branch | 177.02 | 510.27 | 333.25 |
| foliage | 244.46 | 452.81 | 208.35 |
| root (+stump) | 379.34 | 597.98 | 218.64 |
| total tree | 1267.65 | 2860.89 | 1593.24 |
| total above ground | 888.31 | 2262.91 | 1374.60 |
| | | | |
| | | | |
| Foliage Class | | | |
| 0 | 81.18 | 118.63 | 37.45 |
| 1 | 163.28 | 334.18 | 170.90 |
| | | | |
| Crs Root A | 216.41 | 351.37 | 134.96 |
| Crs Root B | 97.66 | 196.08 | 98.42 |
| | | | |
| Fine Root A | 56.73 | 44.08 | -12.65 |
| Fine Root B1 | 4.70 | 6.08 | 1.38 |
| Fine Root B2 | 3.84 | 0.37 | -3.47 |
| | | | |
| | | | |
| Values are in grams carbon! | | | |
| | | | |
| Boxes shaded in gray indicate values for entry from field data. | | | |

**Table 5**. Biomass of family BA in the fertilized plots in January 1998 and January 2000. Other information as in Table 1.

| | | | | | |
|---|---|---|---|---|---|
| | Family | Treatment | | YEAR | |
| | BA | F | | 1998-2000 | |
| | | | | | |
| | | | | | |
| | | | | MEASURED | |
| | COMPONENT | 1998 Biomass | 2000 Biomass | 2yr growth | |
| | | | | | |
| | stem | 1681.50 | 4891.67 | 3210.17 | |
| | | | | | |
| | branch | 629.95 | 1004.23 | 374.28 | |
| | | | | | |
| | foliage | 730.53 | 867.46 | 136.93 | |
| | | | | | |
| | root (+stump) | 1138.95 | 1889.27 | 750.32 | |
| | | | | | |
| | total tree | 4180.93 | 8652.63 | 4471.70 | |
| | | | | | |
| | total above ground | 3041.98 | 6763.36 | 3721.38 | |
| | | | | | |
| | | | | | |
| | Foliage Class | | | | |
| | 0 | 375.93 | 298.43 | -77.50 | |
| | 1 | 354.60 | 569.03 | 214.43 | |
| | | | | | |
| | Crs Root A | 623.15 | 1210.77 | 587.62 | |
| | Crs Root B | 362.30 | 632.98 | 270.68 | |
| | | | | | |
| | Fine Root A | 129.95 | 32.82 | -97.13 | |
| | Fine Root B1 | 11.84 | 10.00 | -1.84 | |
| | Fine Root B2 | 11.72 | 2.70 | -9.02 | |
| | | | | | |
| | Values are in grams carbon! | | | | |
| | | | | | |
| | Boxes shaded in gray indicate values for entry from field data. | | | | |
| | | | | | |

**Table 6**. Biomass of family BA in the control plots in January 1998 and January 2000. Other information as in Table 1.

| | | | | |
|---|---|---|---|---|
| Family | Treatment | | YEAR | |
| BA | C | | 1998-2000 | |
| | | | | |
| | | | | |
| | | | MEASURED | |
| COMPONENT | 1998 Biomass | 2000 Biomass | 2yr growth | |
| | | | | |
| stem | 466.83 | 1033.00 | 566.17 | |
| | | | | |
| branch | 177.02 | 250.12 | 73.10 | |
| | | | | |
| foliage | 244.46 | 227.13 | -17.33 | |
| | | | | |
| root (+stump) | 263.80 | 598.38 | 334.58 | |
| | | | | |
| total tree | 1152.11 | 2108.63 | 956.52 | |
| | | | | |
| total above ground | 888.31 | 1510.25 | 621.94 | |
| | | | | |
| | | | | |
| Foliage Class | | | | |
| 0 | 81.18 | 54.45 | -26.73 | |
| 1 | 163.28 | 172.68 | 9.40 | |
| | | | | |
| Crs Root A | 143.16 | 386.55 | 243.39 | |
| Crs Root B | 55.27 | 159.72 | 104.45 | |
| | | | | |
| Fine Root A | 55.19 | 43.90 | -11.29 | |
| Fine Root B1 | 9.55 | 7.73 | -1.82 | |
| Fine Root B2 | 0.63 | 0.48 | -0.15 | |
| | | | | |
| | | | | |
| Values are in grams carbon! | | | | |
| | | | | |
| Boxes shaded in gray indicate values for entry from field data. | | | | |
| | | | | |

**Table 7**. Biomass of family GR in the fertilized plots in January 1998 and January 2000. Other information as in Table 1.

| | | | | | |
|---|---|---|---|---|---|
| | Family | Treatment | | YEAR | |
| | GR | F | | 1998-2000 | |
| | | | | | |
| | | | | | |
| | | | | MEASURED | |
| | COMPONENT | 1998 Biomass | 2000 Biomass | 2yr growth | |
| | | | | | |
| | stem | 1026.50 | 3137.83 | 2111.33 | |
| | | | | | |
| | branch | 378.42 | 642.95 | 264.53 | |
| | | | | | |
| | foliage | 409.10 | 477.80 | 68.70 | |
| | | | | | |
| | root (+stump) | 688.72 | 1462.12 | 773.40 | |
| | | | | | |
| | total tree | 2502.74 | 5720.70 | 3217.96 | |
| | | | | | |
| | total above ground | 1814.02 | 4258.58 | 2444.56 | |
| | | | | | |
| | | | | | |
| | Foliage Class | | | | |
| | 0 | 135.00 | 204.23 | 69.23 | |
| | 1 | 274.10 | 273.57 | -0.53 | |
| | | | | | |
| | Crs Root A | 394.27 | 924.17 | 529.90 | |
| | Crs Root B | 208.98 | 493.88 | 284.90 | |
| | | | | | |
| | Fine Root A | 73.49 | 37.78 | -35.71 | |
| | Fine Root B1 | 6.71 | 4.77 | -1.94 | |
| | Fine Root B2 | 5.27 | 1.52 | -3.75 | |
| | Values are in grams carbon! | | | | |
| | | | | | |
| | Boxes shaded in gray indicate values for entry from field data. | | | | |
| | | | | | |

**Table 8**. Biomass of family GR in the control plots in January 1998 and January 2000. Other information as in Table 1.

| | | | | | |
|---|---|---|---|---|---|
| | Family | Treatment | | YEAR | |
| | GR | C | | 1998-2000 | |
| | | | | | |
| | | | | | |
| | | | | MEASURED | |
| | COMPONENT | 1998 Biomass | 2000 Biomass | 2yr growth | |
| | | | | | |
| | stem | 442.00 | 884.17 | 442.17 | |
| | | | | | |
| | branch | 204.05 | 301.23 | 97.18 | |
| | | | | | |
| | foliage | 298.03 | 274.87 | -23.16 | |
| | | | | | |
| | root (+stump) | 327.79 | 426.56 | 98.77 | |
| | | | | | |
| | total tree | 1271.87 | 1886.83 | 614.96 | |
| | | | | | |
| | total above ground | 944.08 | 1460.27 | 516.19 | |
| | | | | | |
| | | | | | |
| | Foliage Class | | | | |
| | 0 | 113.58 | 50.52 | -63.06 | |
| | 1 | 184.45 | 224.35 | 39.90 | |
| | | | | | |
| | Crs Root A | 175.38 | 267.60 | 92.22 | |
| | Crs Root B | 107.68 | 107.53 | -0.15 | |
| | | | | | |
| | Fine Root A | 38.74 | 43.98 | 5.24 | |
| | Fine Root B1 | 1.74 | 6.13 | 4.39 | |
| | Fine Root B2 | 4.25 | 1.32 | -2.93 | |
| | | | | | |
| | Values are in grams carbon! | | | | |
| | | | | | |
| | Boxes shaded in gray indicate values for entry from field data. | | | | |
| | | | | | |

**Table 9**. Biomass allocation in each compartment for trees from family 81 in the fertilized plots in January 1998. Values represent grams carbon (2 grams biomass = 1 gram carbon). Foliage structure = (total leaf biomass) / 1+ foliage TNC %; foliage TNC = structure * foliage TNC %; wood percent = heartwood percent; wood = total * wood percent; structure = (total - wood) / 1 + tissue TNC %; TNC = structure * tissue TNC %.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | 81 | F | | | 1998 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 344.72 | 414.05 | | | | |
| | structure | 312.53 | 373.69 | | | | |
| | tnc | 32.19 | 40.36 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 662.65 | 1735.67 | 765.04 | 333.30 | 1098.34 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 630.49 | 1699.97 | 623.50 | 271.64 | | |
| | tnc | 32.16 | 35.70 | 141.54 | 61.66 | | |
| | | | | 69.65 | 30.35 | % in horizon | |
| | total root = | 1227.81 | | | | | |
| | crs root % = | 89.46 | | | | | |
| | fine root % = | 10.54 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.103 | | | | | |
| | 1foliage TNC % = | 0.108 | | | | | |
| | branch TNC %= | 0.051 | | | | | |
| | stem TNC % = | 0.021 | | | | | |
| | crsroot TNC % = | 0.227 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 114.95 | 11.54 | 2.98 | 129.47 | | |
| | structure | 114.95 | 11.54 | 2.98 | | | |
| | % in horizon | 88.79 | 8.91 | 2.30 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | Boxes shaded in gray indicate values for entryfrom field data. | | | | | | |

**Table 10**. Biomass allocation in each compartment for trees from family 81 in the fertilized plots in January 2000. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | 81 | F | | | 2000 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 297.65 | 491.75 | | | | |
| | structure | 269.85 | 443.82 | | | | |
| | tnc | 27.80 | 47.93 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 1414.22 | 4942.17 | 1367.66 | 696.32 | 2063.98 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 1345.59 | 4840.52 | 1114.64 | 567.50 | | |
| | tnc | 68.63 | 101.65 | 253.02 | 128.82 | | |
| | | | | 66.26 | 33.74 | % in horizon | |
| | total root = | 2118.35 | | | | | |
| | crs root % = | 97.43 | | | | | |
| | fine root % = | 2.57 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.103 | | | | | |
| | 1foliage TNC % = | 0.108 | | | | | |
| | branch TNC %= | 0.051 | | | | | |
| | stem TNC % = | 0.021 | | | | | |
| | crsroot TNC % = | 0.227 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 45.62 | 5.27 | 3.48 | 54.37 | | |
| | structure | 45.62 | 5.27 | 3.48 | | | |
| | % in horizon | 83.91 | 9.69 | 6.40 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 11**. Biomass allocation in each compartment for trees from family 81 in the control plots in January 1998. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | 81 | C | | | 1998 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 131.20 | 211.60 | | | | |
| | structure | 118.68 | 190.29 | | | | |
| | tnc | 12.52 | 21.31 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 240.82 | 775.17 | 326.94 | 181.96 | 508.90 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 228.70 | 757.74 | 268.87 | 149.63 | | |
| | tnc | 12.12 | 17.43 | 58.07 | 32.32 | | |
| | | | | 64.25 | 35.75 | % in horizon | |
| | total root = | 591.03 | | | | | |
| | crs root % = | 86.10 | | | | | |
| | fine root % = | 13.90 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.106 | | | | | |
| | 1foliage TNC % = | 0.112 | | | | | |
| | branch TNC %= | 0.053 | | | | | |
| | stem TNC % = | 0.023 | | | | | |
| | crsroot TNC % = | 0.216 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 76.15 | 4.66 | 1.32 | 82.13 | | |
| | structure | 76.15 | 4.66 | 1.32 | | | |
| | % in horizon | 92.72 | 5.67 | 1.61 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 12**. Biomass allocation in each compartment for trees from family 81 in the control plots in January 2000. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | 81 | C | | | 2000 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 100.77 | 252.32 | | | | |
| | structure | 91.15 | 226.91 | | | | |
| | tnc | 9.62 | 25.41 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 462.17 | 1611.67 | 554.77 | 277.35 | 832.12 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 438.91 | 1575.43 | 456.23 | 228.08 | | |
| | tnc | 23.26 | 36.24 | 98.54 | 49.27 | | |
| | | | | 66.67 | 33.33 | % in horizon | |
| | total root = | 879.85 | | | | | |
| | crs root % = | 94.58 | | | | | |
| | fine root % = | 5.42 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.106 | | | | | |
| | 1foliage TNC % = | 0.112 | | | | | |
| | branch TNC %= | 0.053 | | | | | |
| | stem TNC % = | 0.023 | | | | | |
| | crsroot TNC % = | 0.216 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 41.93 | 2.17 | 3.63 | 47.73 | | |
| | structure | 41.93 | 2.17 | 3.63 | | | |
| | % in horizon | 87.85 | 4.55 | 7.61 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 13**. Biomass allocation in each compartment for trees from family 91 in the fertilized plots in January 1998. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | 91 | F | | | 1998 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 243.25 | 324.97 | | | | |
| | structure | 220.53 | 293.56 | | | | |
| | tnc | 22.72 | 31.41 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 576.68 | 1539.00 | 702.58 | 303.05 | 1005.63 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 548.70 | 1504.40 | 583.05 | 251.49 | | |
| | tnc | 27.98 | 34.60 | 119.53 | 51.56 | | |
| | | | | 69.86 | 30.14 | % in horizon | |
| | total root = | 1142.07 | | | | | |
| | crs root % = | 88.05 | | | | | |
| | fine root % = | 11.95 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.103 | | | | | |
| | 1foliage TNC % = | 0.107 | | | | | |
| | branch TNC %= | 0.051 | | | | | |
| | stem TNC % = | 0.023 | | | | | |
| | crsroot TNC % = | 0.205 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 123.73 | 9.92 | 2.79 | 136.44 | | |
| | structure | 123.73 | 9.92 | 2.79 | | | |
| | % in horizon | 90.69 | 7.27 | 2.04 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 14**. Biomass allocation in each compartment for trees from family 91 in the fertilized plots in January 2000. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | 91 | F | | | 2000 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 240.88 | 471.10 | | | | |
| | structure | 218.39 | 425.56 | | | | |
| | tnc | 22.49 | 45.54 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 1127.55 | 3883.33 | 1197.00 | 498.13 | 1695.13 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 1072.84 | 3796.02 | 993.36 | 413.39 | | |
| | tnc | 54.71 | 87.31 | 203.64 | 84.74 | | |
| | | | | 70.61 | 29.39 | % in horizon | |
| | total root = | 1757.45 | | | | | |
| | crs root % = | 96.45 | | | | | |
| | fine root % = | 3.55 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.103 | | | | | |
| | 1foliage TNC % = | 0.107 | | | | | |
| | branch TNC %= | 0.051 | | | | | |
| | stem TNC % = | 0.023 | | | | | |
| | crsroot TNC % = | 0.205 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 56.02 | 5.75 | 0.55 | 62.32 | | |
| | structure | 56.02 | 5.75 | 0.55 | | | |
| | % in horizon | 89.89 | 9.23 | 0.88 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 15**. Biomass allocation in each compartment for trees from family 91 in the control plots in January 1998. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | 91 | C | | | 1998 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 81.18 | 163.28 | | | | |
| | structure | 72.74 | 145.79 | | | | |
| | tnc | 8.44 | 17.49 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 177.02 | 466.83 | 216.41 | 97.66 | 314.07 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 168.75 | 457.68 | 175.37 | 79.14 | | |
| | tnc | 8.27 | 9.15 | 41.04 | 18.52 | | |
| | | | | 68.90 | 31.10 | % in horizon | |
| | total root = | 379.34 | | | | | |
| | crs root % = | 82.79 | | | | | |
| | fine root % = | 17.21 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.116 | | | | | |
| | 1foliage TNC % = | 0.120 | | | | | |
| | branch TNC %= | 0.049 | | | | | |
| | stem TNC % = | 0.020 | | | | | |
| | crsroot TNC % = | 0.234 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 56.73 | 4.70 | 3.84 | 65.27 | | |
| | structure | 56.73 | 4.70 | 3.84 | | | |
| | % in horizon | 86.92 | 7.20 | 5.88 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 16**. Biomass allocation in each compartment for trees from family 91 in the control plots in January 2000. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | 91 | C | | | 2000 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 118.63 | 334.18 | | | | |
| | structure | 106.30 | 298.38 | | | | |
| | tnc | 12.33 | 35.81 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 510.27 | 1299.83 | 351.37 | 196.08 | 547.45 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 486.43 | 1274.34 | 284.74 | 158.90 | | |
| | tnc | 23.84 | 25.49 | 66.63 | 37.18 | | |
| | | | | 64.18 | 35.82 | % in horizon | |
| | total root = | 597.98 | | | | | |
| | crs root % = | 91.55 | | | | | |
| | fine root % = | 8.45 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.116 | | | | | |
| | 1foliage TNC % = | 0.120 | | | | | |
| | branch TNC %= | 0.049 | | | | | |
| | stem TNC % = | 0.020 | | | | | |
| | crsroot TNC % = | 0.234 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 44.08 | 6.08 | 0.37 | 50.53 | | |
| | structure | 44.08 | 6.08 | 0.37 | | | |
| | % in horizon | 87.24 | 12.03 | 0.73 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 17**. Biomass allocation in each compartment for trees from family BA in the fertilized plots in January 1998. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | BA | F | | | 1998 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 375.93 | 354.60 | | | | |
| | structure | 338.37 | 319.17 | | | | |
| | tnc | 37.56 | 35.43 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 629.95 | 1681.50 | 623.15 | 362.30 | 985.45 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 601.67 | 1648.53 | 511.61 | 297.45 | | |
| | tnc | 28.28 | 32.97 | 111.53 | 64.85 | | |
| | | | | 63.23 | 36.77 | % in horizon | |
| | total root = | 1138.95 | | | | | |
| | crs root % = | 86.52 | | | | | |
| | fine root % = | 13.48 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.111 | | | | | |
| | 1foliage TNC % = | 0.111 | | | | | |
| | branch TNC %= | 0.047 | | | | | |
| | stem TNC % = | 0.020 | | | | | |
| | crsroot TNC % = | 0.218 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 129.95 | 11.84 | 11.72 | 153.51 | | |
| | structure | 129.95 | 11.84 | 11.72 | | | |
| | % in horizon | 84.66 | 7.71 | 7.63 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 18**. Biomass allocation in each compartment for trees from family BA in the fertilized plots in January 2000. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | BA | F | | | 2000 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 298.43 | 569.03 | | | | |
| | structure | 268.61 | 512.18 | | | | |
| | tnc | 29.82 | 56.85 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 1004.23 | 4891.67 | 1210.77 | 632.98 | 1843.75 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 959.15 | 4795.75 | 994.06 | 519.69 | | |
| | tnc | 45.08 | 95.92 | 216.71 | 113.29 | | |
| | | | | 65.67 | 34.33 | % in horizon | |
| | total root = | 1889.27 | | | | | |
| | crs root % = | 97.59 | | | | | |
| | fine root % = | 2.41 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.111 | | | | | |
| | 1foliage TNC % = | 0.111 | | | | | |
| | branch TNC %= | 0.047 | | | | | |
| | stem TNC % = | 0.020 | | | | | |
| | crsroot TNC % = | 0.218 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 32.82 | 10.00 | 2.70 | 45.52 | | |
| | structure | 32.82 | 10.00 | 2.70 | | | |
| | % in horizon | 72.10 | 21.97 | 5.93 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 19**. Biomass allocation in each compartment for trees from family BA in the control plots in January 1998. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | BA | C | | | 1998 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 81.18 | 163.28 | | | | |
| | structure | 73.07 | 146.70 | | | | |
| | tnc | 8.11 | 16.58 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 177.02 | 466.83 | 143.16 | 55.27 | 198.43 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 168.27 | 457.68 | 120.10 | 46.37 | | |
| | tnc | 8.75 | 9.15 | 23.06 | 8.90 | | |
| | | | | 72.15 | 27.85 | % in horizon | |
| | total root = | 263.80 | | | | | |
| | crs root % = | 75.22 | | | | | |
| | fine root % = | 24.78 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.111 | | | | | |
| | 1foliage TNC % = | 0.113 | | | | | |
| | branch TNC %= | 0.052 | | | | | |
| | stem TNC % = | 0.020 | | | | | |
| | crsroot TNC % = | 0.192 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 55.19 | 9.55 | 0.63 | 65.37 | | |
| | structure | 55.19 | 9.55 | 0.63 | | | |
| | % in horizon | 84.43 | 14.61 | 0.96 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 20**. Biomass allocation in each compartment for trees from family BA in the control plots in January 2000. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | BA | C | | | 2000 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 54.45 | 172.68 | | | | |
| | structure | 49.01 | 155.15 | | | | |
| | tnc | 5.44 | 17.53 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 250.12 | 1033.00 | 386.55 | 159.72 | 546.27 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 237.76 | 1012.75 | 324.29 | 133.99 | | |
| | tnc | 12.36 | 20.25 | 62.26 | 25.73 | | |
| | | | | 70.76 | 29.24 | % in horizon | |
| | total root = | 598.38 | | | | | |
| | crs root % = | 91.29 | | | | | |
| | fine root % = | 8.71 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.111 | | | | | |
| | 1foliage TNC % = | 0.113 | | | | | |
| | branch TNC %= | 0.052 | | | | | |
| | stem TNC % = | 0.020 | | | | | |
| | crsroot TNC % = | 0.192 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 43.90 | 7.73 | 0.48 | 52.11 | | |
| | structure | 43.90 | 7.73 | 0.48 | | | |
| | % in horizon | 84.24 | 14.83 | 0.92 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 21**. Biomass allocation in each compartment for trees from family GR in the fertilized plots in January 1998. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | GR | F | | | 1998 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 135.00 | 274.10 | | | | |
| | structure | 122.28 | 247.83 | | | | |
| | tnc | 12.72 | 26.27 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 378.42 | 1026.50 | 394.27 | 208.98 | 603.25 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 360.40 | 1005.39 | 315.04 | 166.98 | | |
| | tnc | 18.02 | 21.11 | 79.23 | 42.00 | | |
| | | | | 65.36 | 34.64 | % in horizon | |
| | total root = | 688.72 | | | | | |
| | crs root % = | 87.59 | | | | | |
| | fine root % = | 12.41 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.104 | | | | | |
| | 1foliage TNC % = | 0.106 | | | | | |
| | branch TNC %= | 0.050 | | | | | |
| | stem TNC % = | 0.021 | | | | | |
| | crsroot TNC % = | 0.252 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 73.49 | 6.71 | 5.27 | 85.47 | | |
| | structure | 73.49 | 6.71 | 5.27 | | | |
| | % in horizon | 85.98 | 7.85 | 6.17 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 22**. Biomass allocation in each compartment for trees from family GR in the fertilized plots in January 2000. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | GR | F | | | 2000 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 204.23 | 273.57 | | | | |
| | structure | 184.99 | 247.35 | | | | |
| | tnc | 19.24 | 26.22 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 642.95 | 3137.83 | 924.17 | 493.88 | 1418.05 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 612.33 | 3073.29 | 738.45 | 394.63 | | |
| | tnc | 30.62 | 64.54 | 185.72 | 99.25 | | |
| | | | | 65.17 | 34.83 | % in horizon | |
| | total root = | 1462.12 | | | | | |
| | crs root % = | 96.99 | | | | | |
| | fine root % = | 3.01 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.104 | | | | | |
| | 1foliage TNC % = | 0.106 | | | | | |
| | branch TNC %= | 0.050 | | | | | |
| | stem TNC % = | 0.021 | | | | | |
| | crsroot TNC % = | 0.252 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 37.78 | 4.77 | 1.52 | 44.07 | | |
| | structure | 37.78 | 4.77 | 1.52 | | | |
| | % in horizon | 85.73 | 10.82 | 3.45 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 23**. Biomass allocation in each compartment for trees from family GR in the control plots in January 1998. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | GR | C | | | 1998 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 113.58 | 184.45 | | | | |
| | structure | 101.96 | 164.98 | | | | |
| | tnc | 11.62 | 19.47 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 204.05 | 442.00 | 175.38 | 107.68 | 283.06 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 193.96 | 434.18 | 148.75 | 91.33 | | |
| | tnc | 10.09 | 7.82 | 26.63 | 16.35 | | |
| | | | | 61.96 | 38.04 | % in horizon | |
| | total root = | 327.79 | | | | | |
| | crs root % = | 86.35 | | | | | |
| | fine root % = | 13.65 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.114 | | | | | |
| | 1foliage TNC % = | 0.118 | | | | | |
| | branch TNC %= | 0.052 | | | | | |
| | stem TNC % = | 0.018 | | | | | |
| | crsroot TNC % = | 0.179 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 38.74 | 1.74 | 4.25 | 44.73 | | |
| | structure | 38.74 | 1.74 | 4.25 | | | |
| | % in horizon | 86.61 | 3.89 | 9.50 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 24**. Biomass allocation in each compartment for trees from family GR in the control plots in January 2000. Other information as in Table 9.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Family | Treatment | | | YEAR | | |
| | GR | C | | | 2000 | | |
| | | | | | | | |
| | Foliage Class | 0 (current) | 1 (old) | | | | |
| | gC | | | | | | |
| | total | 50.52 | 224.35 | | | | |
| | structure | 45.35 | 200.67 | | | | |
| | tnc | 5.17 | 23.68 | | | | |
| | | | | | | | |
| | gC | branch | stem | crsroot A | crsroot B1 | total crs root | |
| | total | 301.23 | 884.17 | 267.60 | 107.53 | 375.13 | |
| | wood | 0.00 | 0.00 | 0.00 | 0.00 | | |
| | structure | 286.34 | 868.54 | 226.97 | 91.20 | | |
| | tnc | 14.89 | 15.63 | 40.63 | 16.33 | | |
| | | | | 71.34 | 28.66 | % in horizon | |
| | total root = | 426.56 | | | | | |
| | crs root % = | 87.94 | | | | | |
| | fine root % = | 12.06 | | | | | |
| | | | | | | | |
| | wood percent = | 0.00 | | | | | |
| | | | | | | | |
| | 0foliage TNC % = | 0.114 | | | | | |
| | 1foliage TNC % = | 0.118 | | | | | |
| | branch TNC %= | 0.052 | | | | | |
| | stem TNC % = | 0.018 | | | | | |
| | crsroot TNC % = | 0.179 | | | | | |
| | | | | | | | |
| | gC | fine root A | fine root B1 | fine root B2 | total fine root | | |
| | total | 43.98 | 6.13 | 1.32 | 51.43 | | |
| | structure | 43.98 | 6.13 | 1.32 | | | |
| | % in horizon | 85.51 | 11.92 | 2.57 | | | |
| | | | | | | | |
| | Values are in grams carbon! | | | | | | |
| | | | | | | | |
| | Boxes shaded in gray indicate values for entry into TREGRO from field data. | | | | | | |
| | | | | | | | |

**Table 25**. Calculated photosynthesis, respiration, and photosynthesis/respiration for foliage from trees of family 81 in the fertilized plots in May 1998.

| **Family** | **Treatment** | | **FOLIAGE CLASS** | |
|---|---|---|---|---|
| 81 | F | | **old** | |
| Values | | | | |
| | | | Source | |
| Net Ps | 7.1698 | µmol $CO_2$ $m^{-2}$ $s^{-1}$ | SETRES II | |
| Rn | 1 | µmol $CO_2$ $m^{-2}$ $s^{-1}$ | Literature | |
| SLA | 0.019064 | $m^2$ $g_{-1}$ leaf_C | Literature | |
| Photosynthesis | | | | |
| µmol $CO_2$ $m^{-2}$ $s^{-1}$ | mol $CO_2$ $m^{-2}$ $s^{-1}$ | gC $m^{-2}$ $s^{-1}$ | gC $m^{-2}$ $h^{-1}$ | gC $g_{-1}$ leaf_C $hr^{-1}$ |
| 7.1698 | 7.1698E-06 | 8.60376E-05 | 0.30973536 | **0.00590** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | $m^{-2}$ $g_{-1}$ leaf_C |
| Respiration | | | | |
| µmol CO2 $m^{-2}$ $s^{-1}$ | mol CO2 $m^{-2}$ $s^{-1}$ | gC $m^{-2}$ $s^{-1}$ | gC $m^{-2}$ $h^{-1}$ | gC $g_{-1}$ leaf_C $hr^{-1}$ |
| 1 | 0.000001 | 0.000012 | 0.0432 | **0.00082** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | $m^{-2}$ $g_{-1}$ leaf_C |
| | Rn/Pnet Ratio | **0.139473904** | | |

**Table 26**. Calculated photosynthesis, respiration, and photosynthesis/respiration for foliage from trees of family 81 in the control plots in May 1998.

| **Family** | **Treatment** | | **FOLIAGE CLASS** | |
|---|---|---|---|---|
| 81 | C | | **old** | |
| Values | | | | |
| | | | Source | |
| Net Ps | 7.4245 | µmol CO2 m-2 s-1 | SETRES II | |
| Rn | 1 | µmol CO2 m-2 s-1 | Literature | |
| SLA | 0.019064 | m2 g-1 leaf_C | Literature | |
| Photosynthesis | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s-1 | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 7.4245 | 7.4245E-06 | 0.000089094 | 0.3207384 | **0.00611** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| Respiration | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s1- | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 1 | 0.000001 | 0.000012 | 0.0432 | **0.00082** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| | Rn/Pnet Ratio | **0.134689205** | | |

**Table 27**. Calculated photosynthesis, respiration, and photosynthesis/respiration for foliage from trees of family 91 in the fertilized plots in May 1998.

| **Family** | **Treatment** | | **FOLIAGE CLASS** | |
|---|---|---|---|---|
| 91 | F | | **old** | |
| Values | | | | |
| | | | Source | |
| Net Ps | 7.3884 | µmol CO2 m-2 s-1 | SETRES II | |
| Rn | 1 | µmol CO2 m-2 s-1 | Literature | |
| SLA | 0.019064 | m2 g-1 leaf_C | Literature | |
| Photosynthesis | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s-1 | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 7.3884 | 7.3884E-06 | 8.86608E-05 | 0.31917888 | **0.00608** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| Respiration | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s1- | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 1 | 0.000001 | 0.000012 | 0.0432 | **0.00082** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| | Rn/Pnet Ratio | **0.135347301** | | |

**Table 28**. Calculated photosynthesis, respiration, and photosynthesis/respiration for foliage from trees of family 91 in the control plots in May 1998.

| **Family** | **Treatment** | | **FOLIAGE CLASS** | |
|---|---|---|---|---|
| 91 | C | | **old** | |
| Values | | | | |
| | | | Source | |
| Net Ps | 6.6986 | µmol CO2 m-2 s-1 | SETRES II | |
| Rn | 1 | µmol CO2 m-2 s-1 | Literature | |
| SLA | 0.019064 | m2 g-1 leaf_C | Literature | |
| Photosynthesis | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s-1 | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 6.6986 | 6.6986E-06 | 8.03832E-05 | 0.28937952 | **0.00552** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| Respiration | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s1- | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 1 | 0.000001 | 0.000012 | 0.0432 | **0.00082** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| | Rn/Pnet Ratio | **0.149284925** | | |

**Table 29**. Calculated photosynthesis, respiration, and photosynthesis/respiration for foliage from trees of family BA in the fertilized plots in May 1998.

| **Family** | **Treatment** | | **FOLIAGE CLASS** | |
|---|---|---|---|---|
| BA | F | | **old** | |
| Values | | | | |
| | | | Source | |
| Net Ps | 7.6701 | µmol CO2 m-2 s-1 | SETRES II | |
| Rn | 1 | µmol CO2 m-2 s-1 | Literature | |
| SLA | 0.019064 | m2 g-1 leaf_C | Literature | |
| Photosynthesis | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s-1 | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 7.6701 | 7.6701E-06 | 9.20412E-05 | 0.33134832 | **0.00632** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| Respiration | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s1- | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 1 | 0.000001 | 0.000012 | 0.0432 | **0.00082** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| | Rn/Pnet Ratio | **0.130376397** | | |

**Table 30**. Calculated photosynthesis, respiration, and photosynthesis/respiration for foliage from trees of family BA in the control plots in May 1998.

| **Family** | **Treatment** | | **FOLIAGE CLASS** | |
|---|---|---|---|---|
| BA | C | | **old** | |
| Values | | | Source | |
| Net Ps | 6.6144 | µmol CO2 m-2 s-1 | SETRES II | |
| Rn | 1 | µmol CO2 m-2 s-1 | Literature | |
| SLA | 0.019064 | m2 g-1 leaf_C | Literature | |
| Photosynthesis | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s-1 | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 6.6144 | 6.6144E-06 | 7.93728E-05 | 0.28574208 | **0.00545** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| Respiration | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s1- | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 1 | 0.000001 | 0.000012 | 0.0432 | **0.00082** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| | Rn/Pnet Ratio | **0.151185293** | | |

**Table 31**. Calculated photosynthesis, respiration, and photosynthesis/respiration for foliage from trees of family GR in the fertilized plots in May 1998.

| **Family** | **Treatment** | | **FOLIAGE CLASS** | |
|---|---|---|---|---|
| GR | F | | **old** | |
| Values | | | | |
| | | | Source | |
| Net Ps | 7.1762 | µmol CO2 m-2 s-1 | SETRES II | |
| Rn | 1 | µmol CO2 m-2 s-1 | Literature | |
| SLA | 0.019064 | m2 g-1 leaf_C | Literature | |
| Photosynthesis | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s-1 | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 7.1762 | 7.1762E-06 | 8.61144E-05 | 0.31001184 | **0.00591** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| Respiration | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s1- | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 1 | 0.000001 | 0.000012 | 0.0432 | **0.00082** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| | Rn/Pnet Ratio | **0.139349516** | | |

**Table 32**. Calculated photosynthesis, respiration, and photosynthesis/respiration for foliage from trees of family GR in the control plots in May 1998.

| **Family** | **Treatment** | | **FOLIAGE CLASS** | |
|---|---|---|---|---|
| GR | C | | **old** | |
| Values | | | | |
| | | | Source | |
| Net Ps | 7.0137 | µmol CO2 m-2 s-1 | SETRES II | |
| Rn | 1 | µmol CO2 m-2 s-1 | Literature | |
| SLA | 0.019064 | m2 g-1 leaf_C | Literature | |
| Photosynthesis | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s-1 | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 7.0137 | 7.0137E-06 | 8.41644E-05 | 0.30299184 | **0.00578** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| Respiration | | | | |
| µmol CO2 m-2 s-1 | mol CO2 m-2 s-1 | gC m-2 s1- | gC m-2 hr-1 | gC g-1 leaf_C hr-1 |
| 1 | 0.000001 | 0.000012 | 0.0432 | **0.00082** |
| value | 0.000001 | 12 | 3600 | 0.019064 |
| units | mol CO2/µmol CO2 | gC/mol CO2 | s/hr | m-2 g-1 leaf_C |
| | Rn/Pnet Ratio | **0.142578097** | | |

**Table 33**. Initial and final biomass and 2 year carbon gain values from SETRES II field data and the resulting TREGRO model 2 year C gain and the percent estimate for a simulated family 81 tree in the fertilized plots.

| Family | Treatment |
|---|---|
| 81 | F |

| COMPONENT | Measured Initial Biomass | Measured 2yr Biomass | Measured 2yr growth | Model growth | % ESTIMATE |
|---|---|---|---|---|---|
| stem | 1735.67 | 4942.17 | 3206.50 | 3185.11 | -0.67 |
| branch | 662.65 | 1414.22 | 751.57 | 738.13 | -1.79 |
| foliage | 758.77 | 836.75 | 77.98 | 76.22 | -2.26 |
| root (+stump) | 1227.81 | 2206.76 | 978.95 | 957.44 | -2.20 |
| total tree | 4384.90 | 9399.90 | 5015.00 | 4956.90 | -1.16 |
| total above ground | 3157.09 | 7193.14 | 4036.05 | 3999.46 | -0.91 |
| Crs Root A | 765.04 | 1367.66 | 602.62 | 596.54 | -1.01 |
| Crs Root B | 333.30 | 696.32 | 363.02 | 347.54 | -4.26 |
| Total Crs Root | 1098.34 | 2063.98 | 965.64 | 944.08 | -2.23 |
| Fine Root A | 114.95 | 126.76 | 11.81 | 11.89 | 0.65 |
| Fine Root B1 | 11.54 | 12.73 | 1.19 | 1.17 | -1.35 |
| Fine Root B2 | 2.98 | 3.29 | 0.31 | 0.30 | -2.04 |
| Total Fine Root | 129.47 | 142.78 | 13.31 | 13.36 | 0.41 |

**Measured 2yr growth = 2yr biomass - initial biomass**

**Values represent grams carbon!**

**% estimate = ((modeled growth - measured growth)/measured growth)*100**

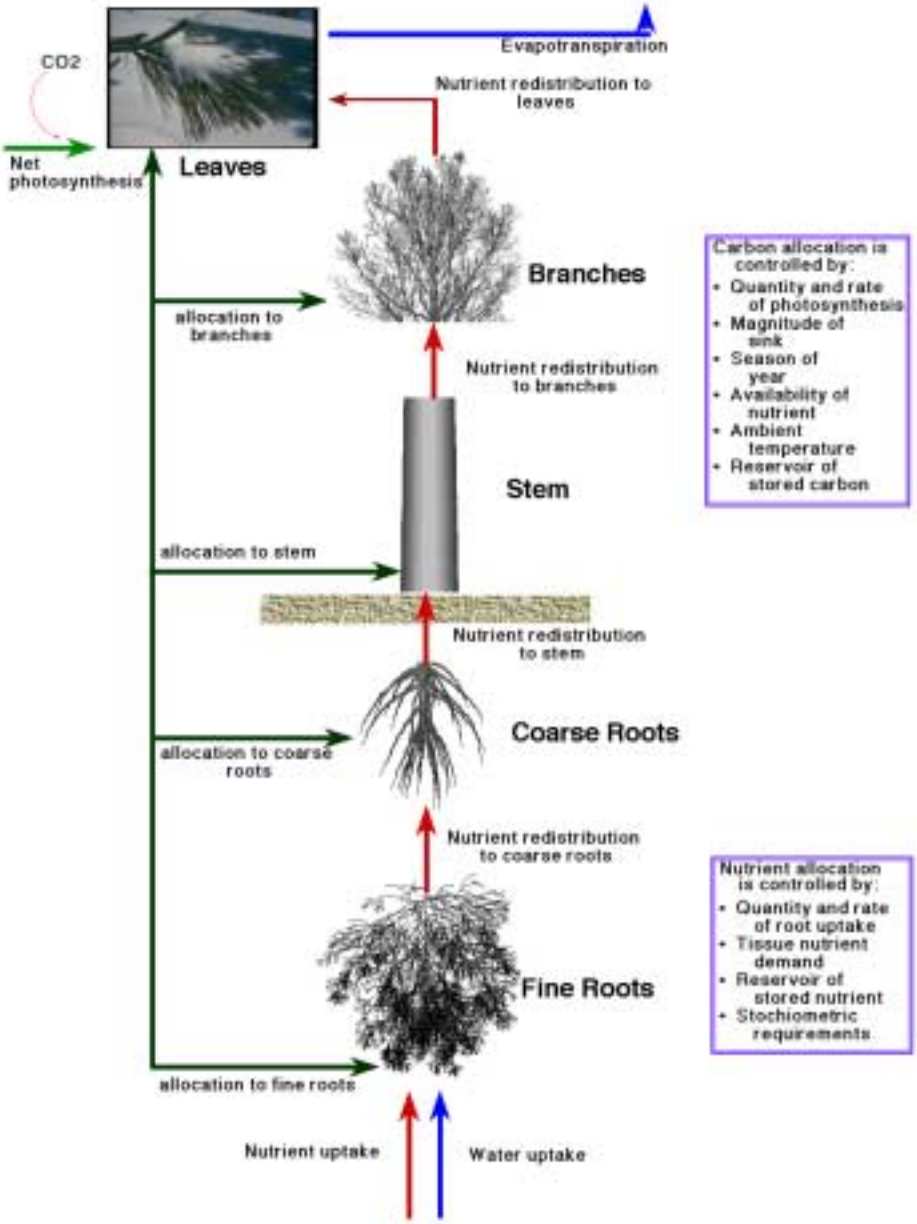


**Figure 1**. Key components, flows, and processes in the TREGRO model.

# CHAPTER 4

## TASK 3. GENETICS AND MOLECULAR BIOLOGY

**S.E. McKeand[1], D. M. O'Malley[2], Y. Y. Kim[3], B.C. Lee[4] and R.L. Wu[5]**

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## OVERVIEW

Genetic variation in tree growth and development could play an important role in explaining the patterns and variation in tree productivity. Tree roots are especially important in tree growth and development because a large portion of the photosynthate produced by a tree is allocated to the roots, especially when soil nutrient levels are low. Roots are the major sink for photosynthate under these conditions. Most of the carbon allocated to roots is respired or lost in fine root turnover. Allocation of carbon to plant parts (foliage, shoots, stem, coarse roots, fine roots) is plastic in response to nutrients, especially nitrogen. Less photosynthate is allocated to roots when nutrients are abundant than when nutrients are scarce. This responsiveness to nutrients is reflected in changes in the partitioning of biomass to plant parts under different nutrient regimes. Plants grown with high nutrient levels are not only larger, but the amount of biomass in different tissues are proportionally different. This responsiveness to changes in environmental conditions can be described as phenotypic plasticity. Phenotypic plasticity occurs when organisms alter their phenotypes to better suit different environmental circumstances. One example could be the production of different leaf morphologies in sun and shade in some trees. Evolutionary models to explain phenotypic plasticity have been proposed, but the role of this mechanism in adaptation is not understood. Plasticity could be under genetic control, or at least subject to genetic constraints. Plasticity to nutrients could play a role in managing forest productivity because fertilizer is becoming widely used in forest tree plantations.

We carried out three studies to address the role of genetics in controlling responsiveness to nutrients. In the first, we studied seedling biomass changes in response to different nutrient regimes in loblolly pine seedlings. In the second, we carried out a genetic dissection of height and diameter growth in an open pollinated family of loblolly pine at 6 years in the field. In the third, we attempted a genetic dissection of seedling biomass partitioning. Analysis is still in progress on the third study.

We determined that seedlings from the eastern Texas xeric ecotype (Lost Pines provenance) of loblolly pine differed in root traits and biomass partitioning from the Atlantic Coastal Provenance mesic ecotype (Wu *et al*. 2000). There were significant differences among ecotypes and among nutrient levels. Our results supported the hypothesis that the differences in productivity between the xeric and mesic loblolly pine ecotypes planted at Scotland County are due to below ground differences in root systems. Based on the results of this study, we determined that seedling biomass partitioning could be analyzed as a set of quantitative traits and that the xeric and mesic ecotypes were sufficiently differentiated that we could expect to find

meaningful differences in these traits segregating within a family parented by an F1 hybrid between the two ecotypes.

We carried out a genetic dissection of height and diameter growth of loblolly pine OP family 7-1037 at the Scotland County field test. We assayed DNA markers in haploid DNA samples corresponding to 7-1037 trees in the field. This allowed us to detect several quantitative trait loci (QTLs) with average effects in trees at selection age (6 years in the field). Average effects are directly related to breeding value and few studies have defined QTLs this way. To do this, we overcame several challenging problems. We developed DNA marker methods to enable us to efficiently make maps from megagametophyte samples. Megagametophytes from field grown trees have years of field data associated with them. These samples cannot be replaced with equivalent samples and earlier methods had a prohibitively high failure rate. We acquired better software for gel scoring and data analysis. The biggest problem we faced was the small amount of phenotypic variation that is under genetic control in field grown half-sib families. To deal with this, we developed spatial analysis methods to reduce environmental variation and enhance genetic resolution for our study. Originally, we had planned to analyze two families, but we had to scale our effort back to a single family. The QTLs that we detected controlled a small proportion of the phenotypic variation in height and diameter. We had intended to characterize root traits from a sample of trees that had different QTL genotypes. In principle, the above-ground productivity of trees with different QTL genotypes could be due to differences in belowground traits. However, the magnitude of the QTL effects is not large enough to make such an investigation feasible. A large number of root systems would have to be sampled to detect effects that are expected to be small, and the effort required is not feasible. The genetic dissection is described in a draft of a manuscript in preparation for journal submission in January (Kim *et al*. 2001).

We initiated a genetic dissection of seedling biomass partitioning in an open pollinated family of loblolly pine selection 5-1065. The seedlings were planted in sand-filled pots and grew outdoors during the summer and fall of 1999. The seedlings were harvested, measured, and parts were separated for biomass analysis. We obtained large root systems in the 10 inch diameter, 20 inch tall pots, but the processing was extremely laborious. DNA samples were made from the megagametophytes and AFLP DNA markers prepared from the DNA. The analysis of this family is in progress and will be completed before spring 2001. We hope to find QTLs for biomass partitioning and address the issue of pleiotropy, i.e., do genes that control root traits affect partitioning in other plant parts as well?

## TASK 3. GENETICS AND MOLECULAR BIOLOGY

# I. GENETIC DISSECTION OF HEIGHT AND DIAMETER GROWTH IN A HALF-SIB FAMILY OF LOBLOLLY PINE

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## INTRODUCTION

The genetic basis of quantitative trait variation is now understood to involve a small number of genes with relatively large effects (i.e., quantitative trait loci, QTLs), and many genes with small effects. The availability of large numbers of genetic markers have made it possible to detect QTLs and to estimate their effect. Within F2 families obtained through interspecific hybridization, QTL effects are large and genetic markers explain a large portion of the phenotypic variation. Large QTL effects have also been found in families obtained by crossing divergently selected lines. However, QTLs are unlikely to explain much of the genetic or phenotypic variation in families obtained by crossing non selected parents within species. Little is known about the magnitude of effect and gene frequency of QTLs in natural populations. QTLs with large effects are likely to be rare in outbred populations, but these genes could play a central role in adaptation and breeding.

QTLs effects vary greatly in mapping populations replicated across environments, with differences in developmental stage, or in different genetic backgrounds. These observations have led some researchers to the conclusion that many QTLs are "unstable". However, the statistical power of methods to detect QTLs is low (Beavis, others). Even though a QTL with favorable effect could be present, the probability of rejecting the null hypothesis of no QTL effect is small. The statistical criteria for detecting QTLs are stringent because there are statistical problems for detection. The number of genetic markers to be tested for phenotypic effects is often larger than the sample size for the mapping family. The markers are not independent and the significance level must be adjusted higher due to the large number of tests. Permutation methods are used to determine the experiment-specific critical values for test statistics that correspond with empirically determined P-values. One permutation-based criterion for QTL detection is significance at the $P = 0.05$ level genome-wise (i.e., one spurious QTL peak by chance for each 20 genome maps studied). Generally, nominal P-values in the range of 0.005 to 0.001 are needed to meet this standard (LOD 2.5 to 3.0). The most powerful statistical method for QTL detection is composite interval mapping. However, small changes in data or marker map locations can have a large impact on the statistical significance of the QTLs detected. Furthermore, the joint estimation of effects can be affected by the large number of possible QTL genotypes in some mating designs. Thus, experiments to detect QTLs within species require large effort and often have low certainty with respect to the QTL effects that are characterized.

Most tree breeding programs are based on selection for general combining ability (i.e., breeding value). Breeding value is the average effect of an individual's gamete when mated to a large sample of the population. QTLs detected in dairy cattle have average effects because they are estimated using large, half-sib families (granddaughter design). Average effects are dependent on the additive and dominant effects of the gene as well as its frequency in a population. Low frequency QTLs with large dominant effects will have the greatest average effect. The magnitude of the average effect of a QTL within a half-sib family can be large compared with the additive genetic variance of population. QTLs with average effects are expressed in many different genetic backgrounds, but are more difficult to detect because half-sib families contain 3/4 of the additive and all of the dominance genetic variance of the entire population. QTL detection within full-sib families or selfed families is less difficult because the within family variances are smaller. However, these QTL effects could be specific to the family studied, and have little breeding value.

Pine has a special feature that enables estimation of QTL effects in half-sib families. The seed of gymnosperms contains haploid tissue (megagametophyte) derived from a single megaspore. This haploid tissue has the same genotype as the gamete contributed to the embryo by the seed parent. If the megagametophyte tissue of the seed is collected at the time of germination, then the DNA markers contributed to the seedling by the seed parent can be determined unambiguously. Marker analysis is much simpler for this situation compared with inference of one parent's contribution relative to a broad sample of gametes from many different individuals in the population.

Selection age for loblolly pine in the southern US is 6 to 8 years. The height of trees at this age is ~ 7 meters, with phenotypic standard error ~1.0 meter. Heritability (narrow sense) for height and diameter is ~ 0.2. If heritability is 0.5, then the expected contribution of additive genetic variance from the seed parent to the phenotypic variance of the half sib family is only ~ 14% assuming the infinitesimal model (O'Malley and McKeand 1995). Thus, the amount of variance available to be explained by genetic markers is expected to be small. However, if quantitative traits are controlled by a small number of QTLs with relatively large effects and many genes with very small effects, then genetic variance could vary greatly among families depending on whether a QTL was segregating in that family. The magnitude of QTL effects that are detectable is approximately 0.5 to 2 phenotypic standard errors.

Detecting average effect QTLs in half-sib families of pine requires consideration of approaches to increase the power and resolution of QTL mapping. Large mapping populations are needed to detect the expected small QTL effects, but the increased experimental effort is costly. The individuals close to the mean of the family make little contribution to the detection of QTL effects. Selective genotyping is a method that reduces experimental effort by using only the individuals from the tails of the trait distribution. Through selective genotyping, the number of individuals characterized using molecular markers can be reduced by half with only a small reduction in the power of detection. Another problem involves phenotypic assessment. In forest trees, the large plot size in field trials can be responsible for violation of the assumption of environmental uniformity even within plots. Systematic environmental trends associated with gradients in soil nutrients and moisture can inflate the phenotypic variance. Statistical methods

for spatial analysis can remove some of the variance associated with nonrandom environmental effects within large plots.

We have used amplified fragment length polymorphism (AFLP) markers in this study of average effect QTLs. AFLP markers are based on the selective amplification of a sample of restriction fragments throughout the genome. Many of these fragments are polymorphic and show simple, dominant patterns of inheritance. AFLPs provide large numbers of highly repeatable, highly multiplexed DNA markers with little effort required for marker development AFLP methods require only a small amount of DNA but generate a large number of markers. The dominant inheritance of AFLPs presents no disadvantage for marker genotyping using megagametophyte (1N) DNA samples.

Physiological studies, mutagenesis or transformation experiments can identify genes that could affect the phenotype of an organism (i.e. candidate genes). An alternative approach to QTL analysis is based on candidate genes rather than genetic markers. Marker analysis assumes that the phenotypic effect is controlled by an unknown gene that is located in a map interval between two markers. However, it is difficult to locate QTLs precisely enough to identify the gene sequence responsible for the phenotypic effect (i.e. positional cloning). Furthermore, large effort is needed to carry out QTL "scans" of the entire genome. The candidate gene approach creates markers within a candidate gene, then seeks to determine whether these markers explain significant amounts of phenotypic variance. This kind of approach has been used with milk protein genes in dairy cattle (). The candidate gene approach does not require as stringent criteria for detection of effects as QTL mapping, but is subject to concern that the effects are due to closely linked loci. Very large sample sizes would be needed to obtain enough recombinants that would isolate the effects to map intervals smaller than 1 cM. The effect of the candidate gene could be validated by nonstatistical means, such as changes in gene expression associated with different marker types at the candidate gene locus. Another approach is to compare the map locations of ESTs with the locations of QTLs.

We carried out a molecular marker dissection of height and diameter growth at 6 years of age for the open pollinated family from loblolly pine second generation selection 7-1037. One of the parents of 7-1037 is first generation selection 7-56. 7-56 is an elite clone in the NCSU:Industry Cooperative Tree Improvement Program. 7-56 has one of the highest breeding values for production traits of all of the selections in the breeding population. The other parent is 7-51, which has been culled from the program due to low breeding value. The goal of this study was to detect QTLs with average effects in a half-sib family of loblolly pine. We chose to work with the 7-1037 OP family rather than the 7-56 OP family because any rare QTLs with high breeding value should segregate in 7-1037 OP. To do this, we carried out selective genotyping and applied the random field model to remove nonrandom environmental effects from our phenotypic data (i.e., detrending). We used AFLP markers to scan a large portion of the genomic map of loblolly pine. Selection 7-1037 inherited the cad null gene from selection 7-56. In selfed family 7-56, the cad null gene had a large effect on growth and showed significant overdominance. A previous study using family 7-1037 OP planted at another location showed that the *cad* null gene had an average effect on growth. In our current study, we found several QTLs that affected height and diameter, as well as an effect on height that was associated with the *cad* null gene.

## MATERIALS AND METHODS

### *Plant materials and field trial*

We studied progeny from the open-pollinated family of loblolly pine second generation selection 7-1037 from the North Carolina State University:Industry Cooperative Tree Improvement Program. The seeds were obtained from ramets of 7-1037 grafted into Federal Paperboard's seed orchard at Lumberton, NC (now owned by International Paper, Inc.). The parents of 7-1037 were first generation selections 7-56 and 7-51. We obtained samples of 7-56 OP seeds and needles from several sources. We also obtained 7-51 OP seeds. However, 7-51 has been culled from the breeding program and no ramets of that clone could be found among the Coop breeding materials. The seedlings of 7-1037 were germinated during July 1993. Megagametophyte tissue was removed from each seedling and stored at –80°C for DNA extraction. The seedlings were hardened off in the fall of 1993 and planted at a study site in Scotland County during the winter of 1993-1994. Nantucket pine shoot tipmoth was controlled by insecticide application during the first 2 years. Weeds were controlled by mowing. Seedlings that were extensively damaged by insects or deer browsing were excluded from the study.

The field test was laid out as a split-split plot design. The first split involved fertilizer application vs. control. The soil is sand with low soil nutrients. The second split involved seed source, with 5 families from a drought hardy Texas provenance and 5 families from the Atlantic Coastal Plain provenance. The plots consisted of 100 progeny from a single OP family, planted in ten rows of ten trees on 5 foot by 7 foot spacing. The fertilizer applications were designed for optimum nutrition based on yearly foliar nutrient analyses. The field trial included 10 replications of the split-split plots, with 20,000 study trees and 16,000 border row trees. The fertilized and control plots were separated by 8 fertilized border rows and 8 control border rows to minimize movement of fertilizer from one plot to another and to ensure that trees on the edge of the fertilized plots experienced a uniform environment with respect to nutrients.

### *DNA extraction and AFLP marker methods*

DNA was extracted from 500 megagametophytes corresponding with OP seedlings of 7-1037 planted in a field test at Scotland County, NC. The megagametophytes were removed from the seedlings shortly after germination. The 25 tallest and 25 smallest trees at 4 years were selected from each 100 tree plot (10 plots, 1000 trees total in family). The final sample size was 343, with losses due to damaged or dead trees in the field plots or poor DNA prep yields from the megagametophytes. We used a modified Qiagen DNEasy protocol for DNA purification. The AFLP methods were optimized for LiCor automated fluorescent DNA sequencing instruments. AFLP markers were generated from 7 primer pairs following Remington *et al.* (1999).

When we began this project, AFLP methods were not robust enough to accomplish our objectives. The methods developed over the past 3 years have made mapping using AFLPs much more feasible. The Qiagen DNEasy methods now make DNA extraction much more reliable for megagametophytes. The high quality and consistent DNA yields greatly increased the quality of the AFLP fragments obtained from megagametophytes and from foliage.

Microsatellite markers were amplified from loblolly pine genomic and chloroplast DNA to verify the identity of the parents for the 7-1037 family planted at Scotland County. An M13 tailed primer strategy was used to label the fragments during PCR amplification.

***AFLP Polymorphisms and genetic markers***

The methods for construction of genetic maps assume DNA markers have a heritability of 1.0. The AFLP band polymorphisms that we scored were well-defined and intense and should be readily repeatable. Polymorphisms that are useful as markers should show simple Mendelian segregation ratios when transmitted from parent to progeny. However, significant departure from expected segregation ratios does not alone suffice as evidence that a polymorphism should not be used as a marker. Markers should also be mapable and this more complex model makes it possible to detect departures from heritability 1.0. Scoring errors result in an apparent excess of double crossovers within an ordered linkage group. The gel image was rechecked for apparent double crossovers deduced after mapping. We carried out a $Chi^2$ test for departure from 1:1 segregation, computed as the squared difference between number of band present and band absent marker phenotypes, divided by the number of progeny scored.

***Map construction***

Markers were grouped and ordered using MapMaker MacIntosh version 2 (Dupont, Wilmington, Deleware). To deal with the problem of unknown phase, we duplicated the dataset, then recoded the band absent as band present, and vice versa. The recoded data set was merged with the original data so that every combination of coupling and repulsion can be tested for every marker. The grouping criterion was LOD 10. Two linkage groups are expected for each chromosome due to the methods we used (i.e., one map for each homologue). Marker order was determined using the First Order command. Framework linkage maps were constructed following markers were dropped until the RIPPLE command yielded interval support > 3.0 for all permutations of order for each group of 3 adjacent markers.

***Statistical methods***

QTL Cartographer was used to detect and to map QTL effects. Regression analysis, as implemented in SAS JMP software, was used to analyze pairwise interactions of QTLs and to determine the amount of phenotypic variation explained by the markers associated with QTLs. Principal components analysis was carried out using SAS JMP software.

***Spatial analysis***

We used a random filed model to remove the possible nonrandom environmental effect existed in field experiment. The best polynomial regression model that predicted phenotypic measurements using row and column variables were fitted to each plot. Correlation analysis showed that the residuals were not correlated, so no model was required for the residual matrix. We used the Studentized residuals from these regressions as detrended phenotypic data.

## RESULTS

### *Phenotypic measurements*

Tree heights for family 7-1037 OP were measured using a height pole. Regressions of height on row and column position variables for each plots explained from 7.3 to 42.8% of within plot phenotypic variance, strongly suggesting that a departure from the assumption of plot uniformity. We carried out spatial analysis to detrend the phenotypic data. The Studentized residuals from the spatial analysis comprise a second variable related to height. The mean height for the entire study (N = 781) was 636.8 cm, with standard error 70.8 cm. The trees that were marker genotyped were chosen from the 25 tallest and 25 shortest trees in each 100 tree plot, based on the 4 yr height measurements. The mean height of the 323 progeny used for selective genotyping was 632.69 cm, with standard error 79.80 cm. The height distribution of the selective genotyped trees at 6 years did not significantly depart from a normal distribution using Shapiro-Wilk's test. The mean of the detrended height was close to 0, and the standard error was close to 1, as expected. The detrended data showed significant departure from the normal distribution, suggesting that the selective genotyping worked, generating a bimodal distribution of heights.

The diameter at breast height (DBH) was measured using a diameter tape. Regressions of height on row and column position variables for each plots explained from 12.6 to 26.2% of within plot phenotypic variance, strongly suggesting a departure from the assumption of plot uniformity. We carried out spatial analysis to detrend the phenotypic data. The Studentized residuals from the spatial analysis comprise a second variable related to DBH. The mean height for the entire study (N = 783) was 93.1 cm, with standard error 27.1 cm. The trees that were marker genotyped were chosen based on the extreme height measurements in year 4 (see above). The mean DBH of the 323 progeny used for selective genotyping was 91.72 cm, with standard error 18.20 cm. The DBH distribution of the selective genotyped trees at 6 years significantly departed from a normal distribution using Shapiro-Wilk's test. The mean of the detrended DBH was close to 0, and the standard error was close to 1, as expected. The detrended data did not show a significant departure from a normal distribution, suggesting that the selective genotyping did not work so well for detrended data.

Height and DBH are both measures of the size of a tree, and they tend to be highly correlated. We carried out a principal component analysis based on the correlation matrix data for these two phenotypic variables. The first principle component (PC1) explained 90.1% of the variation in these two variables. We used PC1 as a means to combine information on the two traits for a variable for overall size. The test for departure from normality using PC1 was significant at the $P < 0.0001$ suggesting that the selective genotyping worked well, generating a bimodal distribution for this variable.

### *Inheritance*

Family 7-1037 OP was produced from open-pollinated seeds of selection 7-1037. The paternal and maternal parents of 7-1037 were 7-51 and 7-56 respectively. The identity of the family was confirmed by analysis of both nuclear and chloroplast microsatellites. AFLP DNA

fragments were generated from 7 primer pairs for 343 DNA preps from megagametophyte samples (haploid) of 7-1037 OP. The number of polymorphisms scored was 265. The proportion of polymorphisms that departed from the expected 1:1 segregation ratio with $P < 0.05$ was 0.20. We checked on the quality of the bands scored for the polymorphisms with distorted segregation ratios. We concluded that the distorted ratios were not due to low quality band scoring. We analyzed the AFLP bands in parents of selection 7-1037. Of the polymorphic bands consisting of framework maps, 51were unambiguously present in 7-56 and 17 in 7-51, but the inheritance of 53 of the bands could not be determined by inspection. We concluded that most of the DNA bands could be used as markers, despite the high proportion of distorted segregation ratios.

***Linkage analysis***

A genomic map was constructed for the 265 AFLP markers. The markers were grouped using LOD 10, yielding 24 linkage groups. Groups were combined based on knowledge of marker locations on the 7-56 map to yield 20 linkage groups (Remington *et al*. 1999). Framework maps were constructed for each linkage group, using the criterion of interval support > 3.0. The total framework map length was 1869 cM, roughly equivalent to the estimated map length of 7-56. The average framework marker spacing was 18.5 cM. The marker density was 1 marker per 7 cM. The location of the *cad* locus was determined by PCR amplification of a length polymorphism in the promoter region of the gene, following Wu *et al*. (1999).

***QTL analysis***

We carried out a molecular marker dissection of phenotypic variation associated with 5 traits: height, diameter at breast height, the detrended height and diameter, and the first principal component of height and diameter. Single marker analysis showed 22 markers that explained a significant amount of phenotypic variation, using an empirical $\alpha = 0.05$ genome-wise P-value obtained by permutation. Interval mapping and composite interval mapping were used to more precisely locate phenotypic effects and to increase the statistical power for detection of QTLs. These analyses revealed 6 map regions that had meaningful and/or significant effects. The QTL on linkage group 4 was significant at the 0.05 level genome-wise for height using interval mapping and composite interval mapping. This QTL peak was among the 5 largest peaks for every trait and mapping method. The QTL on linkage group 11 was never significant at the $P < 0.05$ level genome-wise, but was among the 3 largest peaks for every trait and mapping method. The QTLs for linkage group 7 and 10 were never significant at the $P < 0.05$ level genome-wise. These QTLs had peaks that were among the 3 largest for two diameter traits and for PC1. The QTL associated with cad was significant at the $P < 0.05$ level genome-wise for detrended height, but there was no meaningful peak associated with *cad* for any other trait or mapping method. Thus, we recognized 5 putative QTLs, 3 associated with diameter and height, 2 associated with diameter, and one associated with height.

Multiple regression analysis using the markers associated with these QTLs explained 3 to 11% of the phenotypic variance in our sample for the 5 traits. There was little evidence of pairwise interactions of the 5 putative QTLs. QTLs on linkage groups 7 and 10 had an interaction that was significant at the $P < 0.01$ level, but there were 50 pairs of markers tested

over the 5 traits. ANOVA results showed significant plot by marker genotype interaction for cad. No other putative QTL showed this kind of interaction with the field environment. The largest QTL effect was the association of height with linkage group 4. This QTL effect was 39.56 cm compared with the overall mean of 636.78 cm. The square root of the additive genetic variance would be approximately 35 cm assuming heritability = 0.2. The breeding value of the largest QTL we detected would be approximately 1 $\sigma_A$. The two major QTLs for height together have an effect of 0.89 $\sigma$ and a breeding value of 1.8 $\sigma_A$. However, selective genotyping cause effects to be overestimated. (We plan to apply a method to correct for this upward bias.)

We aligned the 7-1037 linkage groups where the putative QTLs were located with the genomic 7-56 AFLP map of Remington *et al.* (1999). Linkage group 4 contained the largest 7-1037 QTL effect. This linkage group corresponded with 7-56 linkage group 3 which also contained a QTL effect on height in the selfed family. The 7-56 linkage group 3 QTL was located in a distinctly different location from the 7-1037 linkage group 4 QTL on the aligned maps. The effect of cad on height agreed in both families, but other correspondences were observed. In every case for 7-1037, the positive effect on the traits was contributed by 7-56. The marker density of the 7-1037 map was insufficient to determine which homolog of the 7-56 map was contributed in the regions of the 7-56 QTLs observed in the selfed family.

**DISCUSSION**

We analyzed associations of AFLP markers with the production traits of height and diameter using field measurements at 6 years. Six years is the age for trait evaluation and selection for loblolly pine breeding, which was based on juvenile:mature trait correlations. Rather than carrying out marker analysis on the whole mapping population (~1000 individuals), we selectively genotyped individuals from the extremes of the distribution for height at 4 years. The trees were severely impacted by Nantucket pine shoot tip moth during the first two years, which obscured the expression of height and diameter growth to some degree. The selective genotyping based on heights at four years was effective. The 6 year trait distributions were generally bimodal. The spatial analysis and environmental detrending was removed nonrandom environmental variation from the trait data, reducing bimodal appearance of the detrended height and detrended dbh distributions. The spatial analysis removed as much as 25% of the phenotypic variance from some plots. Through direct field observation, we verified that trait patterns of height and diameter were correlated with location in some plots. Field observation showed noticeably larger diameters for trees adjacent to empty positions resulting from mortality in the early years of the study. (Mortality during year one was replaced with filler trees.) Adjustment of dbh was not attempted to account for increased diameter growth near empty positions. Growth at the Scotland County site was good on the fertilized plots, but the overall lower fertility and dryness of the sandy soils probably had a small negative impact on tree growth relative to rich, sandy loam sites like a previous 7-1037 trial at Lumberton, NC.

We used AFLP markers to construct a genomic map for 7-1037. Our AFLP methods have advanced greatly during the time of this grant. We can now routinely obtain high quality AFLP markers in large numbers from conifer megagametophytes. We encountered some problems with the analysis of AFLPs in 7-1037 OP and its parents. First, the number of markers with ratio that departed from the expected 1:1 was very large. Some of these markers probably

are segregating in a 3:1 ratio. The only way to get a 3:1 ratio in megagametophytes from 7-1037 is for the gene sequence that encoded for that AFLP fragment to occur at two loci on different chromosomes and for both sites to be heterozygous. We also noted that parent 7-56 seems to have contributed a much larger proportion of the AFLP bands segregating in 7-1037 megagametophytes than parent 7-51. In addition, we observed so many cases of apparently homozygous band present AFLP fragments that were not represented in both of the parents, 7-51 and 7-56, that for a time, we questioned the identity of the family planted at Scotland County. To finally validate the identity of 7-1037 and its parentage, we used recently developed microsatellite markers. Selective genotyping could also contribute to departures from Mendelian ratios. In other mapping samples of 7-1037 OP, high levels of segregation distortion were not observed.

We detected 5 QTLs for height and diameter traits in OP family 7-1037 at Scotland County. The selective genotyping approach that we used will cause effects to be overestimated but the approach will not bias the parameter estimates obtained by likelihood methods (Ronin *et al.* 1998). There are methods we plan to apply to correct for the overestimation of effects. The magnitude of the overestimation is likely to be relatively small (less than a factor of 1.5). Markers associated with the 4 QTLs and *cad* explained a highly significant proportion of the variation for all traits ($P < 0.01$). Adjusted $R^2$ was greatest for dbh and for the first principal component of dbh and height, 0.097 and 0.088 respectively. We found more QTLs and explained more variance for diameter than for height, although the heritability of diameter is probably a little smaller than for height. Only *cad* had an effect on height alone. The other QTLs all influenced height as well as dbh. The detrending appeared to be more successful for height than for dbh in removing systematic environmental variance. The *cad* null mutation had a highly significant effect only for detrended data analyzed using composite interval mapping. Roughly speaking, the markers account for a mean difference less than 1 phenotypic standard error.

Remington and O'Malley (2000) reported 4 QTLs for height in selfed family 7-56. We aligned the linkage groups from 7-1037 and its elite parent 7-56, and determined that only the cad null mutant allele was most likely inherited from 7-56. However, all positive QTL effects in 7-1037 OP originated in 7-56. One possibility is that 7-1037 did not inherit the favorable allele from the 7-56 loci. Unfortunately, the marker density on our map is insufficient to distinguish the inheritance of 7-56 homologs so we do not know which 7-56 allele was transmitted to 7-1037 for each 7-56 QTL. If the 7-56 alleles were common in the population, then their average effect would be small, even though they could have a large effect within the 7-56 selfed family. The average effect QTLs detected in 7-1037 could be attributed to 7-56 loci that are homozygous, thus were not segregating in the 7-56 selfed family. 7-56 was selected for breeding value and it is possible that part of its superor performance is due to homozygosity for some rare favorable QTL alleles. The power to detect QTLs is generally small and the possibility exists that different sets of QTLs would be detected in replicated mapping experiment involving these two families. More effort will be needed to decide among the explanations for the lack of concordance in QTLs between 7-1037 and its elite parent 7-56.

The *cad* null mutation is a recessive loss of function mutation that has a major impact on lignin composition and bonding patterns. The mutant allele was found only in selection 7-56 and

its descendants in the NCSU:Industry Cooperative Tree Improvement Program. The level of *cad* mRNA in homozygotes was < 1% compared with normal homozygotes, and the level of mRNA in heterozygotes was approximately 50%. A QTL effect on height associated with the *cad* null mutation was detected for family 7-1037 at the Scotland County field site. The effect was significant at the $P < 0.05$ level genome-wise for the detrended height trait using composite interval mapping, but not for other traits or mapping methods. The single marker analysis was significant at the nominal $P < 0.05$ level in the raw height data. The *cad* null effect had previously been detected in selfed family 7-56 and OP family 7-1037 planted at Lumberton, NC (Remington and O'Malley 2000, Wu *et al*. 1999). The 7-56 selfed family had a large QTL peak for height centered on *cad*. This QTL effect was overdominant (heterozygote superiority). In principle, a rare overdominant QTL would be expected to contribute to breeding value.

At Lumberton, NC, the *cad* null heterozygotes in OP family 7-1037 differed from the homozygotes by 14% in volume at 4 years of age. The effect of the *cad* null mutation in 7-1037 OP at Scotland County is much smaller at 6 years after planting. The Lumberton site was more fertile and less xeric than the Scotland County site suggesting genotype x environment interaction. The *cad* null mutation QTL was unusual among the QTLs studied because it showed significant genotype x plot interaction at Scotland County. Genotype by plot interaction approached significance ($P < 0.10$) in the analysis of the Lumberton data. We were unable to discern any pattern to the interaction. The difference in effect between the two planting sites and the genotype x plot interaction suggests that the *cad* null mutation affects developmental homeostasis. *Cad* and other genes are targets for genetic engineering to alter lignin content and quality by reducing gene expression. Our results affirm the need for field evaluations before deployment of genetically engineered trees.

*Cad* is one of several candidate genes that could have variants that influence wood properties. Genetic engineering of lignin biosynthetic enzymes has altered lignin content and composition. Some of the engineered mutations have pleiotropic effects on growth, but our work with the cad null mutation is the only work that demonstrated a similar effect on growth for *cad* heterozygotes. Homozygotes for the cad null mutation grow more slowly and probably die at a young age (< 10 years). Most loss of function mutations have no apparent phenotype for heterozygotes compared with normal homozygotes. Preliminary work that we carried out has shown a difference between in lignin between *cad* null heterozygotes and normal homozygotes for family 7-1037 (Jay Scott, NCSU). The DFRC method cleaves the β-O4 bond that comprises ~50% of the chemical bonds in lignin. Wood from heterozygotes at Lumberton had ~50% less guaiacyl monomeric units released from the lignin compared with control homozygotes. The heterozygote wood from Scotland County had ~20% less monomeric units released. There was no quantitable difference in lignin content or wood density. One weakness of the candidate gene approach and QTL mapping in general is that the phenotypic effects associated with the candidate gene are chromosome substitution effects; the QTL cannot be dissociated from closely linked loci on the same chromosome. The chemical differences among genotypes helps to validate the effect of the *cad* null on lignin in heterozygotes, and makes it more plausible that the cad null mutation has a pleiotropic effect on growth. One possible mechanism for heterozygote effect for this kind of knockout mutation is haploinsufficiency, where a single functional copy of a gene is unable to fully accomplish its physiological role.

The economic potential genetic modifications like the *cad* null to influence energy and chemical use is significant. Preliminary pulping studies we carried out have shown a large difference in Kraft processing time for cad null heterozygote wood. There is an ~ 8 kappa difference in lignin for a cad null heterozygote pulp cooked to the normal end point compared with a control normal heterozygote pulp cooked to the same time under the same conditions. This kappa difference scales to a ~ $4 saving in chemical and energy costs per ton of paper produced. The cad null gene is already widely distributed in loblolly pine breeding programs in descendants from selection 7-56. Open pollinated families from 7-56 have been widely planted throughout the South, probably occupying tens of thousands of acres. Half of these trees are heterozygotes for the *cad* null mutation. Many years will be required to deploy genetically engineered trees to the field because of the testing needed as well as the perceived need to engineer sterility as well as the trait. The benefit from a naturally occurring mutation could be realized much more quickly through both seed and vegetative propagation. In 1996, the US produced 81.1 million metric tons of paper and paperboard, and 58.2 million metric tons of pulp, with the majority of production located in the South. Pine plantations now total ~ 31,000,000 acres and will be making an increasing contribution to the wood supply. Approximately one billion loblolly pine seedlings are planted each year. (This work will be followed up in another D.O.E. Agenda 2020 project, Chang *et al*. 2001).

Little is known about population level QTL effects due the large effort required to extend genomic mapping from individual families to whole populations. QTLs defined in half-sib families of dairy cattle provide a whole population perspective on QTL effects. Bulls are selected based on genetic tests that document increased productivity of their progeny obtained from mating with a large population of cows. Georges *et al*. (1995) found large QTL effects (0.7 to 1.6 $\sigma$ for low heritability traits ($h^2$ = 0.2 to 0.5) with breeding values of 0.6 to 1.3 $\sigma_A$. Thus, low frequency QTLs with large effects could be difficult to detect in outbred populations, but have the greatest potential to shift the population mean either through natural selection or through breeding. In laboratory populations of *Drosophila*, response to selection in long-term multi generational studies occurs in 2 phases: short term response that depends upon existing variation in the base population, and intermediate to long-term response that depends upon variation generated by mutation. Long term selection response often shows periods of stasis followed by jumps in the trait value, presumably caused by the occurrence and fixation of mutation with large effect. Additive effects of large favorable mutations are in the range of 0.5 to 2 $\sigma$. The magnitude of these effects is too small to be obvious (1 to 4 abdominal bristles in a population which ranges from 13 to 25 bristles), but can be resolved by mapping with molecular markers. Effects of this magnitude that occur in natural populations are suggested by the large variation in response in the first few generations of selection among populations initiated from single pairs of flies.

The QTL effects that we have defined in 7-1037 OP are average effects related to breeding value. While the effects are relatively small as a proportion of the phenotypic variance, they are large compared with the amount of additive genetic variance in the loblolly pine breeding population. Some perspective on QTLs and breeding value can be obtained from a simple model of additive genetic variation in a breeding population. The breeding value of QTLs is much greater for low heritability traits than for high heritability traits. The effort required to detect QTLs with breeding value is large using half sib families. However, selections

made on the basis of breeding value, such as 7-56, are logical places to look for QTLs with breeding value. Because of the way these QTLs are defined, average QTLs effects should be transmitted to other families obtained by crosses with other selection (although there could be interactions with other QTLs). These QTLs could be more readily used for deployment of superior germplasm through vegetative propagation. The efficiency of marker assisted selection remains a matter of debate. Our results suggest that within family phenotypic selection was not very effective in capturing QTL effects from 7-56 in selection 7-1037. However, the 7-56 QTLs could have little breeding value, hence were not detected in 7-1037 OP. Breeding value is a good trait for marker assisted selection because the value of the trait is difficult and expensive to determine. Therefore, it is more efficient to follow the trait using markers. Our results show that it is possible to detect QTLs in a half-sib family, and that the magnitude of the average effects is sufficient to suggest that these QTLs could be especially valuable for tree breeding.

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## TASK 3. GENETICS AND MOLECULAR BIOLOGY

## II. GENETIC DISSECTION OF ROOT ARCHITECTURE AND SEEDLING BIOMASS PARTITIONING IN FAMILY 5-1065

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As we already described in the previous overview section, the objectives of the study were to assess whether seedling biomass partitioning could be analyzed as a set of quantitative traits , and if so, could we expect to find meaningful differences in these traits segregating within a family parented by an F1 hybrid between the xeric and mesic ecotypes. . In principle, the above-ground productivity of trees with different QTL genotypes could be due to differences in below ground traits. However, the magnitude of the QTL effects is not large enough to make such an investigation feasible. A large number of root systems would have to be sampled to detect effects that are expected to be small, and the effort required is not feasible.

We initiated a genetic dissection of seedling biomass partitioning in an open pollinated family of loblolly pine selection 5-1065. The seeds from the OP family were cut at one end of the tail part for stimulating germination and were soaked in $H_2O_2$ on May 2. Germinated seeds were sown in a container with sterilized vermiculite. After one week we started collection of germinated megegametophytes for two weeks. The plantlets were transplanted into pots of 12"(d) x 20"(h) size and irrigated every day for one week. The potted trees were fertilized with 50ppm of 15-16-17 fertilizers three times per week until the tree was harvested. Two weeks after transplanting, the trees were inoculated with mycorrhiza; two months later, tip moth insecticide was sprayed every week. The seedlings grew outdoors during the summer and fall of 1999. After 4 months, the seedlings grew about one foot in height and were harvested. Shoots were separated into stems, branches and needles, and root collar diameter and stem length were measured. Roots were collected on a screen and washed with water. The roots were divided into coarse and fine roots, and the number and length of coarse roots were measured. Tissues were dried at 70°C for one day and dry weights were determined. DNA samples were made from the megagametophytes and AFLP DNA markers prepared from the DNA.

The mean stem length was 16.43cm ± 3.92 cm. The distribution of this phenotypic trait significantly departed from a normal distribution using Shapiro-Wilk's test (W-test). However, stem diameter showed a normal distribution. The mean of the trait was 6.57 ± 1.23 mm. For the AFLP analysis, we used three of AFLP primer combinations which were also used in the 7-1037 QTL mapping study. About 106 polymorphic DNA bands were identified and the AFLP genotypic DNA for 414 haploid samples were already determined. We are still working for identifying more reliable AFLP bands and have a plan to use all the same primer combinations that already were used in our 7-1037 study as well as Remington's study (1999). The analysis of this family is in progress and will be completed before spring 2001. We hope to find QTLs for biomass partitioning and address the issue of pleiotropy,

**PUBLICATIONS RESULTING FROM PROJECT:**


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**Please note:** Certain phases of the project are still in progress; consequently, all phases have not been written for publication and submitted to peer-reviewed journals. We anticipate all remaining publications to be submitted in 2001.