THE SHORT-TERM EFFECTS OF FERTILIZATION ON LOBLOLLY PINE (*Pinus taeda* L.) PHOTOSYNTHESIS, DARK RESPIRATION, AND LEAF AREA

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ABSTRACT

The initial physiological processes leading to enhanced growth of loblolly pine subsequent to fertilization are not clearly understood. Much of the debate revolves around the temporal response of photosynthesis (P_n) to fertilization or even if P_n increases at all due to enhanced nutrition. This study tracked loblolly pine light-saturated photosynthesis (A_{sat}), dark respiration (R_d), volume, height, basal diameter, and leaf area responses in eight clones to fertilization (112 kg/ha N) over the course of a growing season in the field. Measurements were conducted intensively before and after fertilization in order to track the initial physiological changes prior to any changes in growth in the fertilized seedlings.

The results showed that fertilization does increase P_n rates although there was no significant effect on R_d rates during the study. The fertilized seedlings mean A_{sat} rates were significantly higher on three sampling dates and remained higher throughout most of the sampling period. At the end of the growing season, the fertilized seedlings had a 30.5% higher projected crown area than the controls and 48% greater mean volumes. Physiological and growth responses were significantly different among clones with some showing large and others showing little or no response to fertilization. These results support the hypothesis from Gough et al. (2004b) that post-fertilization increases in P_n create extra photoassimilate used in building larger leaf areas. These larger leaf areas contribute to higher canopy photosynthesis levels, which leads to an increase in dry matter production.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION AND JUSTIFICATION

Over the last several decades, forest managers have been striving to increase the productivity of their forested lands to meet the needs of a growing society. Fertilization, along with several other silvicultural techniques, is being used to help increase forest productivity on plantations across the United States. It is well known that, in many areas, nutrients are a major limiting factor in forest productivity. Nitrogen and phosphorus are typically considered crucial elements in determining the productivity of forest species (Helms 1976). In unfertile soil types, such as sandy coastal soils typical of the Southeast, fertilization is a very practical way of increasing the nutrient content of the soil. Loblolly pine (*Pinus taeda* L.) stands in the Southeast are often fertilized due to their positive biological and economic responses to nutrient applications (Jokela and Stearns-Smith 1993, Colbert and Allen 1996). Given this fact, 1.9 million hectares of loblolly pine have been fertilized as of 1997 (Jokela and Long 1999).

Although fertilization often results in increased pine tree biomass, the physiological mechanisms for this rise in productivity are still unclear. Teskey (1987) stated that specific leaf photosynthesis rate, respiration rate of foliage tissue, leaf area, and surface area of a tree are responsible for governing net carbon gains. Several studies have focused on gas exchange and leaf area in fertilized forests, but the results have been inconsistent. For example, Murthy et al. (1996) noted a significant difference in net photosynthesis (P_n) of young foliage due to fertilization. However, no increase in P_n was found in loblolly pines that had increased leaf N content and chlorophyll content in a study by Zhang et al. (1997). Some studies have suggested that the primary reason productivity increases is due to increased leaf areas and stem wood in fertilized loblolly pine stands (Teskey et al. 1987, Vose and Allen 1988). However, Teskey (1987) later stated that P_n could also be a determinant due to a study by Hepp and Brister (1982) where needle biomass increased less than above-ground biomass although above-ground biomass and foliar biomass were increasing linearly with increasing site index. Similarly, current research shows no clear pattern in respiration after fertilization. Several studies have shown a positive relationship between fertilization and respiration (Kellomaki and Wang 1997, Zhang et al. 1997, Strand 1996), while others have shown there is no relationship (Lavigne et al. 2001, Roberntz and Stockfors 1998, Schaberg et al. 1996). Hence, the purpose of this study is to clarify the physiological mechanisms involved in increasing loblolly pine biomass with an emphasis on leaf specific photosynthesis, respiration, and leaf area.

Gough (2003) initially studied this topic on loblolly pine seedlings in a greenhouse environment. Gough planted seedlings in a relatively infertile soil from the North Carolina Sandhills region (sandy, siliceous, thermic Psammentric Hapludult [Wakulla series], USDA Forest Service, unpublished data). He reported that following N fertilization, foliar N concentrations increased above the control's and remained that way for approximately 50 days. Foliar N concentrations differed the greatest 28 days after treatment application with fertilized plants averaging 1.7% N and controls averaging 0.76% N. Gough found that the N levels returned to control levels around 146 days after fertilization. Also, Gough reported light-saturated photosynthesis (A_{sat}) levels that were statistically greater in the N fertilized loblolly pines than in the controls 6 days after the treatment application. A_{sat} levels in controls ranged from 1.5 µmol $m^{-2}s^{-1}$ to 4 µmol $m^{-2}s^{-1}$, while fertilized foliage levels ranged from 1.5 µmol $m^{-2}s^{-1}$ to 6 µmol $m^{-2}s^{-1}$ and were almost always higher than the control levels. A_{sat} levels remained high throughout most of the study but began to decrease towards control levels over the last 100 days. Gough also stated that chlorophyll fluorescence indicated an increase in electron transport chain efficiency 22 days after fertilization. He found higher carboxylation rates of Rubisco in fertilized seedlings as compared to non-fertilized controls. These data demonstrate that N fertilization can enhance photosynthesis quickly in loblolly pine. Furthermore, Gough noted that, 4 weeks after the initial increase in photosynthetic capacity, aboveground biomass in the seedlings was statistically higher in the fertilized loblolly pines. More specifically, the fertilized seedlings' ground diameters and heights were greater. Also, projected leaf areas at the end of the study were 36.5% greater in the fertilized seedlings. Hence, Gough hypothesized that increased N uptake into foliage led to an initial increase in photosynthetic capacity, which helped create extra photoassimilate to be used in creating larger leaf areas. Afterwards, the seedling would have higher overall photosynthesis due to larger amounts of photosynthetic tissue and this would lead to increased above-ground biomass. However, Gough noted that his findings in the greenhouse may not translate to a field setting (Gough 2003).

Among several other variables, the amount of solar radiation intercepted by trees plays a large role in determining tree growth. Vose and Allen (1988) noted that the quantity of solar radiation captured is a function of leaf number, angle, and dose. Teskey et al. (1987) noted a strong, linear relationship between leaf biomass and productivity for loblolly pine of different ages across a broad land range. Leaf area data can be correlated to many different functions,

such as canopy respiration and transpiration (Vose and Allen 1988). To date there are no rapid methods for determining an index of leaf area on developing loblolly pine seedlings that incorporate the latest in digital photography and computer analysis.

Based on the above information, this study was designed with the following three objectives:

1. To identify the effects of nutrient additions on above-gas exchange in field-grown loblolly pines (of several clonal varieties) as measured by photosynthesis and dark respiration.
2. To identify the effects of nutrient additions on above-ground growth of field-grown loblolly pine (of several clonal varieties) as measured by changes in height, diameter, canopy size, leaf area, and foliar C and N levels.
3. To identify a rapid method to determine leaf area on young loblolly pines using digital photography and computer analysis.

The hypotheses related to the study objectives from Gough (2003):

1. After fertilization, increased N uptake into the needles will lead to an increase in photosynthetic capacity.
2. The increase in leaf specific photosynthesis rates will create extra photoassimilate to be used in building larger leaf areas.
3. Photosynthesis rates per unit leaf area will eventually return to near the control rates, but the fertilized seedlings will still have more overall photosynthesis due to more photosynthetic tissue.
4. The fertilized seedlings will produce more above-ground biomass due to all or part of these mechanisms.

CHAPTER 2. LITERATURE REVIEW

The following literature review discusses a broad range of aspects encompassing this project. The first section will provide an overview in carbon sequestration and the overall carbon (C) cycle. Beyond the carbon section is a review of fertilization and its effects on foliar nutrients, photosynthesis, dark respiration, biomass allocation, leaf area, and clonal variations. Also, there is a comprehensive review of several popular leaf area determination methods.

Atmospheric CO_2, Forests, and Carbon Sequestration

Over the last few decades, the apparent rise in atmospheric CO_2 has become an increasing global concern as more fossil fuels are being consumed and land use changes occur. Forests are an important component of the global C cycle, accounting for about 33% of the earth's land area (Kramer 1981). Out of the 10^8 Pg C on the planet, the terrestrial biosphere contains an estimated 560 Pg C and the atmosphere an estimated 750 Pg C (Kvenvolden 1993, Sundquist 1993). Furthermore, the terrestrial biosphere has been estimated to account for 70% of the atmospheric carbon fixation per year (Waring and Schlesinger 1985). Given these facts, it is important to understand how trees interact with the atmosphere as sources and sinks for CO_2. Griffin et al. (1985) stated that temperate to sub-tropical zone forests can be an important atmospheric C sink. Thus, a detailed understanding of how net photosynthesis and respiration change given current forest management practices are an important aspect that warrants further investigation (Murthy et al. 1996, Jokela and Martin 2000).

Effects of Fertilization on Tree Physiology

Fertilization and Current Forest Management

Future effective forest management for improved tree productivity is dependent on having a better understanding of how trees respond to nutrient availability (Chandler and Dale 1995). Nitrogen and P are typically considered particularly crucial elements in determining the productivity of forest species (Helms 1976). Fertilization is a practical method of enhancing deficient soil nutrients in infertile soil types, such as sandy coastal plain soils. Loblolly pine stands in this region are often fertilized due to their positive biological and economic responses to nutrient applications (Jokela and Stearns-Smith 1993, Colbert and Allen 1996). The North Carolina State Forest Nutrition Cooperative (2000) notes that 75% of the planted lands in the region are planted in pine and almost 647,500 hectares of planted pine were fertilized in 1999.

Effects of Nitrogen (N) Fertilization on Foliar Nutrients

Literature concerning N fertilization and foliar nutrient concentrations is abundant and consistently reports increased foliar N concentrations in response to N fertilization. Gough et al. (2004a) reported higher foliar N concentrations for fertilized 14-year-old loblolly pines over the controls for all months sampled in a field study. Monthly mean foliar N concentration values in the fertilized stand ranged from 1.2% to 1.4% dry weight, while the control values ranged from 1.0% to 1.2% dry weight (Gough et al. 2004a). In a greenhouse study, Gough et al. (2004b) found significantly higher foliar N contents in fertilized loblolly pines than in the unfertilized controls. The highest difference in foliar N was found 28 days after the fertilizer treatment was applied. Foliar N values ranged from 0.76% N in the controls to almost 1.7% N in the fertilized seedlings (Gough et al. 2004b). A light intensity and N fertilization study conducted in Oklahoma showed a 40% increase in foliar N concentrations for shaded and unshaded loblolly pine branches subjected to N fertilization (Zhang et al. 1997). Murthy et al. (1996) found a 25% to 34% increase in foliar N concentrations in 1-year-old loblolly pine seedlings given fertilizer and irrigation treatments. Also, Murthy et al. (1996) found a statistically significant increase in foliar N concentrations for all months over unfertilized treatments. In irrigation and fertilization experiments, Axelsson and Axelsson (1986) found increased N concentrations in Scots pine (*Pinus sylvestris* L.) needles, which lasted 1 year and then decreased slightly and leveled off. Nitrogen concentrations in the needles ranged from 1.6% to 1.7% N by dry weight. Kellomaki and Wang (1997) also found increased foliar N concentrations in Scots pine needles during a branch-in-bag experiment on 25- to 30-year-old, naturally seeded trees. Munger et al. (2003) noted a strong positive relationship between fertilization and foliar N concentrations during a competition control and annual fertilization study in loblolly pines at two different study sites. The fertilized pines had significantly higher foliar N concentrations ($p<0.0001$) and the difference between treatments increased with stand age ($p<0.02$) (Munger et al. 2003).

Some other factors that affect foliar N concentrations are crown position and needle age. Schoettle and Smith (1999) found that foliar N varied from 0.64 to 1.31 g N m^{-2} throughout the crown of lodgepole pine (*Pinus contorta ssp. latifolia* Engelm.) with the highest amounts residing in the upper third. Also, foliar N was found to decrease with needle age (Schoettle and Smith 1999). Zhang and Allen (1995) found similar results in loblolly pine; however, N concentrations did not significantly differ across crown positions although N content increased

with crown height. Zha et al. (2002) noted similar results in foliar N trends during a long-term elevated CO_2 concentration and temperature study in Scots pine. Their findings showed needle N decreased along a vertical transect with most of the drop occurring in the lower half of the crown. Also, foliar N concentrations decreased horizontally from the outer to inner canopy. These results were consistent between the control and all of the treatments when foliar N concentrations were expressed on an area basis. However, when expressed on a mass basis there were no obvious trends in foliar N (Zha et al. 2002).

Nitrogen fertilizer and N uptake can also have an impact on the concentrations of other essential elements in tree leaves as well as chlorophyll content. Elements N, P, K, Ca, and Mg are some of the most important macronutrients used by a plant to create macromolecules or be used as cofactors for enzyme activity. They serve in cell structures, chlorophyll, and enzyme regulation and clearly affect overall growth (Kramer and Boyer 1995). Chlorophyll is important in "absorbing" light energy, and it plays a central role in photosystems I and II. Literature dealing with chlorophyll content and nutrient concentrations show somewhat varying results on both topics. For instance, Zhang and Allen (1996) found N fertilization increased Ca concentrations, but decreased Mg and P concentrations with no effect on K concentrations in loblolly pine. Foliar nutrient proportions were 100:13:70:11:10 for N:P:K:Ca:Mg in the control trees and 100:8:47:8:7 for N:P:K:Ca:Mg in the fertilized trees (Zhang and Allen 1996). However, Zhang et al. (1997) found in their light intensity and fertilization study of loblolly pine that N fertilization decreased Ca concentrations, but had similar effects to the previous study on concentrations of the other macronutrients. A study by Jach and Ceulemans (2000) on Scots pine showed that chlorophyll content paralleled changes in N concentration. However, Schaberg et al. (1997) found that N fertilization had no significant effect on chlorophyll content in mature montane red spruce (*Picea rubens* Sarg.). Zhang et al. (1997) found higher leaf chlorophyll content due to N fertilization, but the increase was not proportional to the change in leaf N content.

Effects of Fertilization on Photosynthesis

The short-term physiological changes in photosynthesis associated with N fertilization are not very well understood and not extensively covered in current literature. However, there have been several recent studies on the general relationship between N fertilization and photosynthesis that bear attention. Literature generally shows that net photosynthesis favorably

responds to N fertilization or increased foliar N concentrations over the long-term. This is a logical relationship since photosynthetic reactions need proteins that are associated with foliar N (Evans 1989). Nine out of 14 journal articles surveyed showed an increase in net photosynthesis either all or partially due to the effects of N fertilization (Chandler and Dale 1995, Murthy et al. 1996, Kellomaki and Wang 1997, Murthy et al. 1997, Strand 1997, Roberntz and Stockfors 1998, Schoettle and Smith 1999, Samuelson 2000, Lavigne et al. 2001). The other five articles showed no significant difference or varying differences in net photosynthesis due to N fertilization (Teskey et al. 1994, Schaberg et al. 1997, Zhang et al. 1997, Munger et al. 2003, Gough et al. 2004a). Much of the variation may be explained by differences in experiments or experimental conditions, such as needle age, temperature, relative humidity, light levels, CO_2 concentration, and foliar N content (Evans 1989, Strand 1996, Schoettle and Smith 1999). Also, the amount of time between fertilization and the start of P_n sampling may have a distinct effect on the results (Gough 2003).

Murthy et al. (1997) found that fertilization had a significant effect on mean light saturated net photosynthesis rates (A_{sat}) in loblolly pine. In fact, the mean A_{sat} rates for fertilized loblolly pines ranged from about 2.4 µmol $m^{-2}s^{-1}$ to 8.9 µmol $m^{-2}s^{-1}$ at their peak in March, while the controls ranged from about 2.3 µmol $m^{-2}s^{-1}$ to 7.3 µmol $m^{-2}s^{-1}$ at their peak in April. However, over the course of the 9 months, the mean A_{sat} rates for the fertilized pines were always higher than the non-fertilized pines. In Murthy's study, the A_{sat} measurements were conducted monthly, a year after the fertilization treatments were commenced. Murthy et al. (1997) also found a seasonal variation in mean A_{sat} rates for the loblolly pines. Rates rose from a low in January to their peak in March or April, decreased greatly between May and June, and then rose slightly in August before decreasing again in September. Roberntz and Stockfors (1998) found similar results in their study of Norway spruce [*Picea abies* (L.) Karst] where needle nitrogen concentration explained up to 60% of the variation in A_{sat} rates. While foliar N concentrations increased from 0.2 to 0.4 mg cm^{-2}, A_{sat} of shoots increased from a low of approximately 6 µmol $m^{-2}s^{-1}$ to a high of 15 µmol $m^{-2}s^{-1}$. However, in this experiment the fertilizer was applied daily in a liquid form with drip irrigation and measurements were taken in Sept. 1994 and again in 1995 (Roberntz and Stockfors 1998). Strand (1997) had similar results when studying photosynthetic rates and nutrient content in Norway spruce although the positive relationship between foliar N and A_{sat} was much weaker and varied by season. Strand (1997)

also used liquid fertilization with drip irrigation during the measurement period, but he only measured A_{sat} 9 days over a 130 day period. In another experiment using pot-grown Sitka spruce [*Picea sitchensis* (Bong.) Carr.] given increasing amounts of N fertilizer, net photosynthesis increased with increasing N concentration up to 14 mg l^{-1} (Chandler and Dale 1995). However, Chandler and Dale (1995) noted that net photosynthesis did not increase beyond this concentration, possibly due to a limiting light intensity, which was not saturating. In the experiment, Chander and Dale (1995) measured P_n a year after their fertilization treatments, but they sampled leaf primordia from the year before as well as the current year. Schoettle and Smith (1999) examined the relationships between light, photosynthesis and N in lodgepole pine and found a positive correlation between foliar N and A_{sat} in young needles. However, no significant relationship was found between foliar N and A_{sat} in older needles (Schoettle and Smith 1999). No fertilization treatment was applied during or before the sampling period, which lasted 2 months. (Schoettle and Smith 1999). Kellomaki and Wang (1997) also discovered an increase in A_{sat} rates in Scots pine during a fertilizer and elevated CO_2 branch-in-bag experiment. Kellomaki and Wang (1997) stated that N fertilization helped increase needle N concentration on an area basis, which was closely correlated with an increase in A_{sat} rates despite the treatments of differing CO_2 concentrations. Kellomaki and Wang (1997) also measured P_n a year after fertilization for this study. Lavigne et al. (2001) found slightly higher P_n rates among fertilized balsam fir (*Abies balsamea* L.) than in nearby unfertilized balsam fir during a sink:source experiment where several different experiments were run on 1-year-old foliage. Although Lavigne et al. (2001) began taking measurements within a month of fertilization, P_n measurements were only conducted over a 2-day period. Samuelson (2000) found a significant difference ($p \leq 0.05$) between high and low N fertilization treatments during a leaf physiology and growth study of loblolly pine and slash pine (*Pinus elliottii* Engelm.). The seedlings that received the high N treatment (264 ppm N solution NH_4NO_3) had a higher mean P_n rate than the low N treatment (50 ppm NH_4NO_3). Mean values were 4.0 µmol $m^{-2}s^{-1}$ for high N and 2.4 µmol $m^{-2}s^{-1}$ for low N seedlings of both species. Fertilizer was applied weekly in solution to the high N seedlings and biweekly to the low N seedlings. Also, P_n measurements were initiated 4 months after the fertilizer treatments were applied to the seedlings (Samuelson 2000).

Teskey et al. (1994) found differing results from the previous literature while studying P_n changes in slash pine in Florida. The purpose of the study was to determine the effect of climate

and fertilization on P_n under field conditions. Teskey et al (1994) began fertilizer applications a year before the study and continued them at 3-month intervals during the measurement period. P_n measurements were conducted at 3-week intervals over the course of a year. The results showed mean P_n rates were variable and inconsistent between the fertilized and unfertilized treatments although fertilized rates tended to be slightly higher. The mean P_n rates were not statistically different between the fertilized (1.76 µmol $m^{-2}s^{-1}$) needles and control (1.58 µmol $m^{-2}s^{-1}$) needles over the course of a year. Also, similar results were found by Zhang et al. (1997) during a light intensity and N availability experiment on non-improved loblolly pines in a field in Oklahoma. A treatment of 200 kg/ha N was applied 3 months before P_n measurements were conducted. Net photosynthesis rates were only slightly different between treatments and only differed by 0.4 µmol $m^{-2}s^{-1}$. Schaberg et al. (1997) found no change in P_n rates, chlorophyll fluorescence, or chlorophyll content in a study where N fertilizer was added to plots containing mature montane red spruce. However, relatively low fertilizer rates varying between 0 kg N/ha/yr and 31.4 kg N/ha/yr, were applied to plots eight consecutive years before measurements were conducted in this study. Munger et al. (2003) found similar A_{sat} results between fertilized and unfertilized loblolly pines at two different sites in the southeastern United States. Neither site on the coastal plain nor piedmont of Georgia had significant differences between treatments when the data was compiled. A_{sat} rates ranged between 1 and 6 µmol $m^{-2}s^{-1}$ for each site. In addition, the treatment pines were subject to an annual regimen of fertilizer, so A_{sat} measurements were always taken within a few months after fertilization (Munger et al. 2003).

In an experiment involving fertilized and non-fertilized 14-year-old loblolly pines in North Carolina, Gough et al. (2004a) noted an inconsistent relationship in net photosynthesis over a period of 12 months. Gough et al. (2004a) conducted monthly A_{sat} measurements 1 year after fertilization treatments in the stand ceased. Net photosynthesis rates for fertilized stands were only significantly different from controls only in February. Mean photosynthesis rates were higher in the controls than in the fertilized plots in subsequent months. However, Gough et al. (2004a) stated that nutrient additions may have significantly increased foliar P_n rates for only a short time after fertilization in order to create extra photosynthetic tissue. Thus, increased LAI as a result of fertilization may have been the main factor in greater productivity of the N treatment (Gough et al. 2004a).

Effects of Fertilization on Dark Respiration

The interactions between N fertilization and needle respiration appear to be highly variable in the literature. However, most of the literature surveyed shows either increases in respiration due to N fertilization or no effect on respiration. It is possible that some of the variation in the literature is due to differing darkness acclimation periods, measuring times, or other experimental factors. Strand (1997) took dark respiration (R_d) measurements on Norway spruce needles after 10 minutes in the dark. He found a positive relationship between foliar N concentrations and R_d rates during the autumn and early winter of 1992. However, in September 1993, the relationship in current year needles was more dependent on the rate of photosynthesis and not as much on the N content of the needles (Strand 1997). In a branch-in-bag experiment on Scots pine, R_d rates significantly increased due to high N treatment and time (Kellomaki and Wang 1997). The rates were estimated using A_n/C_i curve slopes as described in Brooks and Farquhar (1985). The mean respiration rate for unbagged controls growing in the high N treatment was 1.347 µmol m^{-2} s^{-1} and the mean rate for the normal N treatment was 1.147 µmol m^{-2} s^{-1} (Kellomaki and Wang 1997). Zhang et al. (1997) noted a slight increase in loblolly pine needle respiration rates due to N fertilization during a light intensity and N availability study. R_d rates for the fertilized pines in light and shade were 0.52 and 0.27 µmol m^{-2} s^{-1} while rates for the unfertilized pines in light and shade were 0.42 and 0.25 µmol m^{-2} s^{-1}. However, the increases were not statistically significant for the treatments. In this experiment, R_d was measured on needles after 30 minutes of acclimation in an infrared gas analyzer with a dark cloth over the cuvette (Zhang et al. 1997).

In another branch-in-bag experiment conducted on Norway spruce, no relationship was found between needle N concentration and respiration (Roberntz and Stockfors 1998). Respiration rates were instead found to be correlated with other needle nutrient concentrations, such as P, B, and Mg. Furthermore, the bag had a negative relationship with respiration rates during the experiment. The respiration rates ranged between 0.45 µmol m^{-2} s^{-1} and 1.3 µmol m^{-2} s^{-1} for the control and in-bag spruce needles. These measurements were conducted after 1 hour of dark acclimation using an oxygen electrode technique that measured oxygen consumption over a 20-minute period on a group of 40 needles (Roberntz and Stockfors 1998).

In an experiment by Lavigne et al. (2001) dealing with sink:source balance in balsam fir, fertilizer treatments had no effect on respiration. The R_d rate was approximately 2.2 µmol m^{-2} s^{-1}

for the fertilized treatment and approximately 2.1 μmol m^{-2} s^{-1} for the unfertilized treatment (Lavigne et al. 2001). Schaberg et al. (1997) found that N fertilization increased foliar N concentrations, but there was no significant correlation between foliar N concentrations and respiration rates in red spruce trees in Vermont. In this report and the previous one, R_d measurements were recorded with an infrared gas analyzer after a few minutes of dark acclimation under a thick cloth. Schaberg et al. (1997) states although respiration rates were generally increased by fertilization, the cause for this relationship is unknown and may be indirectly related to increased N.

One article surveyed did show a decrease in R_d due to N additions during a leaf physiology and growth study of loblolly and slash pines (Samuelson 2000). The study consisted of a high N treatment and a low N treatment. The high N treatment received fertilizer every week while the low N treatment received a smaller dose biweekly. Measurements were conducted on 7-month-old seedlings between 2:00am and 5:00am to avoid any light related photorespiratory inhibition using a LI-6400 infrared gas analyzer. Her findings showed statistically significant ($p<0.05$) decreases in R_d due to the high N treatment in the loblolly and slash pines seedlings. Samuelson (2000) notes that these findings are in contrast with many other studies, but she suggests the lower needle R_d rates may increase the carbon pool available for transport to other tissue, which will allow greater growth in the high N seedlings.

Effects of Fertilization on Tree Biomass and Productivity

Biomass Allocation

It is generally well known that fertilization increases biomass in loblolly pine on nutrient deficient soils. There have been many studies documenting above-ground and below-ground biomass allocation patterns due to the effects of nutrient additions. Since many tree species are intensively managed for biomass production, it is important to understand growth patterns and how they respond over time (Jokela and Martin 2000). The amount of solar radiation captured, efficiency of the photosynthetic system, respiration, and the allocation of photoassimilate to stemwood all help to determine tree growth (Vose and Allen 1988). However, there is also growing evidence that at least some fertilized pine trees allocate more biomass to above-ground production by decreasing dry matter allocation to fine roots (Axelsson and Axelsson 1986, King et al. 1999). Furthermore, ontogeny, species, available resources, and time of year have all been shown to at least partially affect above-ground biomass allocation patterns (King et al. 1999,

Jokela and Martin 2000). Regardless of these variables, biomass partitioning results in the literature surveyed are fairly consistent for several pine species commonly grown in the southeast.

At the Southeast Tree Research and Education Site (SETRES) in the North Carolina sandhills region, several joint studies examined the biomass production of loblolly pine given nutrient and irrigation treatments over a 4-year period. Albaugh et al. (1998) quantified the biomass partitioning patterns of the loblolly pines. King et al. (1999) determined the stand level allometric relationship among plant parts. All treatments greatly increased the biomass of stems, branches, foliage, taproots, and coarse roots. The only plant part that did not show increases in accumulated biomass was fine roots. Albaugh et al. (1998) found a 52%, 109%, 120%, and 152% increase in stem volume growth over the 4-year period. Also, the fertilized pines had increases in diameter (30%), height (23%), volume (81%), and basal area (68%) over the controls (Albaugh et al. 1998). The result was an 86% increase in total biomass for the fertilized trees over the control trees at the end of 4 years. Analysis showed more biomass was allocated to branches as compared to stems and more biomass was allocated to branches relative to foliage. In addition, more biomass was partitioned to perennial roots relative to perennial shoots in the fertilized loblolly pines (King et al. 1999). In a study by Jokela and Martin (2000), loblolly pine and slash pine seedlings subject to fertilizer and weed control treatments for 10 years had slightly different results. Loblolly pine stemwood, branches, bark, and foliage biomass increased 65.3%, 18.5%, 9.1%, and 7.4% respectively. Slash pine stemwood, branches, bark, and foliage biomass increased 62.8%, 15.3%, 13.0%, and 9.2% respectively (Jokela and Martin 2000). Thus, allocation patterns between these last two studies were similar in regards to branches receiving more biomass than foliage but differed in the fact that stemwood received more biomass than branches.

Leaf Area Index and Biomass Relationships

Vose and Allen (1988) studied the relationship between nutrients and leaf area in loblolly pine. The authors note that nutrients, water, and temperature are the main environmental factors limiting leaf area. Several studies have shown a positive correlation between nutrient additions and leaf area in loblolly pine (Teskey et al. 1987, Vose and Allen 1988, Albaugh et al. 1998, King et al. 1999, Jokela and Martin 2000). It is important to note these factors typically influence photosynthesis as well due to the amount of photosynthetically active radiation larger

leaf areas can intercept (Vose and Allen 1988). Hepp and Brister (1982) have shown there is a relationship between leaf area and net photosynthesis, and Teskey et al. (1987) noted a positive and linear relationship between leaf biomass and productivity in loblolly pine over a broad range of ages and locations. Thus, there is most likely a distinct relationship between nutrients, photosynthesis, and leaf area.

Vose and Allen (1988) exposed three stands of loblolly pine to varying amounts of N and P fertilizer (0, 112, 224, 336 kg N/ha and 0, 28, 56 kg P/ha). Stands were divided into four blocks and randomly assigned treatments except for stand 3, which only had two blocks. After fertilization in the spring, LAI measurements were conducted using needlefall in the stands. The results showed N fertilizer significantly increased the LAIs of stands 1 and 3. Stand 2 showed no response to any of the treatments. However, Vose and Allen (1988) note that the lack of response in LAI could be due to light limitations associated with an already high LAI or leaf area was not limited by nutrients. The fertilized tree LAIs and control tree LAIs varied by less than 10% in this stand. Stand 3 showed the largest increase in LAI in response to N fertilization. LAI ranged from 33% (112 kg N/ha treatment) to 59% (336 kg N/ha treatment) above the control LAI. Also, stands 1 and 3 showed a significant increase in stemwood growth with increasing LAI. After the cumulative data were analyzed, Vose and Allen (1988) noted that stemwood growth and LAI had a strong, positive, linear relationship ($r^2 = 0.75$, $P < 0.01$, $n = 24$). Furthermore, 66 to 99% of the variation in productivity was explained by LAI for this experiment (Vose and Allen 1988). Jokela and Martin (2000) found similar results in their experiment using fertilizer and weed control treatments on loblolly and slash pines in Florida. Besides the control, plots were given treatments of fertilizer, weed control, or a combination of both for 10 years during the 16-year study. The results showed the same relationship between LAI and stemwood growth for both species of pines. LAI explained greater than 95% of the variation between stemwood biomass production and the treatments applied. Hence, canopy leaf area appears to be the link between rates of biomass production and photosynthesis as influenced by environmental factors (Vose and Allen 1988, Jokela and Martin 2000).

The SETRES study in North Carolina also found similar results in LAI and stemwood growth due to fertilizer treatments on the loblolly pine stand over the 4-year study. Albaugh et al. (1998) showed that peak LAI significantly increased each year in reponse to the fertilizer treatment. The annual percent increases in peak LAI, relative to the controls, between 1992 and

1995 were 54%, 65%, 82%, and 100%. The change was correlated with an increasing stemwood volume growth that rose to 152% above controls by 1995. All of these growth changes led to a total biomass production efficiency increase of 91% due to fertilization. However, Albaugh et al. (1998) noted that there was a discrepancy between the increase in total biomass production efficiency and the shift in biomass allocation from belowground to aboveground components. Hence, the author concluded that net photosynthesis must have increased with the treatment applications in order to achieve such a large increase in production efficiency per unit LAI.

Murthy et al. (1996) also worked in this stand during the 1993 season and found that fertilization was responsible for a 20 to 24% increase in A_{max} in that year's foliage cohort. Thus, an increase in the photosynthetic rate was realized during the same period of time that LAI was beginning to increase rapidly. Gough et al. (2004a) conducted research in the same stand in 1999 and found that P_n rates in the fertilized stand had decreased to near the control stand rates. Thus, Gough et al. (2004a) concluded fertilization may have quickly and briefly increased foliar P_n rates in order to generate C for extra leaf area to be constructed. Then once a peak LAI was achieved, P_n rates down-regulated to near control stand levels. The end results from this process were increased leaf area, greater production efficiency, and higher overall aboveground biomass in the fertilized loblolly pine stands (Albaugh et al. 1998, Gough et al. 2004a).

Methods of Determining Leaf Area

There have been many attempts to accurately determine the LAI of different tree species. These values are important for predicting or calculating carbon sequestration, tree productivity, and other values typically associated with trees (Peper and McPherson 2003). However, problems due to environmental variations, tree architecture, and tree growth habits often mar the accuracy of such attempts (Sampson et al. 2003). For example, loblolly pine is difficult to measure due to its indeterminate growth habit with multiple flushes being produced over the course of a growing season. Also, needle growth can change in response to fertilization or needles can be lost during times of drought or other stresses (Vose and Allen 1988, Sampson et al. 2003).

There are several common approaches to estimating LAI in forested stands. The first includes a destructive harvest of the standing biomass and/or litterfall collections. This method is typically time consuming and labor intensive. Other techniques include using sophisticated equipment, such as a LI-COR LAI-2000 plant canopy analyzer (PCA) or CI-100 Digital Plant

Canopy Analyzer, which give a more instantaneous representation of LAI. These machines use fish-eye lenses or hemispherical sensor heads to obtain digital photos of a tree. These methods require purchasing expensive equipment, but yield results in a shorter amount of time. Furthermore, they require some technical expertise to use and often need specific weather conditions to avoid biased measurements (Peper and McPherson 2003, Sampson et al. 2003). Another method involves taking specific tree measurements and using them in a logistic regression. This method is inexpensive, easy to use, and not very time consuming. A newer approach to determining LAI involves using computer digital image processing. This method requires a two dimensional digital photograph, which can be processes into a unitless quantification of tree crown size. Later the values can be converted into a LAI using conversion factors obtained from the photos and picture taking process (Peper and McPherson 2003).

A study by Sampson et al. (2003) showed that the LI-COR LAI-2000 PCA may not provide an accurate estimate of loblolly pine LAI when used over time. They found that measurements increasingly underestimated the vegetation area index (VAI) as LAI increased. Peper and McPherson (2003) found similar results for the LAI-2000 PCA when used to estimate the LAI of several isolated urban deciduous trees. The LAI-2000 consistently underestimated the "true" LAI values, but it did so with precision. They also found the CD-100 typically underestimated the LAI values and had difficulty getting accurate measurements due to the weather conditions present. The logistic regression method was the only method that typically overestimated the "true" LAI for the urban trees. The computer digital image processing method yielded the best correlations to the true leaf area of *Platanus x acerifolia* (R^2=0.83) and was highly correlated to *Platanus x racemosa* (R^2=0.73) as well. The method required very little time in the field (only two pictures per tree) and photo processing was simple with the use of photo editing software. Peper and McPherson (2003) concluded that this was the most efficient, accurate, and effortless method to use for determining LAI for isolated trees.

Genotypic, Family, or Clonal Variations

Genotypic Physiological Responses due to Fertilization or other Treatments

Tree breeding in the United States represents one way of increasing tree yields and quality. Many forest products companies and research stations have begun to breed trees for superior traits, such as height and diameter growth. Through this selective breeding, they can create superior clone lines that may out-produce their unmodified counterparts due to better

biomass allocation tendencies or growth efficiency. Although research in this area is relatively new, some studies have shown that there is tremendous variation in genotypic responses to different treatments or environmental conditions (Teskey et al. 1987). For example, three studies showed significant or varying genotypic responses in growth or physiological characteristics (Li et al. 1991, McCrady and Jokela 1996, Chang 2002) while one study showed little if any family x treatment response (Samuelson 2000).

Li et al. (1991) notes that identifying variations in biomass allocation among different families may create an avenue for increased biomass production and management. Nitrogen availability greatly affects dry matter partitioning and the resulting growth in many plants. During a N fertilization experiment, Li et al. (1991) found a significant family and family x N interaction on loblolly pine tree biomass. Twenty-three open-pollinated loblolly pine families were grown in a greenhouse under a high (50 ppm) and low (5 ppm) fertilization regime for over 20 weeks. Their analysis showed a significant family effect on biomass with means ranging from 30.0 – 20.4 g in high N and 21.0 – 13.9 g in the low N treatment. Also, a family x N interaction significantly altered total biomass of the loblolly pine seedlings. Some families showed a significantly higher needle and/or stem weight in the high N treatment as compared to the low N treatment. However, each family had significantly less root weight in the high N treatment compared to the low N treatment, suggesting low N seedlings allocate more dry matter to root production. Li et al. (1991) concluded that the families which showed the largest gains in stem and height weight between treatments might help increase forest productivity under several different management conditions.

In a similar N fertilization study on loblolly pine and slash pine seedlings by Samuelson (2000), no significant interactions were found between family and N treatment for P_n, R_d, g_l, or foliar N. However, significant two-way interactions were found for fine root allocation although no other growth characteristics had an interaction. Samuelson (2000) found a similar trend to Li et al. (1991) with reduced allocation to fine roots and an increase in allocation to needles due to the high N treatment. Chang (2002) found slightly different genotypic results during a sweetgum (*Lyquidambar styraciflua* L.) half-sib family and fertilization study. The experiment involved two half-sib sweetgum families and two rates of N and P fertilizer. Measurements were conducted over a 2-month period and significant genotypic responses were found in basal diameter and height growth. Also, one family was shown to have a consistently higher LAI

during the measurement period. Net photosynthesis had a significant three-way interaction (N x P x F) with one family showing higher P_n rates with 100 kg N while the other family had higher P_n rates with 100 kg N and 50 kg P. In conclusion, Chang (2002) notes that selecting families for certain site nutrient conditions could increase overall stand productivity.

The final study surveyed noted growth and crown structure changes in loblolly pine families at different planting densities (McCrady and Jokela 1996). They found significant genotypic variations in foliage amount, distribution, and longevity that resulted in differing growth. Family differences in height explained 38% of the variation in foliage biomass, which varied as much as 60% among families. Also, LAI and foliage biomass genotypic variations were related to the vertical distribution of foliage and standing volume at age 3 ($r=0.59$ and 0.64). McCrady and Jokela (1996) also found the best producing families kept >60% of their foliage biomass in the middle third of the canopy. Furthermore, one genotype with poor performance and a low LAI was shown to have reduced foliage longevity compared to the other families. Hence, McCrady and Jokela (1996) suggest that differences in loblolly pine family production are at least partially due to foliage and crown structure characteristics.

CHAPTER 3. MATERIALS AND METHODS

Study Site Description

The study site was located in Patrick County, Virginia (36° 40' N, 80° 10' W) at the Reynolds Homestead Forestry Research Center on the upper Piedmont province, where the topography consists of gently rolling hills. The elevation varies between 300 and 350 m above sea level. The site was located in the temperate climate zone with warm, humid summers and cool, moist winters. The average minimum temperature is -1.4°C, and the average maximum is 29.2°C. Precipitation is evenly distributed throughout the seasons and averages 1.3 m/year.

The soils consist of the Lloyd clay loam (fine, kaolinitic, thermic Rhodic Kanhapludults), Louisa loam (loamy, micaceous, thermic, shallow Ruptic-Ultic Dystrudepts), and Hiwassee loam series (very-fine, kaolinitic, thermic Rhodic Kanhapludults) are well-drained, deep Ultisols originating from granite, schist, and gneiss parent material. The site has been heavily farmed for two centuries, which has resulted in the loss of essentially the entire original A horizon. Instead there was a reduced profile, with a surface Ap horizon and clayey B horizons mixed in below.

The study site consisted of four blocks, each split into two plots of 25 different loblolly pine clones that were planted May 19, 2003 (See appendix for a map). The site preparations included a treatment of Roundup®, ripping, and shallow cultivation in the planting rows. The clones were evenly spaced 2.4 m apart in rows consisting of five clones that were randomly selected. All rows were evenly spaced 3.0 m apart and the clones were numbered in a serpentine pattern from 1 to 25 starting in the southwest corner of the plot. There was also a row of "buffer" seedlings surrounding and separating the eight study plots. Thus, the total size of each plot was approximately 338.2 m^2. The clones were provided by the Forest Biology Research Cooperative (FBRC) (University of Florida, Gainesville, FL). The parents were selected from the Loblolly Pine Lower Gulf Elite Breeding Population, which contains both Atlantic Coastal and Florida provenances. The study plots were mowed and manually weeded periodically during the growing seasons in order to minimize competition and confounding from a fertilizer weed response. Oust® (Dupont Corp.) and Roundup® (Monsanto Co.) herbicides were also used to clear out competition from the seedling rows. No other environmental variables were controlled during the experiment in order to allow the seedlings to experience typical field-grown conditions.

Nutrient Application

Within each of the four blocks, one plot was randomly chosen for fertilization. The other half was the non-fertilized control plots. Two types of fertilizer were applied to achieve a fertilization level of 112 kg of elemental N per hectare. The fertilizer application consisted of 224 kg/ha of diammonium phosphate (DAP) and 184 kg/ha of ammonium nitrate. Hence, the DAP supplied 47.5 kg N/ha and 23.2 kg P/ha while the ammonium nitrate supplied the other 64.5 kg N/ha. The fertilizer was spread by hand using a banded application technique over the four fertilization treatment plots on May 6, 2004.

Measurements

Prior to application of the fertilizer treatment, growth measurements including height and ground-line diameter were collected on all 200 seedlings. Seedling growth was monitored periodically during the growing season by tracking the changes in the variables above. Also, any dead or dying seedlings were noted and excluded from the study. Three pre-fertilization height measurements were taken starting on December 29, 2003 while two pre-fertilization ground-line diameter measurements were taken starting on March 5, 2004. Volume measurements were calculated by multiplying the measured height by the measured ground-line diameter squared for each seedling on the dates when both data were collected. Measurements were taken intensively during the growing season and less frequently during the winter until sampling ceased in April 2005. Out of the 25 loblolly pine clones, eight clones were chosen for intensive physiological and growth measurements. The following table shows the eight clones from full-sib crosses chosen and their associated parents (note that two clones from each of four full-sib families were used).

Clone ID	*Family 1*	*Family 2*
41021	10-10	22-44
41089	10-10	22-44
42719	7-1011	22-55
42725	7-1011	22-55
43671	7-56	10-5
43694	7-56	10-5
43903	10-10	7-1011
43976	10-10	7-1011

*The first three numbers in the Clone ID refer to the parents and the last two refer to the cross number.

In order to establish the short-term effects of fertilization on loblolly pine physiology, measurements were taken twice, once on April 30, 2004 and once on May 6, 2004 to establish a base of comparison for subsequent measurements after treatment application. Net photosynthesis and a foliar C and N analysis were measured on one new, fully expanded needle fascicle per seedling while R_d was measured using two fascicles per seedling.

Net photosynthesis measurements under saturating light conditions (A_{sat}) were conducted using a LiCor 6400 Portable Photosynthesis System (LiCor Inc., Lincoln, NE). Light-saturated photosynthesis was measured on a fascicle of needles immediately after detachment from the seedling (<3 min). However, literature has shown needle A_{sat} rates are not generally affected by twig removal from the tree for up to 30 minutes (Ginn et al. 1991). The A_{sat} rate was recorded three times per fascicle over a period of 10 seconds and the mean of the three samples was used for subsequent data analysis. Thus, 64 mean A_{sat} values were used for data analysis. All chamber conditions remained at ambient for this study with the exception of photosynthetically active radiation (PAR) = 1600 µmol $m^{-2}s^{-1}$ and [CO_2] = 360 µmol/mol. Hence, the data collected was reflective of the environmental conditions experienced by the field grown seedlings while the set chamber values served as a basis for comparison to Gough et al. (2004b).

Dark respiration measurements were conducted using the same basic methods as the A_{sat} measurements. However, R_d measurements were conducted monthly between 11:00pm and 1:00am, just following, or prior to, A_{sat} measurements on 6 of the same clones. The chamber conditions remained the same as above although the LiCor blue/red chamber light was turned off (PAR = 0 µmol $m^{-2}s^{-1}$) and [CO_2] = 400 µmol/mol. The higher CO_2 concentration was chosen based on previous field observations of CO_2 levels between 400 and 500 ppm during the early morning hours. After A_{sat} and R_d measurements, each removed fascicle diameter was measured using digital calipers and then the A_{sat} fascicles were enclosed in an envelope for later tissue nutrient analysis. Two fascicles were used to measure R_d for each clone and those were discarded after each measurement. The following equation was used to calculate A_{sat} on a per leaf area basis given the information collected (Ginn et al. 1991):

$$LA_l = (n*l*d) + (\pi*d*l)$$

where n = number of needles per fascicle, d = fascicle diameter, and l = needle length in chamber. The A_{sat} fascicles were stored and used to obtain foliar C and N concentrations. All needles were oven dried at 65 °C and ground in a Wiley mill prior to C and N analysis. The C

and N analysis was conducted by the USDA Forest Service Southern Research Station laboratory (Research Triangle Park, NC) using a Carlo-Erba elemental analyzer (Model NA 1500; Fison Instruments, Danvers, MA).

Post-fertilization measurements of A_{sat} were conducted immediately following a soaking rain within a week after application. Measurements were conducted in the exact same fashion as the pre-fertilization measurements. Initially, measurements were conducted twice a week so that the short-term physiological changes in foliar C and N concentrations and A_{sat} were not missed. However, measurements were taken at less frequent intervals once the effects from the fertilizer began to stabilize. Net photosynthesis measurements ceased in April 2005, one year after treatment initiation, and R_d measurements were taken through most of the growing season until October 2004.

Total Soil N

Total soil N samples were collected to a depth of 10 cm with a 2.5 cm diameter push-tube at the base of the same seedling clones measured for R_d. Soil samples were collected weekly just after fertilization and then lessened to monthly once the total soil N content began to stabilize during the fall and winter. Any small roots were removed from the soil by hand and #10 sieve before being analyzed. Also, the six soil samples were combined into a single sample for each plot. Total soil N was analyzed by dry combustion at 900 ºC using a vario MAX CNS elemental analyzer (elementar Americas, Inc.). Values are reported as % N and were not scaled up to a kg/ha basis because measurements were only taken within the rooting zone of the seedling.

Leaf Area Determination Method

Digital photographs were taken following each P_n sampling period, with an Olympus C2500L camera (Olympus America Inc., Melville, NY) between 1:00 pm and 4:00 pm while the sun was overhead or at a slight angle. Initially, two large red posterboards were taped together to provide a solid and uniform backdrop to set behind the seedlings. As the seedlings grew larger, a red folding plywood backdrop was used to create a uniform background. A small white square measuring 100 cm^2 was taped to the red backdrop to be used as a reference area in subsequent projected leaf area calculations. The pictures were taken from the north side of the seedlings looking south at approximately 0.5 to 1.5 m away from the seedling depending on its height and width. All of the pictures were taken in the same order by block on each sampling date between

June and December 2004. Initially, photographs were taken biweekly in order to track any immediate changes in leaf area and lessened to monthly with the onset of winter.

Adobe Photoshop® 6.0 (Adobe Systems Inc., San Jose, CA) was used to open and analyze each picture stored on the computer hard-drive. Computer analysis involved cleaning out the uniform red background in order to leave nothing but the seedling stem and canopy as well as the small white reference square. The numbers of pixels in the isolated seedling and the reference square were determined separately in Adobe Photoshop. These values were then transferred to a formula in Excel where the pixel counts were converted into an area value (cm^2). This was accomplished by cross-multiplying the two pixel counts with the reference square area of 100 cm^2. A more detailed description of the leaf area determination process can be found in the appendix.

Cumulative Total Carbon and Leaf Area Efficiency Determinations

Several variables were calculated using the data collected in order to establish a link between the physiological and growth responses seen during the study. An estimate of total amount of C fixed by each seedling over the growing period was calculated using the following general formula:

$$A_{sat} \text{ rate} * \text{ILA} * \text{Time} = \text{Cumulative total carbon (g)}$$

where ILA was the index of leaf area estimate and Time was the number of days between each successive A_{sat} measurement. A 12 hour day for photosynthesis was assumed for the A_{sat} rates over time to more accurately represent the conditions seen by the seedlings in the field. These values were all totaled at the end of the of the growing season to provide an estimate of the total C fixed in grams (g) given the specific A_{sat} rates and leaf areas experienced over time for each seedling from June until December 2004.

An estimate of the mean leaf area efficiency of each seedling was calculated using the ILA values and volumes. Each seedlings volume at the end of the growing season was divided by its corresponding ILA value in order to determine how much volume could be produced given a certain leaf area. Thus, the values provided an estimate of which seedlings produced more volume with less leaf area or vice-versa from June until December 2004. Initially, these measurements were taken biweekly and then lessened to monthly with the onset of winter.

Statistical Analysis

The study design was a completely randomized block with a split plot. A fertilized plot and a control plot made up each of four main blocks. The split plot was the eight different loblolly pine clones. The analysis was conducted using the PROC GLM procedure in SAS (SAS Institute, Cary, NC). Analysis of variance was used to determine any significant differences in the sampled variables during and at the end of the growing season. The following general ANOVA table was used:

Factors	*Degrees of Freedom*
Block (B)	3
Fertilization (F)	1
Whole plot error (B x F)	3
Split plot – Clone (C)	7
Clone x Block	21
Clone x Fertilization	7
Split plot error (C x B x F)	21

Also, a time series analysis was conducted to observe the effect of fertilization, time, time x fertilization, clone, clone x fertilization, and time x clone x fertilization on the variables measured over several time periods. The following general ANOVA table was used:

Factors	*Degrees of Freedom*
Block (B)	3
Fertilization (F)	1
Whole plot error (B x F)	3
Split plot – Clone (C)	7
Clone x Block	21
Clone x Fertilization	7
Split plot error (C x B x F)	21
Date (D)	23
Date x Fertilization	23
Date x Clone	161
Split-split plot error (D x F x C)	161

An alpha value of 0.05 was used for all tests except for the mean separation test used on the leaf efficiency variable, which had an alpha level of 0.1. Multiple linear regression analysis was used to determine if there were any significant environmental effects on A_{sat} and R_d. Also, appropriate transformations were used if the variance assumptions were not met in the A_{sat}, R_d, and growth data. For example, a log transformation was used for A_{sat} rates, volumes, and cumulative total carbon.

CHAPTER 4. RESULTS

Physiological Responses

Photosynthesis

A time series analysis of the 24 sample dates showed a significant fertilizer x date interaction ($p=0.0026$) and clone x date interaction ($p<0.0001$) (Table 1). Separate analysis of variance (ANOVA) runs by date showed significantly higher mean A_{sat} rates in the fertilized seedlings on 3 occasions: May 25 ($p=0.034$), June 16 ($p=0.078$), and September 24 ($p=0.064$) (Figure 1). Mean A_{sat} rates between treatments varied initially, but quickly settled into a pattern with the fertilized seedlings having higher A_{sat} rates than the controls for most of the growing season.

Analysis of the mean clonal A_{sat} values by date showed 9 sampling days with significantly different rates ($p\leq0.05$) (Figure 2A, Table 2). Clone 43694 consistently had the highest mean A_{sat} rates throughout most of the study. Also, clones 43671 and 43903 had several dates where they ranked first in mean A_{sat} rate (Figure 2B). Clones 41021, 42725, and 43976 tended to have mid-value A_{sat} rates while clone 41089 generally had the lowest A_{sat} rankings.

Dark Respiration

The time series analysis of R_d revealed no significant differences. Date was the only significant factor in the time series analysis ($p<0.0001$, Table 1). Furthermore, the ANOVA by date showed no significant sampling dates (Figure 3). Both treatments showed a general trend of higher mean R_d rates in the early summer and fall and lower R_d rates during the late summer. Then mean R_d rates began to increase in the control seedlings and fertilized seedlings with the onset of winter.

Growth Variable Responses

Volume, Height, and Basal Diameter

After fertilization, all of the biomass variables sampled increased above the controls and remained that way throughout the rest of the sampling period (Figures 4-6). By the end of the growing season (November 15, 2004) the mean fertilized and unfertilized growth values were: 1396.16 vs. 944.15 cm^3 (volume), 138.2 cm vs. 125.4 cm (height), and 30.1 mm vs. 26.4 mm (basal diameter). Hence, the fertilized seedlings had 48% more volume growth, 10% more height growth, and 14% more basal diameter growth than the controls.

Table 1. The partial ANOVAs from the time series analysis and P values for A_{sat}, ILA, foliar N, leaf area efficiency, cumulative total carbon, and volume across both treatments sampled from May 2004 until April 2005 excluding the two pre-fertilization dates.

Source	df	F value	P value	Source	df	F value	P value
Light-saturated net photosynthesis				*Dark Respiration*			
Fert	1	7.06	0.0766	Fert	1	0.53	0.5178
Clone	7	3.27	0.0076	Clone	5	1.77	0.15
Clone*Fert	7	1.87	0.10	Clone*Fert	5	2.02	0.11
Date	23	463.83	<0.0001	Date	4	71.23	<0.0001
Date*Fert	23	2.05	0.0026	Date*Fert	4	1.26	0.288
Date*Clone	161	1.60	<0.0001	Date*Clone	20	0.63	0.8851
Date*Fert*Clone	161	0.71	0.996	Date*Fert*Clone	20	0.77	0.7475
Foliar N				*Index of Leaf Area*			
Fert	1	4.96	0.112	Fert	1	2.18	0.235
Clone	5	2.48	0.032	Clone	7	3.1	0.01
Clone*Fert	5	1.26	0.29	Clone*Fert	7	2.14	0.06
Date	19	26.54	<0.0001	Date	11	182.28	<0.0001
Date*Fert	19	1.47	0.089	Date*Fert	11	6.92	<0.0001
Date*Clone	133	0.83	0.912	Date*Clone	77	1.74	0.0003
Date*Fert*Clone	133	0.68	0.9968	Date*Fert*Clone	77	1.19	0.139
Volume				*Leaf Area Efficiency*			
Fert	1	0.55	0.512	Fert	1	0.04	0.85
Clone	7	1.5	0.19	Clone	7	2.49	0.032
Clone*Fert	7	0.5	0.83	Clone*Fert	7	0.53	0.80
Date	21	1524.83	<0.0001	Date	11	133.95	<0.0001
Date*Fert	21	9.83	<0.0001	Date*Fert	11	2.06	0.022
Date*Clone	147	1.63	<0.0001	Date*Clone	77	0.85	0.814
Date*Fert*Clone	147	1.5	0.0003	Date*Fert*Clone	77	0.97	0.562
Cumulative Total C							
Fert	1	0.68	0.47				
Clone	7	1.86	0.10				
Clone*Fert	7	1.09	0.388				
Date	11	5616.77	<0.0001				
Date*Fert	11	13.23	<0.0001				
Date*Clone	77	1.61	0.0016				
Date*Fert*Clone	77	0.66	0.9872				

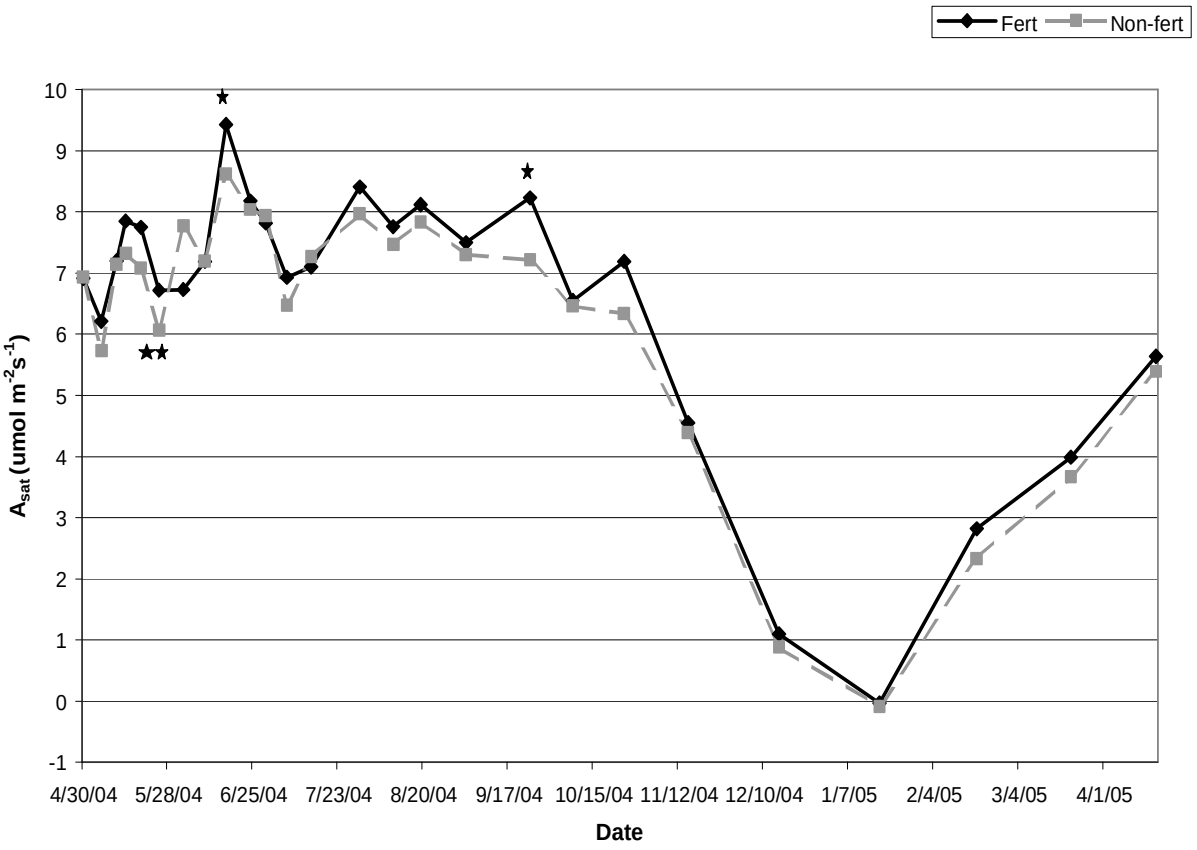
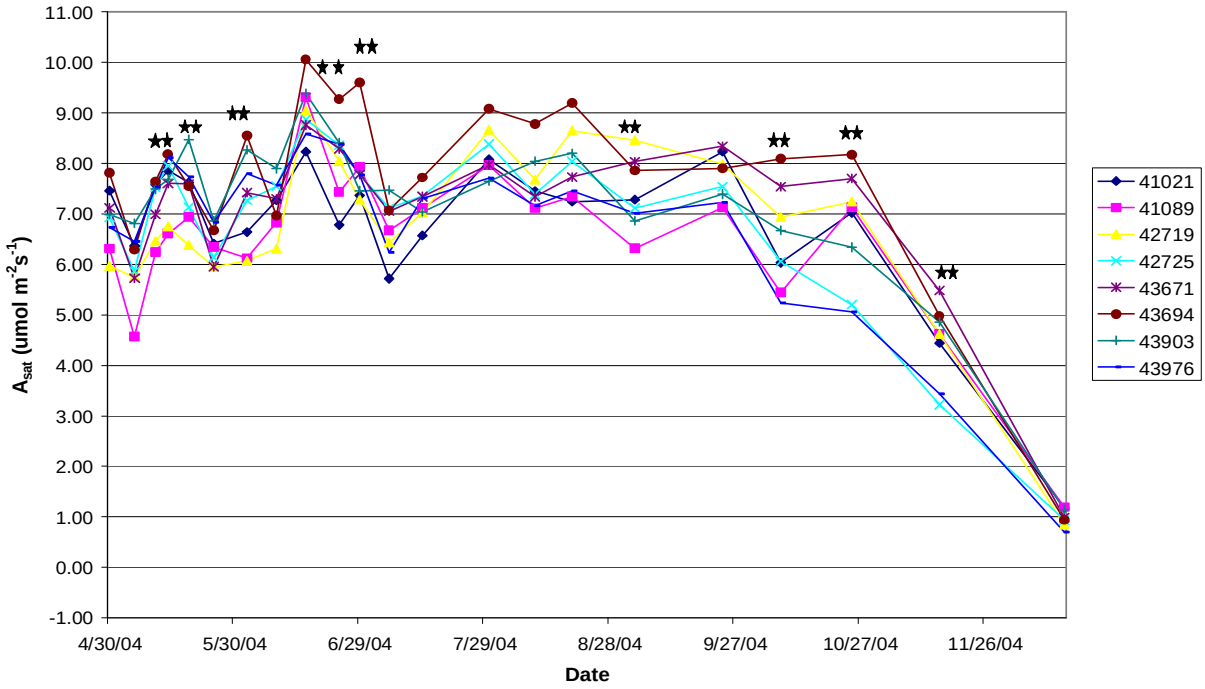


Figure 1. Mean A_{sat} rates (μmol $m^{-2}s^{-1}$) for fertilized and unfertilized seedlings from April 2004 until April 2005 sampled in a 2-year-old loblolly pine plantation in the Piedmont of Virginia. Two stars denote significance at an alpha level of ≤0.05 and one star denotes significance at an alpha level of ≤0.1.

A



B

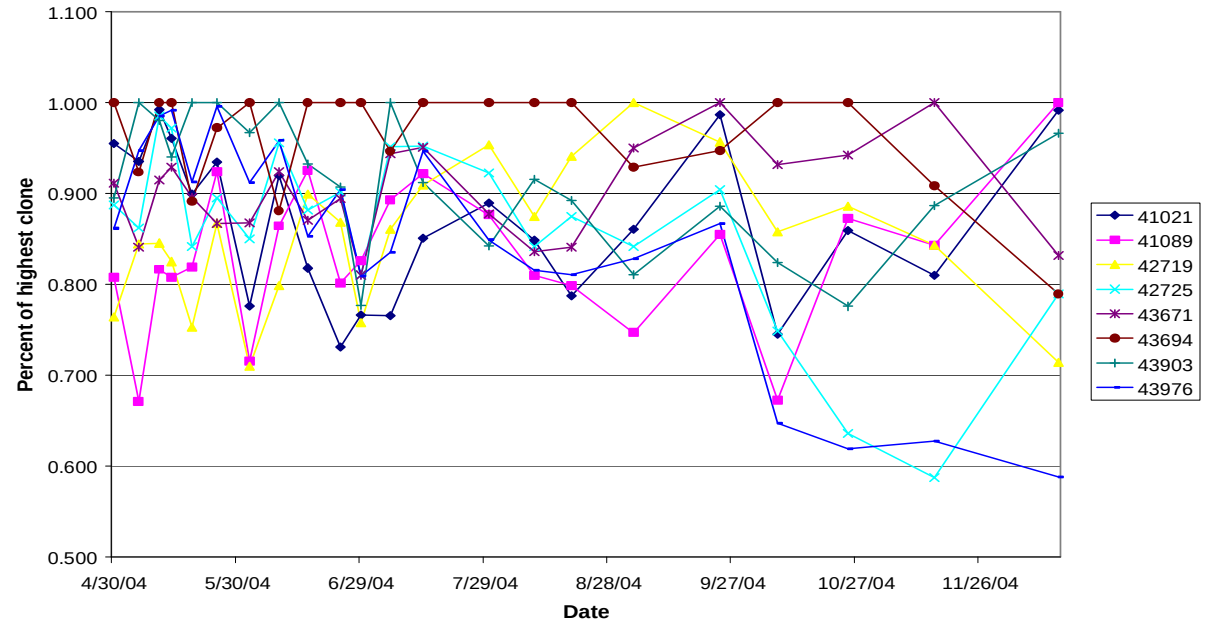


Figure 2. The mean clonal A_{sat} rates ($\mu mol\ m^{-2}s^{-1}$) by date (A) and the mean clonal A_{sat} percent rankings by date (B) during the growing season from April 2004 until December 2005. Two stars denote significance at an alpha level of ≤0.05 and one star denotes significance at an alpha level of ≤0.1.

Table 2. Clonal A_{sat} mean separation results for the significant dates shown in Figure 2A based on a Duncans MRT with an alpha level of 0.05.

	5/14/04		5/19/04		6/2/04		6/24/04		6/29/04	
41021	7.86	C	7.62	AB	6.64	BC	6.78	C	7.36	B
41089	6.61	A	6.94	BC	6.12	C	7.43	BC	7.93	B
42719	6.75	BC	6.38	C	6.07	C	8.05	ABC	7.28	B
42725	7.95	AB	7.13	AB	7.27	ABC	8.36	AB	7.78	B
43671	7.60	ABC	7.59	AB	7.42	ABC	8.29	AB	7.78	B
43694	8.18	A	7.55	AB	8.55	A	9.27	A	9.60	A
43903	7.69	ABC	8.47	A	8.27	A	8.41	AB	7.46	B
43976	8.11	A	7.73	AB	7.80	AB	8.38	AB	7.77	B
	9/3/04		10/8/04		10/25/04		11/15/04			
41021	7.28	ABC	6.03	CD	7.02	AB	4.44	B		
41089	6.32	C	5.44	D	7.13	ABC	4.62	AB		
42719	8.46	A	6.94	ABC	7.24	AB	4.62	AB		
42725	7.12	BC	6.06	CD	5.20	C	3.22	C		
43671	8.04	AB	7.54	AB	7.70	AB	5.48	A		
43694	7.86	AB	8.09	A	8.17	A	4.98	AB		
43903	6.86	BC	6.67	BC	6.34	BC	4.86	AB		
43976	7.01	BC	5.24	D	5.06	C	3.44	C		

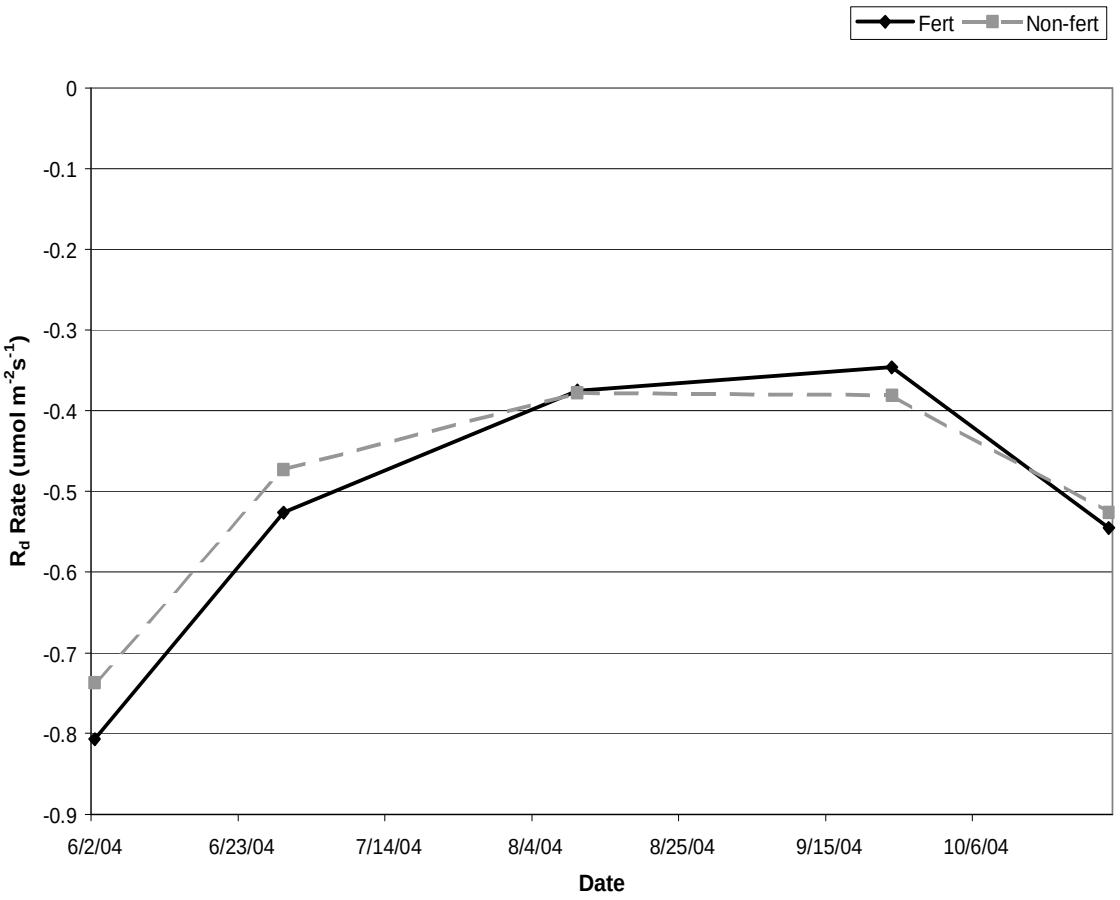


Figure 3. Monthly mean clonal R_d rates (µmol $m^{-2}s^{-1}$) by treatment from June until October 2004. There were no significant differences between fertility treatments on any individual dates.

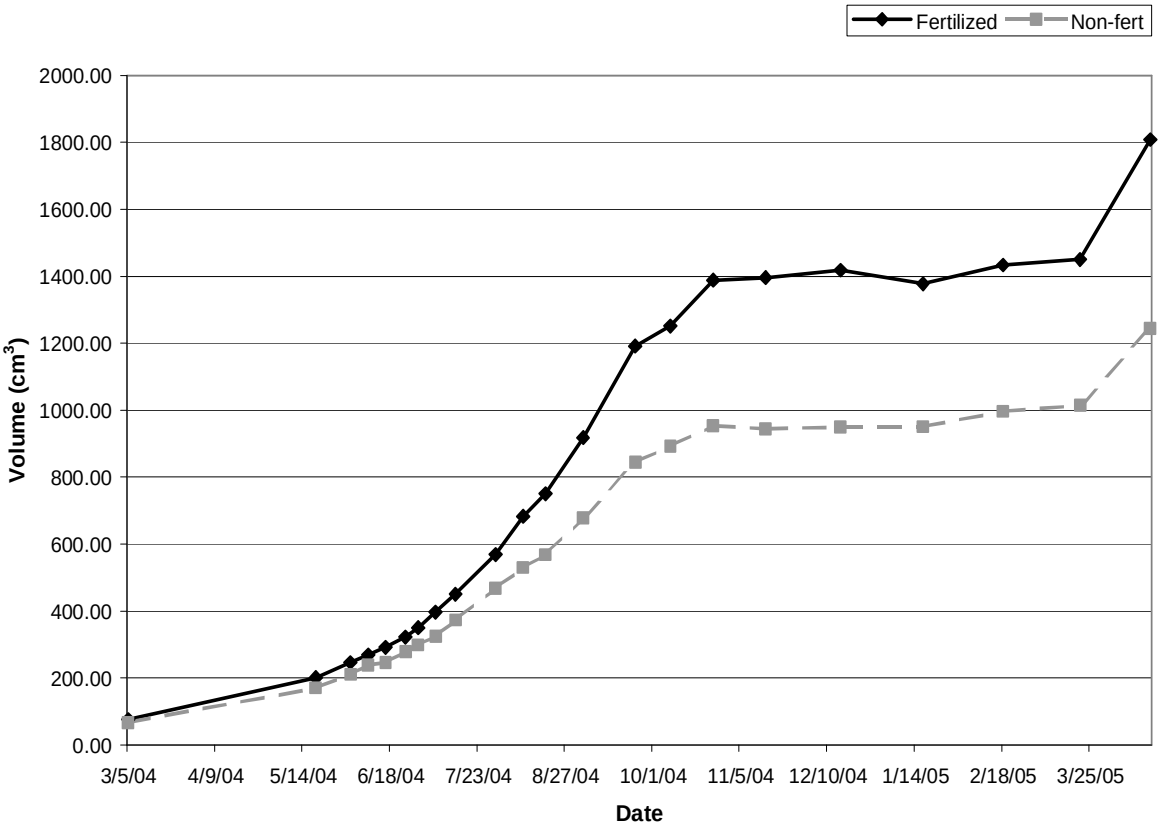


Figure 4. Mean volumes (cm^3) by treatment from March 2004 until April 2005. There were no significant differences between fertility treatments on any individual dates.

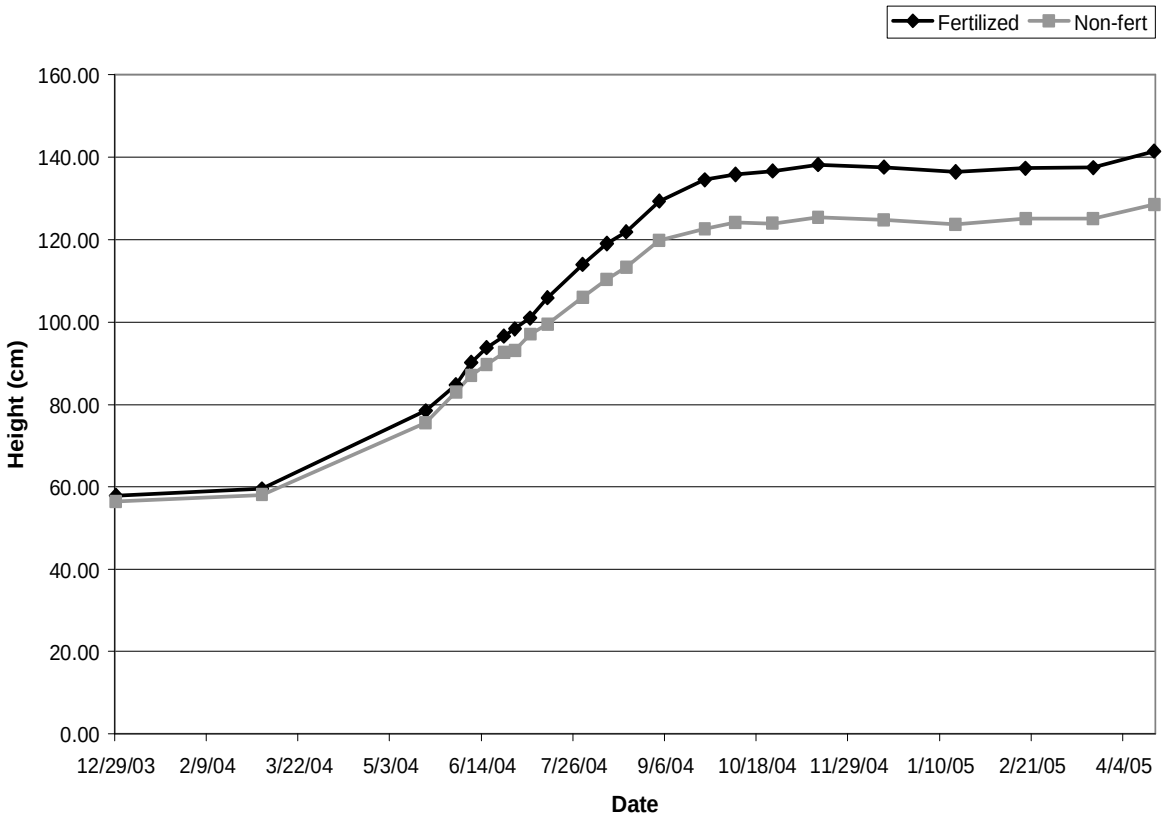


Figure 5. Mean heights (cm) for fertilized and unfertilized seedlings from December 2003 until April 2005. There were no significant differences between fertility treatments on any individual dates.

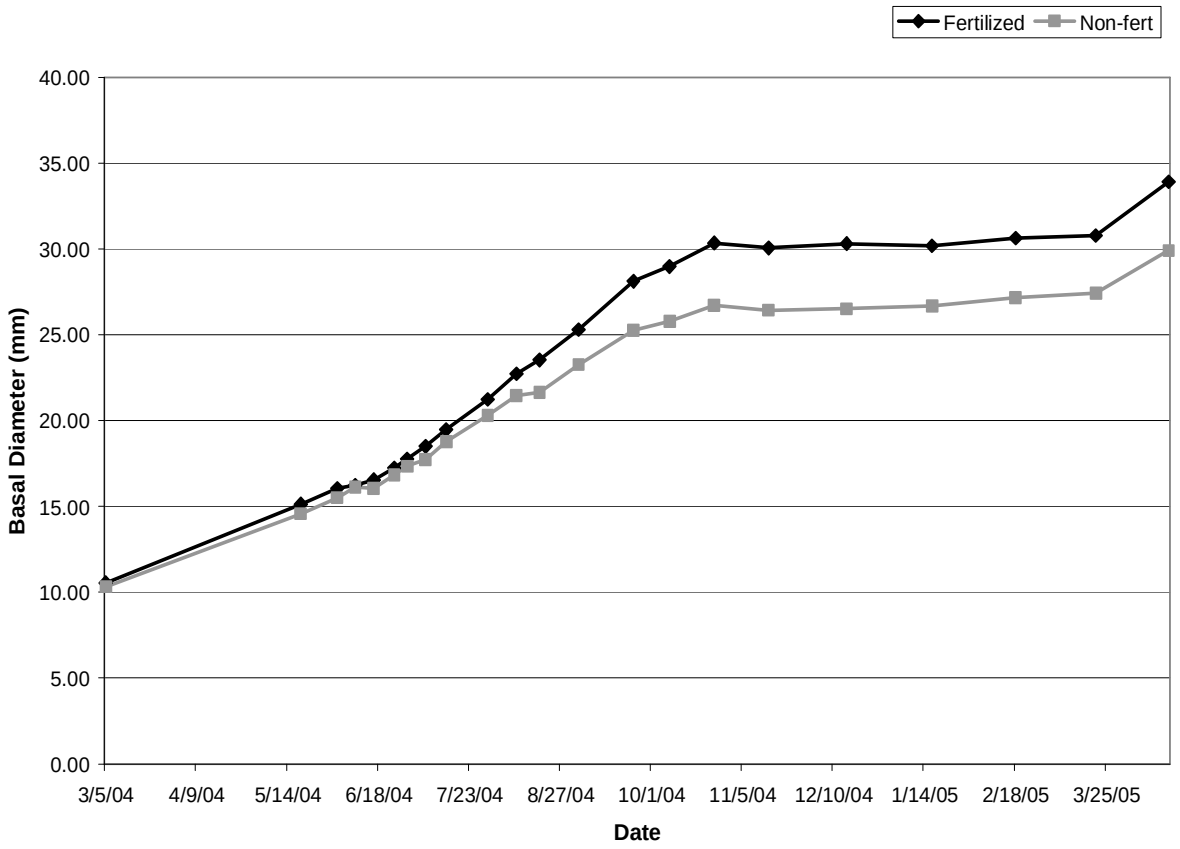


Figure 6. Mean basal diameter (mm) for fertilized and unfertilized seedlings from April 2004 until April 2005. There were no significant differences between fertility treatments on any individual dates.

A time series analysis of volume showed a significant date x fertilization x clone interaction ($p=0.0003$) (Table 1). When volume growth was displayed over time for each clone and fertilizer treatment it was clear that not all clones responded to fertilization by the same degree (Figure 7A-B). For example, clone 42725 was the smallest clone at the end of the growing season in control plots; however, when fertilized, it showed a dramatic (154%) increase in growth. Clones 43694 and 43903 also responded well to fertilization. In contrast, clones 41021, 41089, 42719, and 43671 responded little or not at all to fertilization.

Physiological and Growth Responses

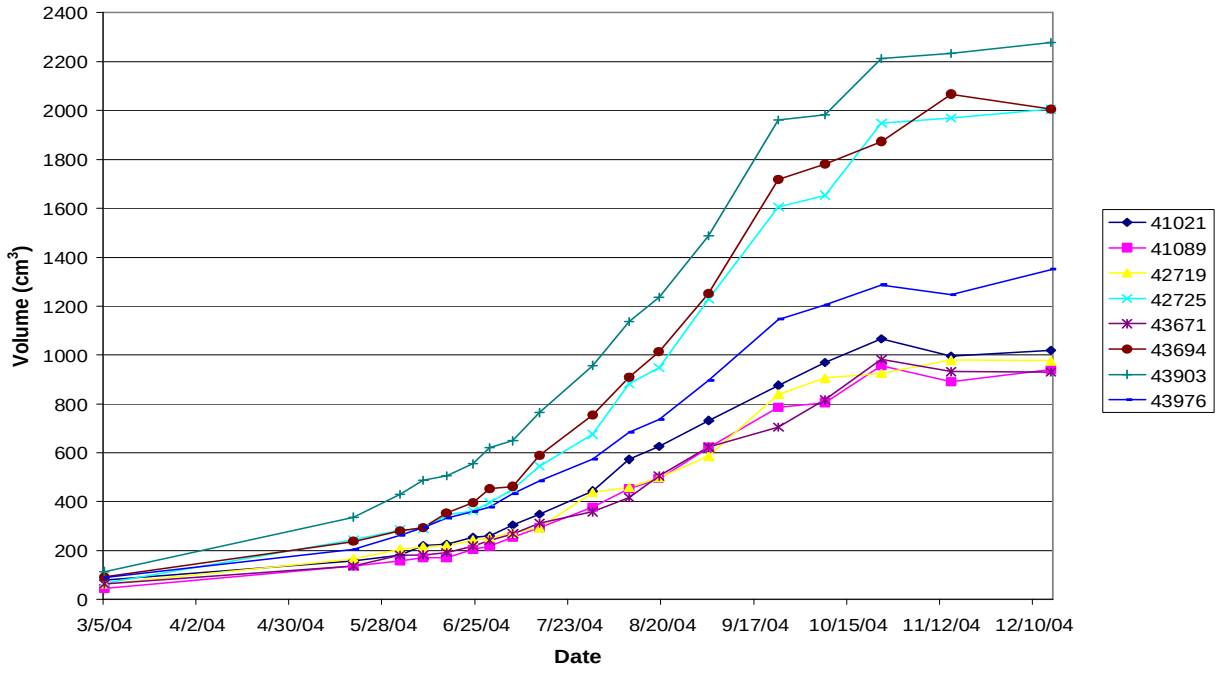
Foliage Response

Needle N content had a slightly significant fertilizer x date interaction when examined in a time series analysis ($p=0.09$). The mean foliar N% for the fertilized seedlings was 1.67% while the control mean was 1.49%. The maximum and minimum values were nearly the same for both treatments with most days being above the critical value of 1.2% for needle N content. The individual ANOVA's by date showed four dates where foliar N was significantly higher ($p\leq0.05$) in the fertilized seedings and three dates where it was slightly higher ($p\leq0.1>0.05$). Overall, the fertilized seedlings foliar N contents were higher than the controls on all but one sampling date (Figure 8). The time series analysis also showed a significant clonal effect on N% ($p=0.03$). Further investigation showed clone 43694 had a significantly higher foliar N content than any of the other clones (Figure 9). Furthermore, clone 43694 had the highest mean N content over most of the growing season while the other seven clones remained tightly bunched at slightly lower N levels.

Soil N data collected in conjunction with another study showed mean fertilized soil N content was mostly below the control levels except for approximately 25 days just after fertilization (Figure 10). In fact, mean control soil N levels were significantly higher ($p<0.05$) than the fertilized levels on two dates (October 25, March 21) and slightly significantly higher ($p=0.06$) on one date (August 19). Despite this fact, foliar N levels were almost always higher in the fertilized seedlings than the controls during this study. However, a multiple linear regression of the growing season A_{sat} rates and foliar N levels showed a small, but significant correlation ($R^2= 0.09$, $p<0.0001$) over the measurement period (Figure 11).

Multiple linear regression analysis was used to elucidate the relationship between the growing season mean clonal A_{sat} rates and the end of the year volumes. The correlation was

A



B

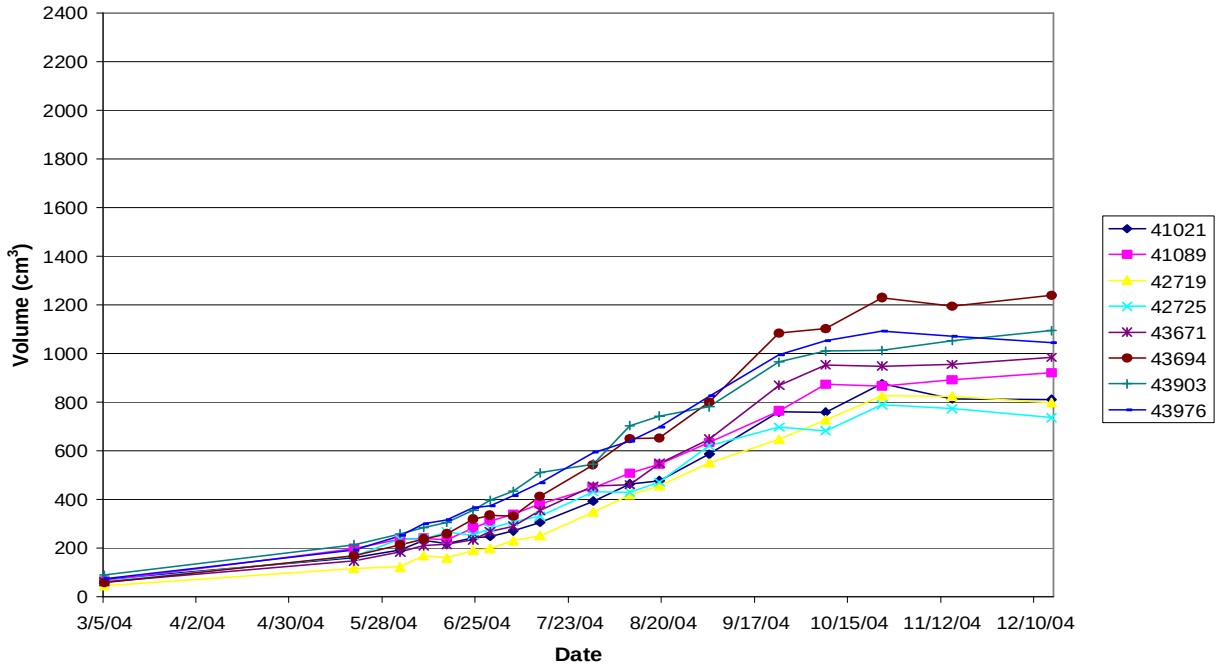


Figure 7. Mean clonal volumes (cm^3) for fertilized (A) and non-fertilized (B) seedlings from April 2004 until December 2004.

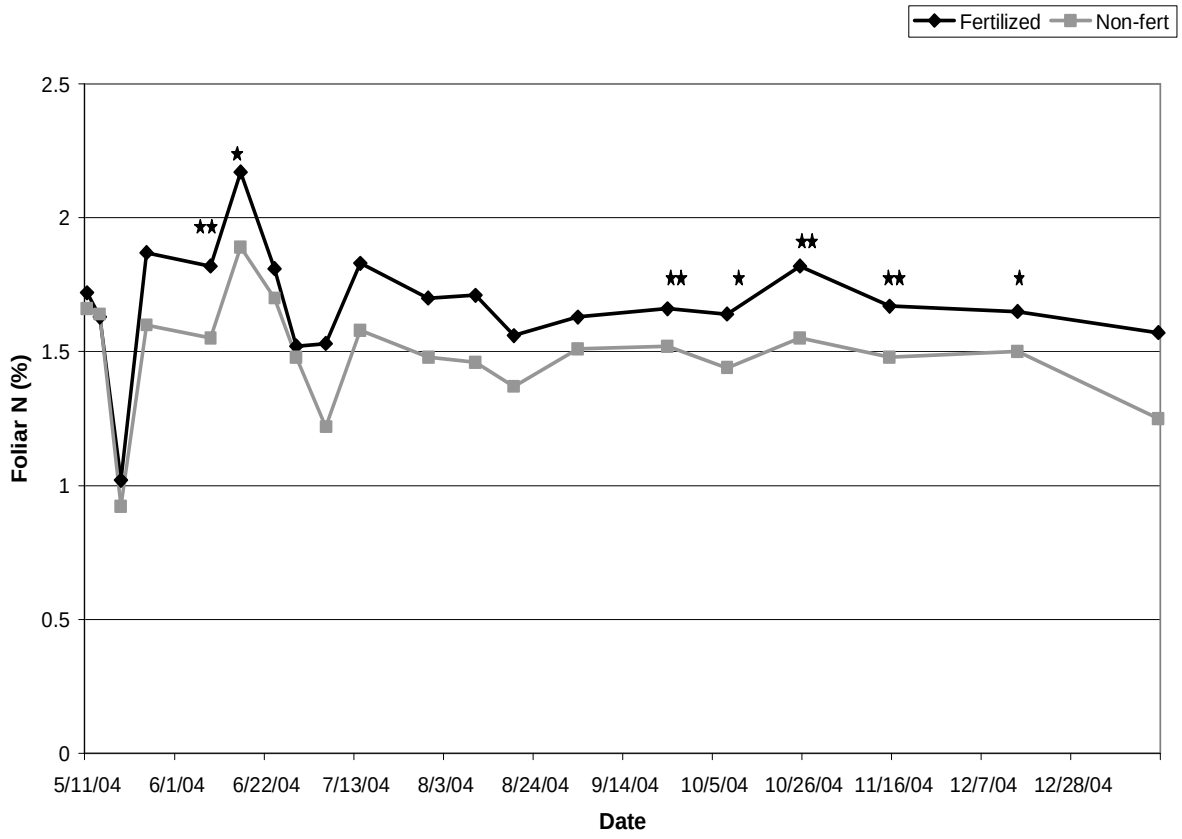


Figure 8. Foliar N % by treatment from May 2004 until December 2004. Two stars denote significance at an alpha level of ≤0.05 and one star denotes significance at an alpha level of ≤0.1.

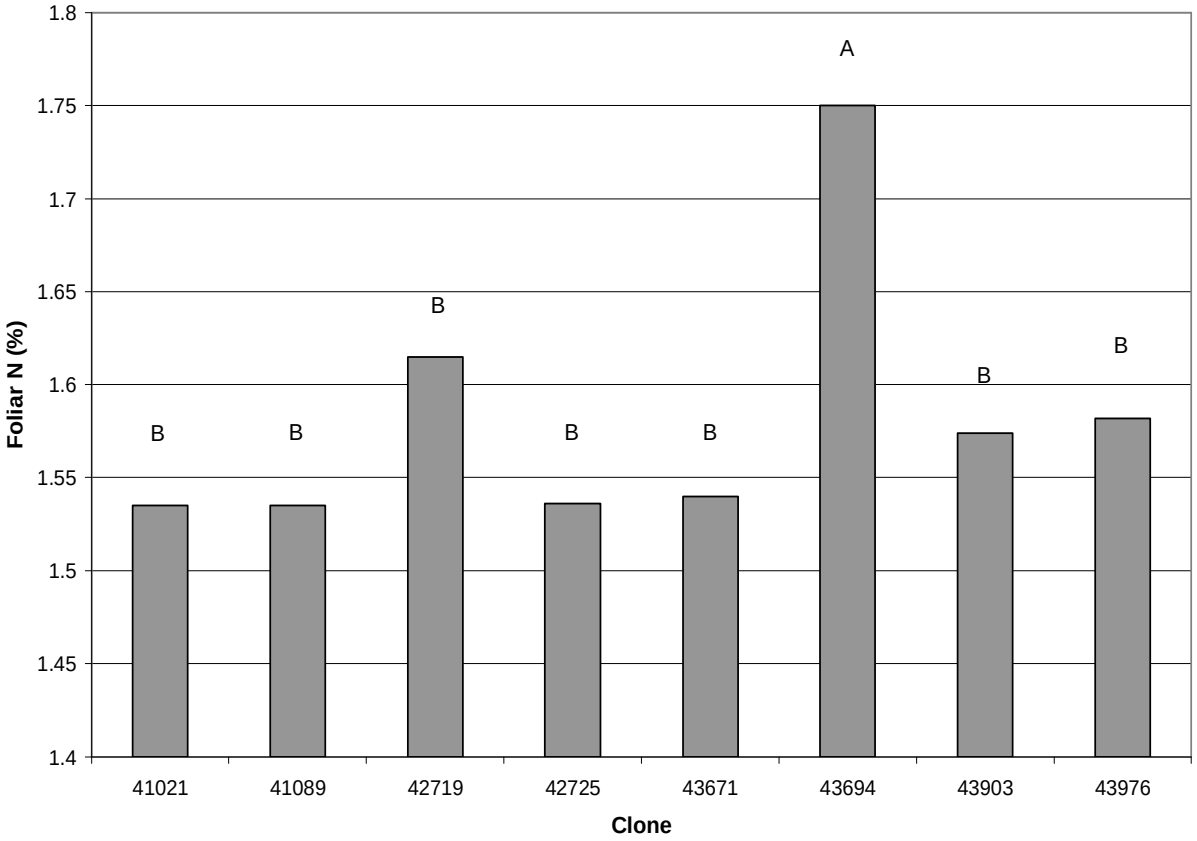


Figure 9. Mean clonal foliar N % over the sampling period. Different letters denote significant differences at the 0.05 alpha level.

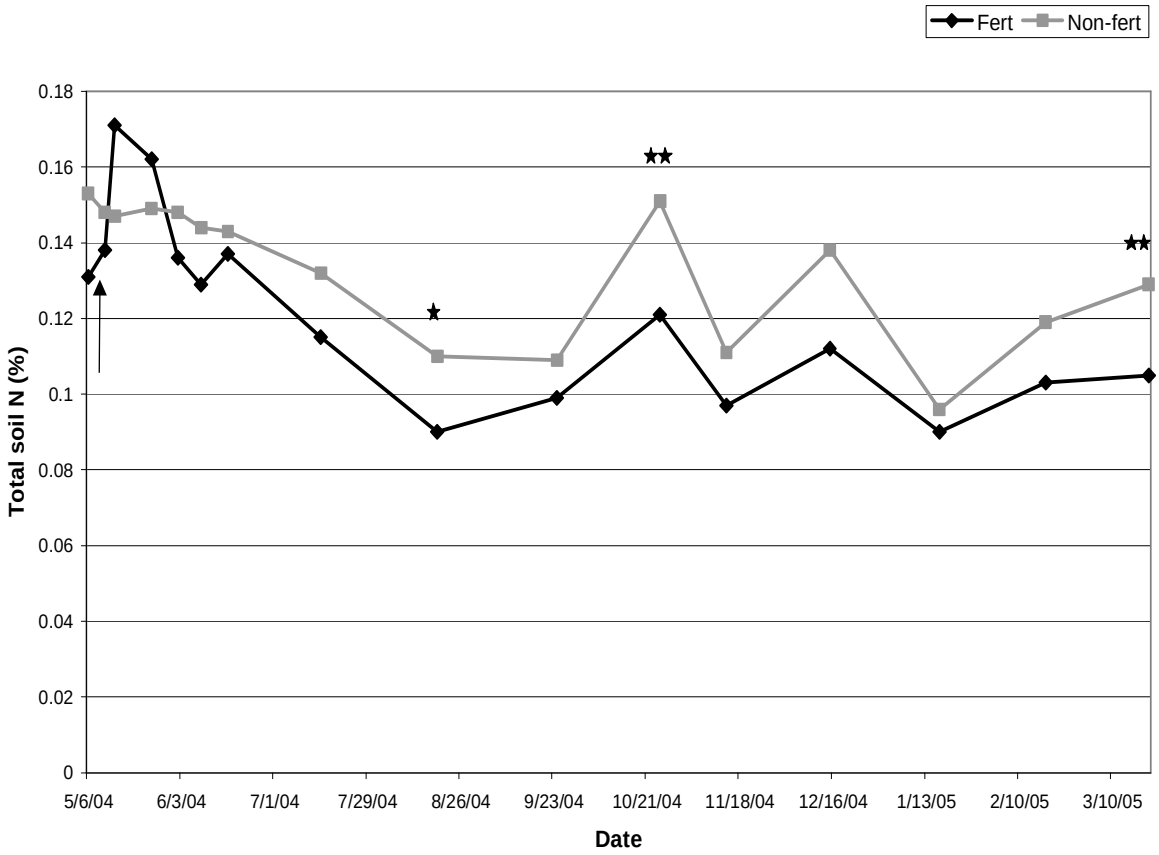


Figure 10. Mean total soil N content (%) for fertilized and control seedlings by date from May 2004 until April 2005. Two stars denote significance at an alpha level of ≤0.05 and one star denotes significance at an alpha level of ≤0.1. A black arrow indicates when fertilization occurred in early May.

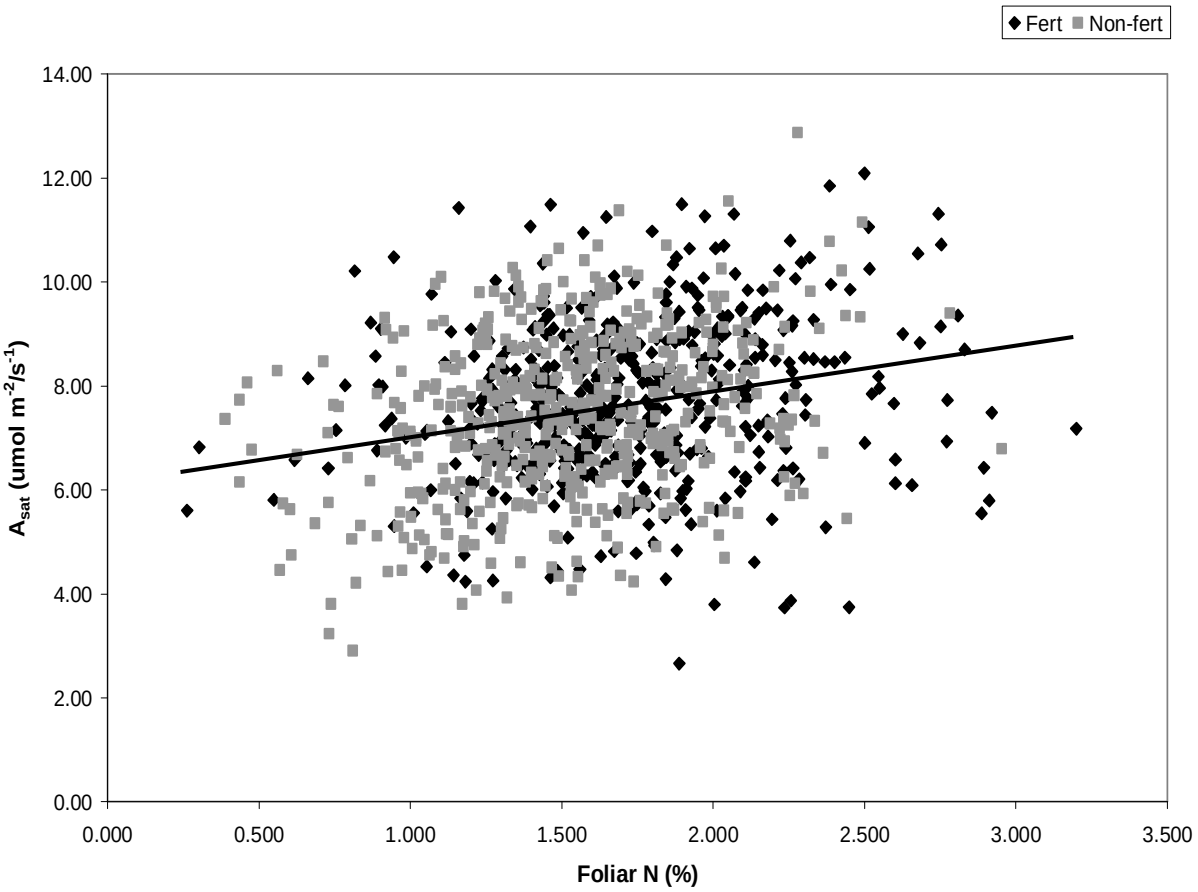


Figure 11. A scatterplot of the relationship between A_{sat} and foliar N for fertilized and unfertilized seedlings for most of the growing season (May 2004 until September 2004).

significant (p=0.0088) with A_{sat} rates explaining 40% (R^2=0.3975) of the variance in volumes. The mean clonal responses were variable with some clones showing a large response in both variables while others showed only a volume response, A_{sat} response, or neither (Figure 12). For example, clones 43694, and 43903 showed relatively large fertilization responses in A_{sat} and volume. These clones were largely responsible for the overall trend seen in Figure 12. However, clones 42725, 41021 and 43976 only showed a volume response while clone 41089 only showed an A_{sat} response. Furthermore, clone 43671 basically showed no response to fertilization in either variable while clone 42719 showed a large negative A_{sat} response to fertilization.

At the end of the growing season, the mean ILA was 30.5% larger in the fertilized seedlings. This ILA was not meant to provide exact leaf area measurements, but was used to compare the projected leaf areas between seedlings in the study. The fertilized seedlings mean index of leaf area was 0.684 m^2 and the control mean ILA was 0.524 m^2. A time series analysis showed a significant fertilization x date interaction (p<0.0001) and a significant clone x date interaction (p=0.0003). The fertilization x date interaction is evidenced by the differing slopes between treatments in Figure 13. The fertilized seedlings had a slightly higher slope leading to higher mean ILAs on each sampling date. Analysis of variance by date showed that at their peak on the October 25 sampling date there was a slightly significant difference (p=0.1) in the mean fertilized seedling ILAs and the controls. At the end of the growing season there was a significant fertilization x clone interaction (p=0.04). Further inspection revealed clone 42725 had a highly significant leaf area response due to fertilization (p=0.02) (Figure 14). The mean ILA for the fertilized 42725 clones was 1.08 m^2 while the control mean was 0.63 m^2 smaller at 0.45 m^2. A comparison of the fertilizer response between the other clones yielded no significant results. However, clones 43903, 43694, and 41021 had comparatively larger fertilized responses than clones 41089, 42719, 43671, and 43976, which had little or no response over the growing season.

Analysis of variance by date showed there were significant differences between at least two clones on all but the first sampling date (Figure 15, Table 3). At the end of the growing season, (November 15, 2004) clone 43903 had a significantly larger mean index of leaf area than 43671, 42719, 41089, and 41021, which had the lowest mean indexes of leaf area (Table 3). Clones 43694 and 43976 had mediocre mean indexes of leaf area and were not significantly

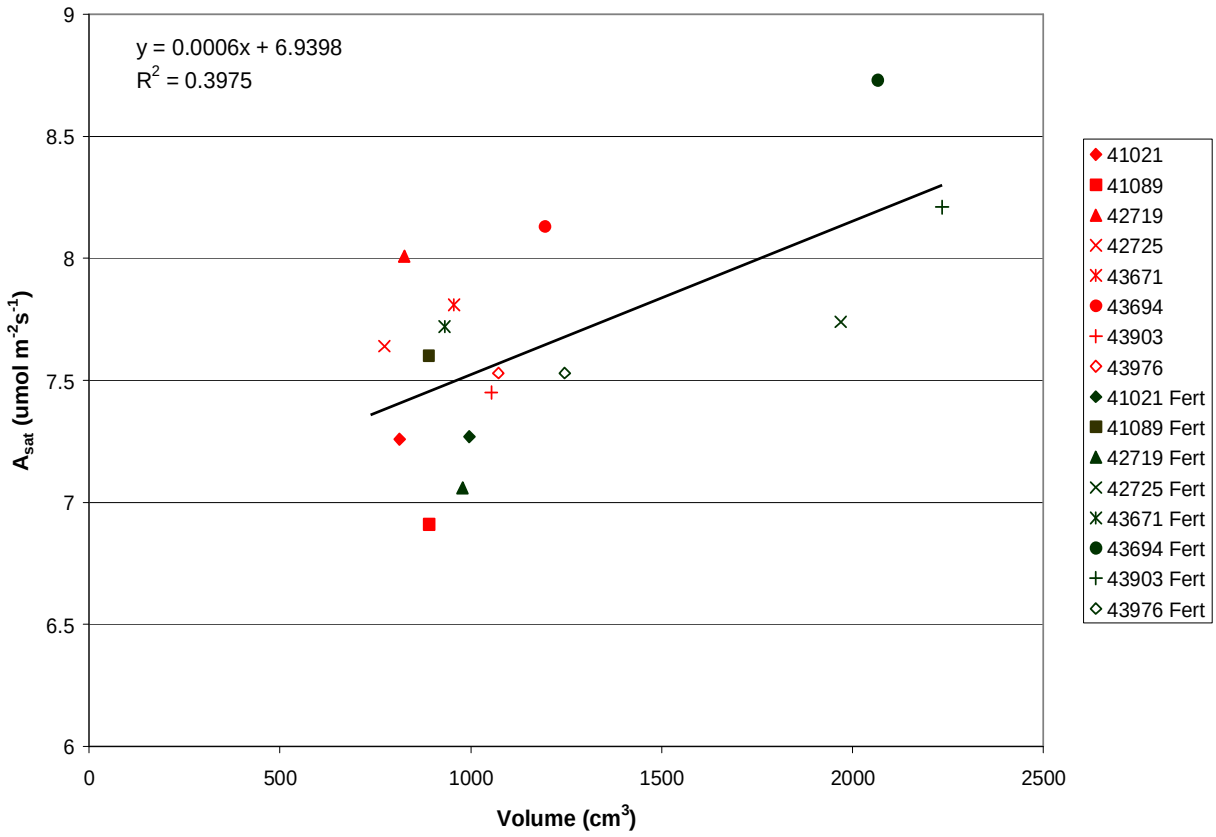


Figure 12. Multiple linear regression analysis of the mean clonal A_{sat} rates (µmol $m^{-2}s^{-1}$) during the growing season and the end of the year mean clonal volumes (cm^3).

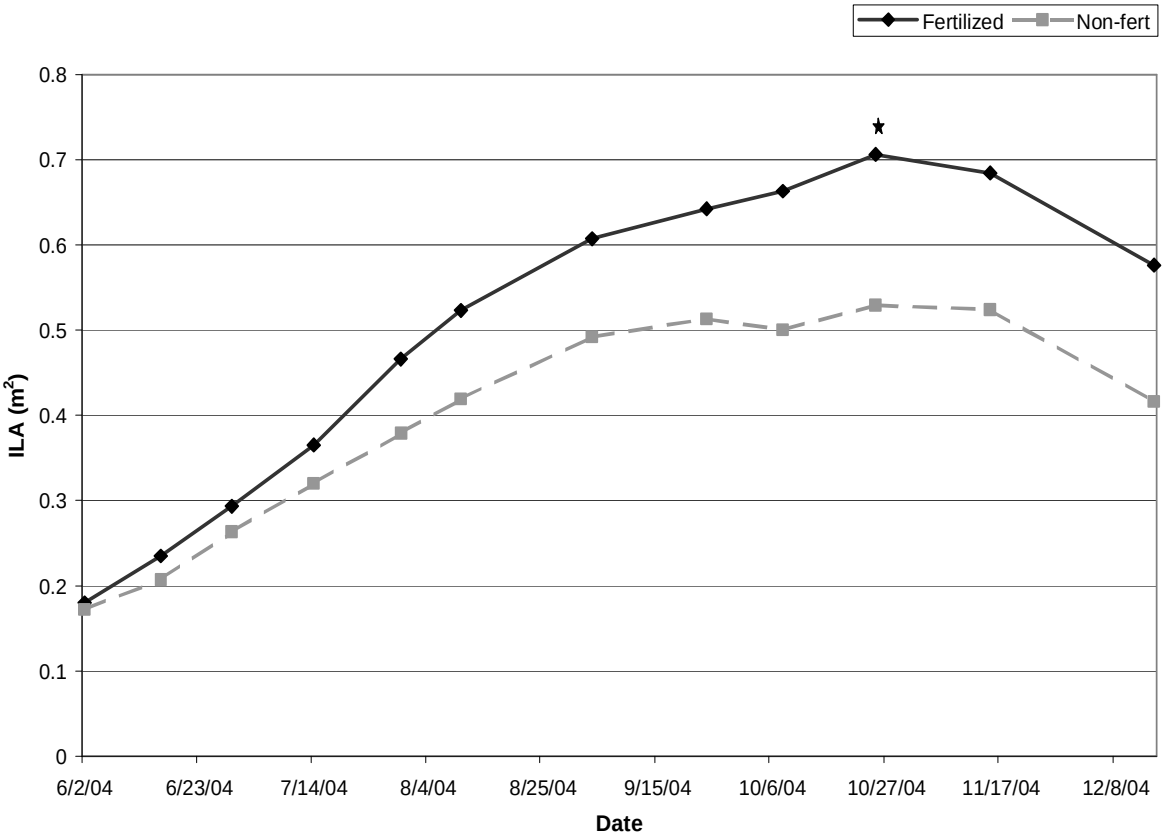


Figure 13. Mean ILA by treatment from June 2004 until December 2004. Two stars denote significance at an alpha level of ≤0.05 and one star denotes significance at an alpha level of ≤0.1.

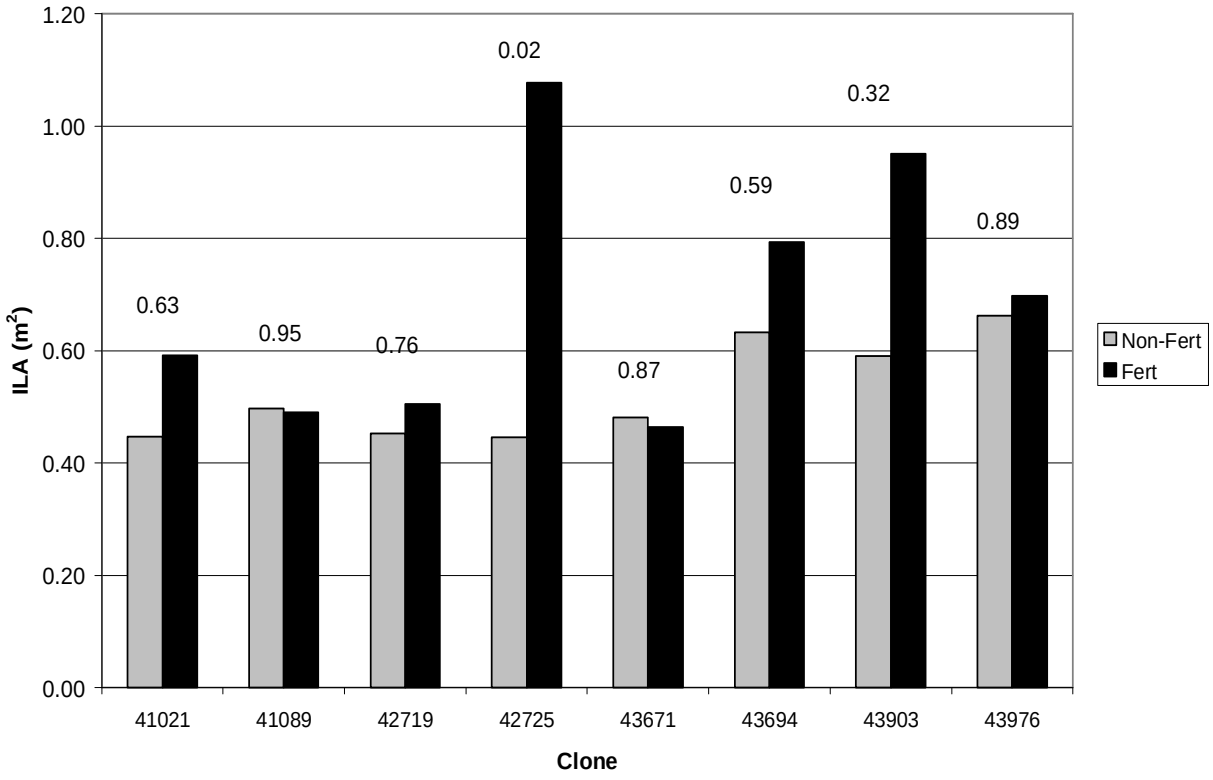


Figure 14. Mean clonal ILA for fertilized and unfertilized seedlings at the end of the year (Nov. 15, 2004).

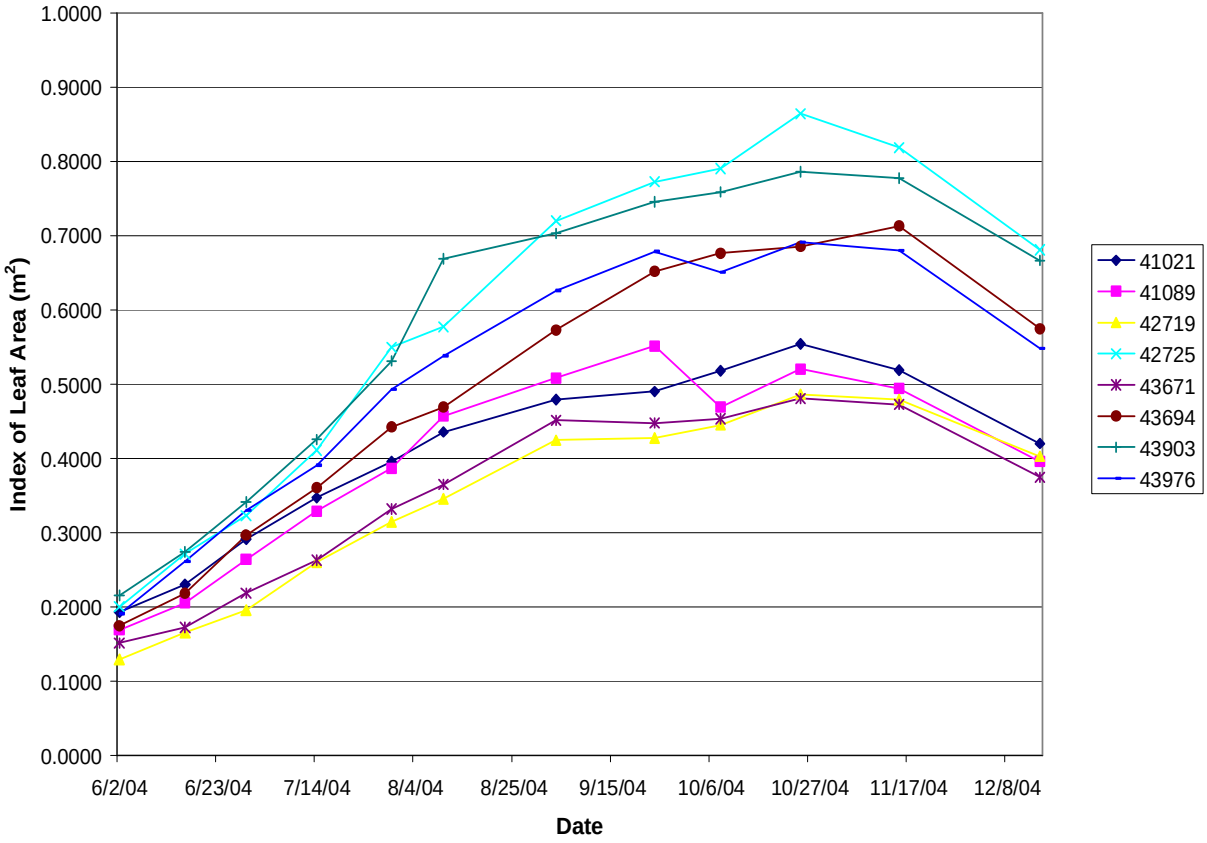


Figure 15. Mean clonal ILA values by date from June 2004 until December 2004. All dates were significantly different at the 0.05 alpha level except for the first sampling date (June 2, 2004).

Table 3. Mean separation of clones on significant index of leaf area dates from June until December 2004. Different letters denote significance at the 0.05 alpha level.

	6/16/04		6/29/04		7/14/04		7/30/04		8/10/04		9/3/04	
41021	0.230	AB	0.291	AB	0.347	AB	0.396	ABC	0.436	BC	0.479	BC
41089	0.205	AB	0.264	AB	0.329	AB	0.387	BC	0.457	BC	0.508	ABC
42719	0.165	B	0.196	B	0.260	B	0.315	C	0.346	C	0.425	C
42725	0.271	A	0.323	AB	0.411	AB	0.550	AB	0.577	ABC	0.720	AB
43671	0.172	B	0.219	B	0.263	B	0.332	C	0.365	BC	0.451	BC
43694	0.218	AB	0.297	AB	0.361	AB	0.442	ABC	0.469	BC	0.573	ABC
43903	0.274	A	0.341	A	0.426	A	0.531	A	0.669	A	0.703	A
43976	0.261	A	0.330	A	0.391	A	0.493	AB	0.538	AB	0.626	ABC
	9/24/04		10/8/04		10/25/04		11/15/04		12/15/04			
41021	0.490	B	0.518	B	0.554	ABC	0.519	B	0.420	BC		
41089	0.552	B	0.469	B	0.520	BC	0.494	B	0.396	C		
42719	0.428	B	0.445	B	0.487	C	0.479	B	0.403	BC		
42725	0.773	AB	0.790	AB	0.864	AB	0.818	AB	0.681	AB		
43671	0.448	B	0.453	B	0.481	C	0.473	B	0.375	C		
43694	0.652	AB	0.676	AB	0.685	AB	0.713	AB	0.574	ABC		
43903	0.745	A	0.759	A	0.786	A	0.777	A	0.667	A		
43976	0.678	AB	0.651	AB	0.691	ABC	0.680	AB	0.548	ABC		

different from any of the clones. Clone 42725 had a mean index of leaf area that was also not significantly different from any other clones although it had one of the largest mean ILA values. However, this clone had the largest fertilizer response of any clone, which contributed to a wide standard error.

Cumulative Total Carbon

An estimate of the total amount of carbon generated from the various A_{sat} rates and leaf areas over time was calculated for each seedling on the sampling dates when the data was available. Thus, an index of the cumulative total carbon gained by each loblolly pine clone over the growing season was created to integrate the physiological and growth data. A time series analysis showed significant fertilizer x date and clone x date interactions ($p<0.0001$, $p=0.00016$) (Table 1). In a similar pattern to the growth data, the fertilized seedlings mean cumulative total carbon amounts were greater than the controls on most sampling dates (Figure 16). The fertilized seedlings mean cumulative total carbon amounts continued to grow at a slightly higher rate than the control mean with each passing date contributing to the significant fertilizer x date interaction. There was no significant fertilizer effect, clone effect, or fertilizer x clone interaction when the ANOVAs were examined by date although the fertilizer effect was approaching slight significance by the end of the growing season ($p=0.18$).

The end of the growing season fertilizer response by clone graph revealed a very similar pattern to volume and ILA with clone 42725 having a significantly larger fertilizer response than any other clone ($p=0.05$) (Figure 17). In fact, the fertilized mean cumulative total carbon amount clones was 129% larger than that of the control. Although statistically not significant, clones 41021, 42976, 43903, and 43694 had relatively larger fertilized mean cumulative total carbon amounts than the non-fertilized controls. Clones 41089, 42719, and 43671 showed little response or even a slight negative response to fertilization in the case of clone 41089.

A mean separation test showed two early dates (June 6 and 29) with significant clonal differences ($p\leq0.05$) (Figure 18, Table 4). However, these significant clonal differences faded by late summer and were not present at the end of the growing season.

Leaf Area Efficiency

An estimate of leaf area efficiency was created to elucidate the ability of each clone to generate extra growth (volume) based on their corresponding projected leaf areas. Hence, an estimate of leaf area efficiency was created to see if any clones were able to accumulate more

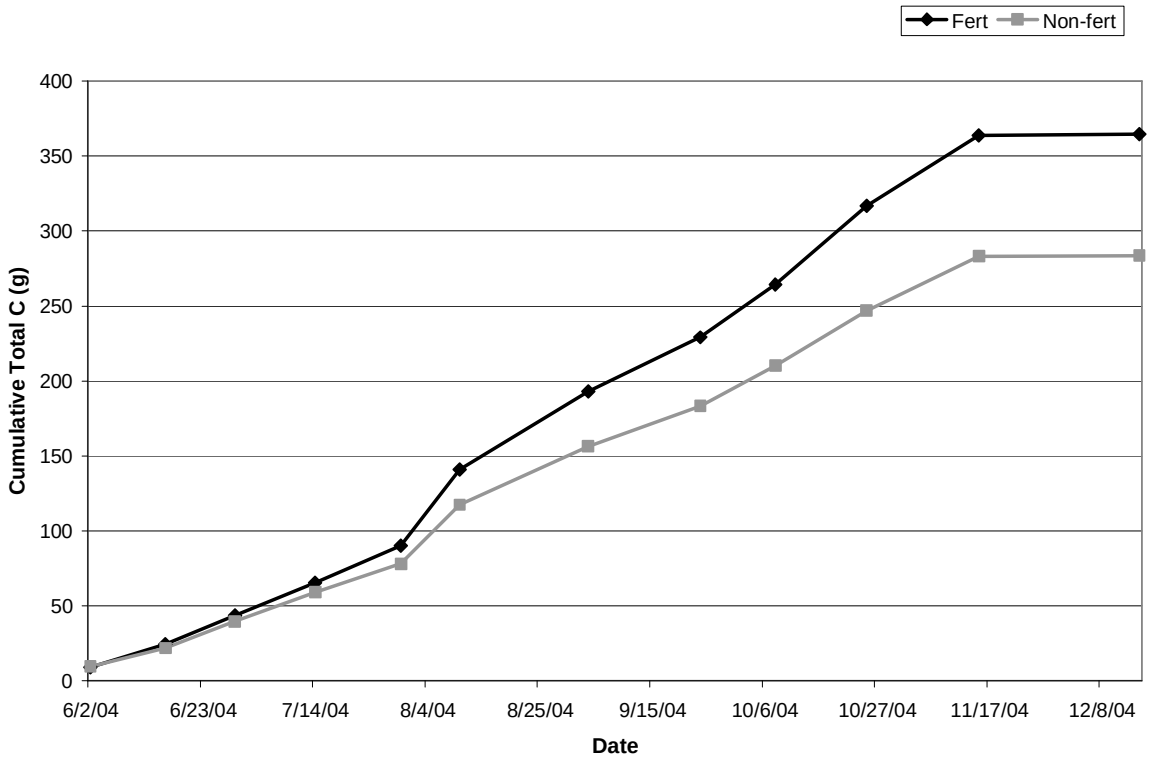


Figure 16. Mean cumulative total carbon amounts (g) for fertilized and unfertilized seedlings from June 2004 until December 2004. There were no significant differences between fertility treatments on any individual dates.

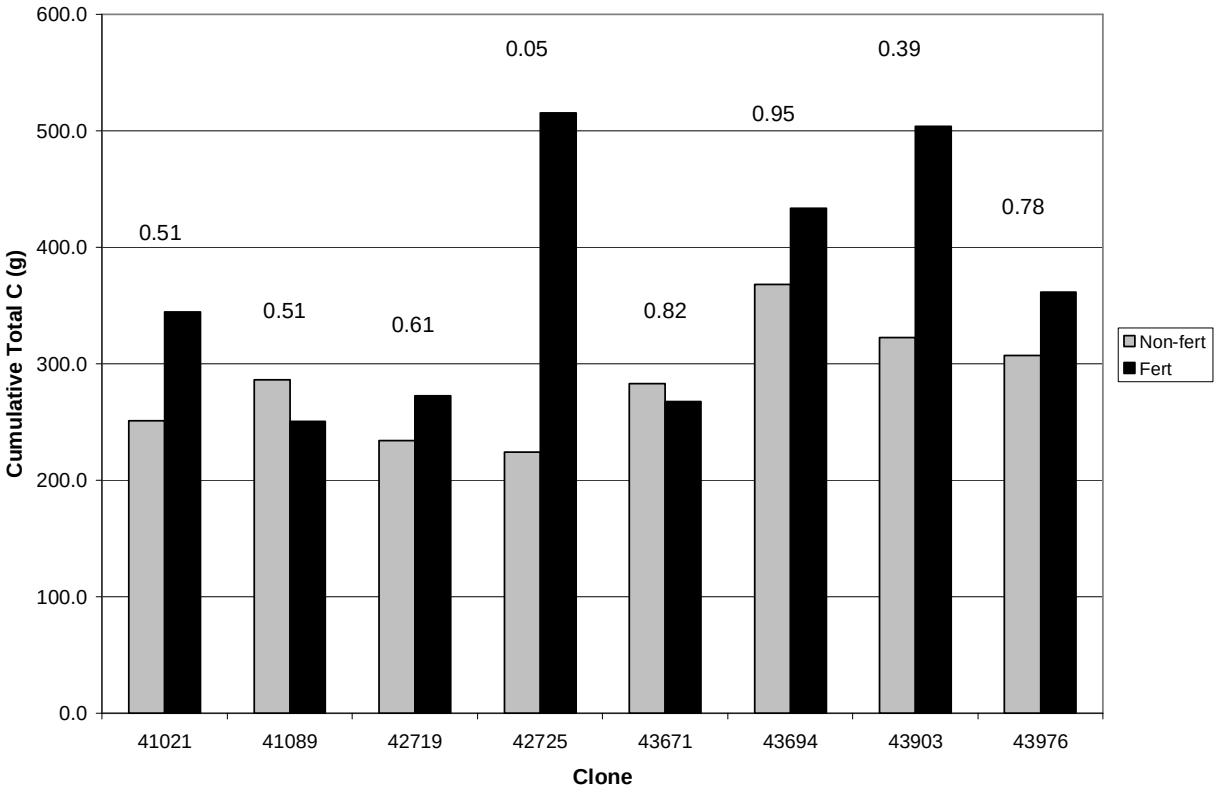


Figure 17. Mean clonal cumulative total carbon amounts (g) for fertilized and unfertilized seedlings at the end of the year (Nov. 15, 2004).

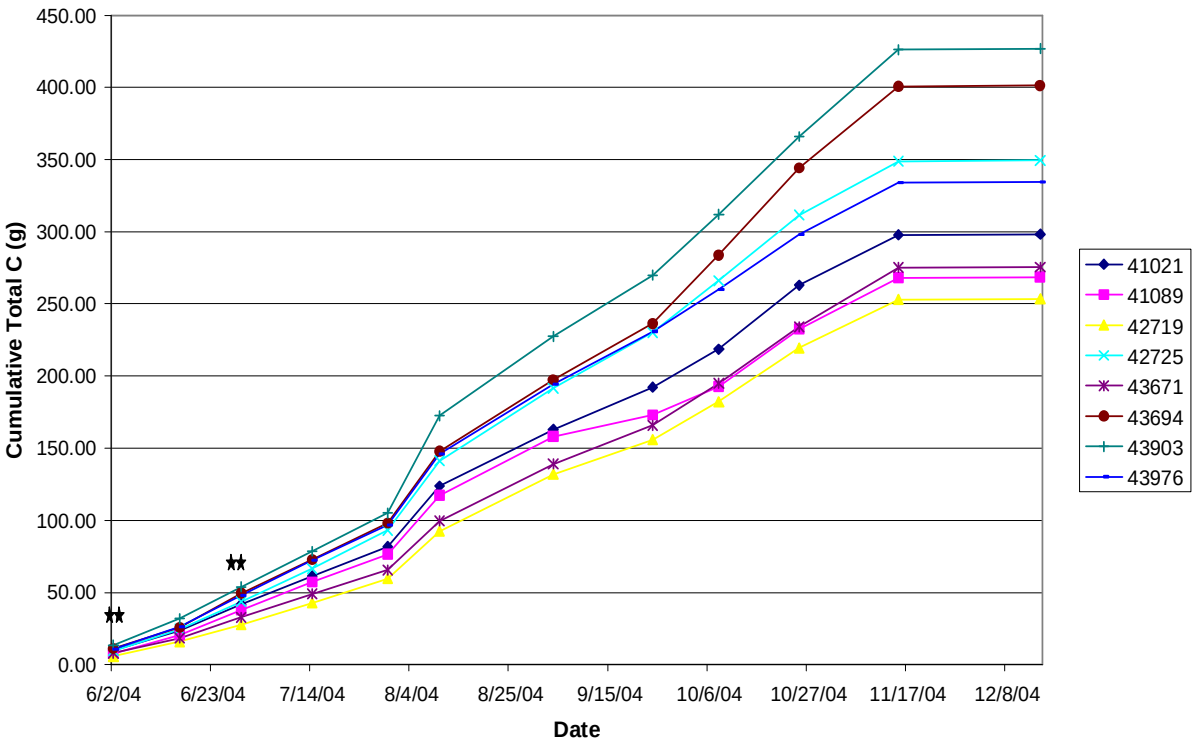


Figure 18. Mean clonal cumulative total carbon amounts (g) by date from June 2004 until December 2004. Two stars denote significance at an alpha level of ≤0.05 and one star denotes significance at an alpha level of ≤0.1.

Table 4. Mean separation of clones on significant cumulative total carbon dates in June 2004. Different letters denote significance at the 0.05 alpha level.

	6/2/04		6/29/04	
41021	9.75	AB	41.84	AB
41089	7.54	AB	37.91	AB
42719	5.73	B	27.74	B
42725	9.55	AB	43.71	AB
43671	8.10	AB	32.78	AB
43694	11.02	AB	49.56	A
43903	13.68	A	54.00	A
43976	10.81	AB	47.88	A

carbon per unit leaf area. A time series analysis showed a significant fertilizer x date interaction ($p=0.022$) and a clonal effect ($p=0.032$) on mean leaf area efficiency (Table 1). In a similar trend to cumulative total carbon there were no significant fertilizer effects when individual ANOVAs by date were examined. Nevertheless, the fertilized seedlings mean leaf area efficiency rate grew higher at an increasing rate compared to the mean control rate, which lead to the fertilizer x date interaction (Figure 19). When the clonal effects were examined at the end of the year, a Duncans MRT test with an alpha level of 0.1 showed clones 43694 and 43903 to be significantly more efficient than clones 43976 and 41021 (Figure 20). Overall clonal differences were very small and were not significant in most cases. However, clones 43903 and 43976 share the same parents, but they differed significantly in mean leaf area efficiency with clone 43903 being 21% more efficient than its full-sibling clone.

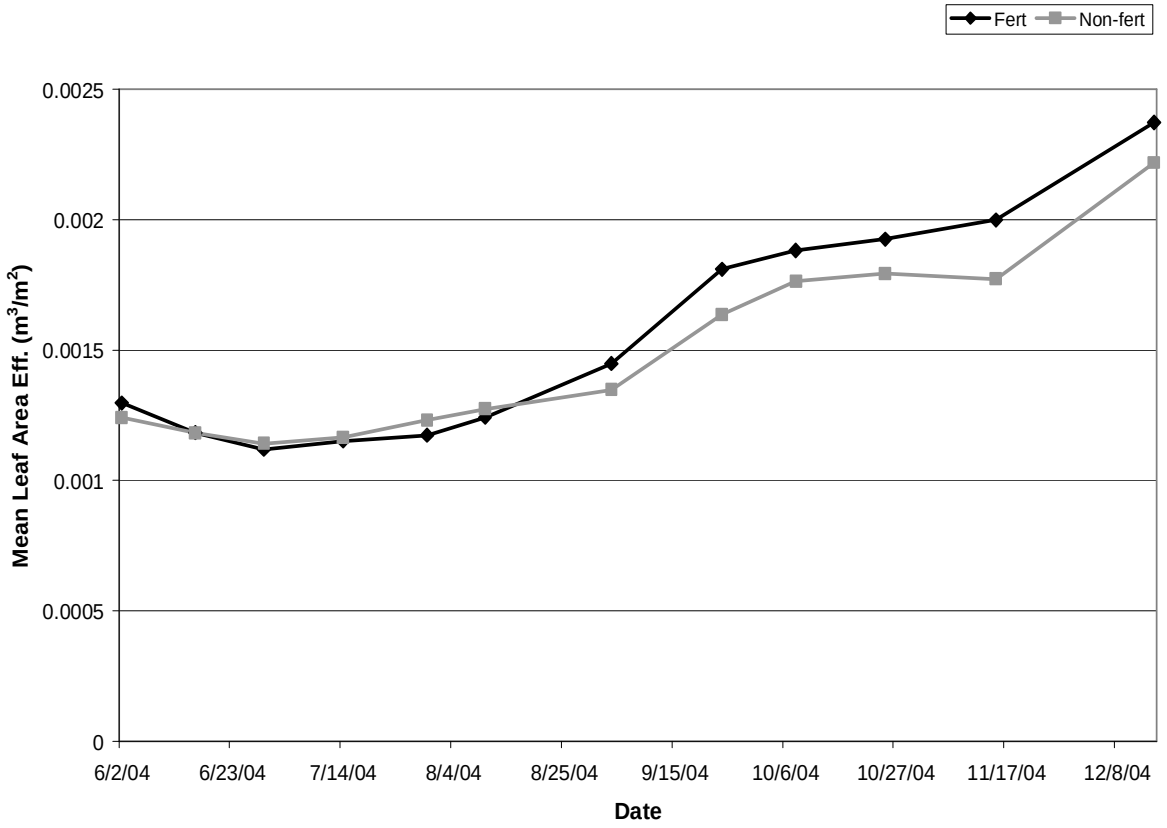


Figure 19. Mean leaf area efficiency for fertilized and unfertilized seedlings from June 2004 until December 2004. There were no significant differences between fertility treatments on any individual dates.

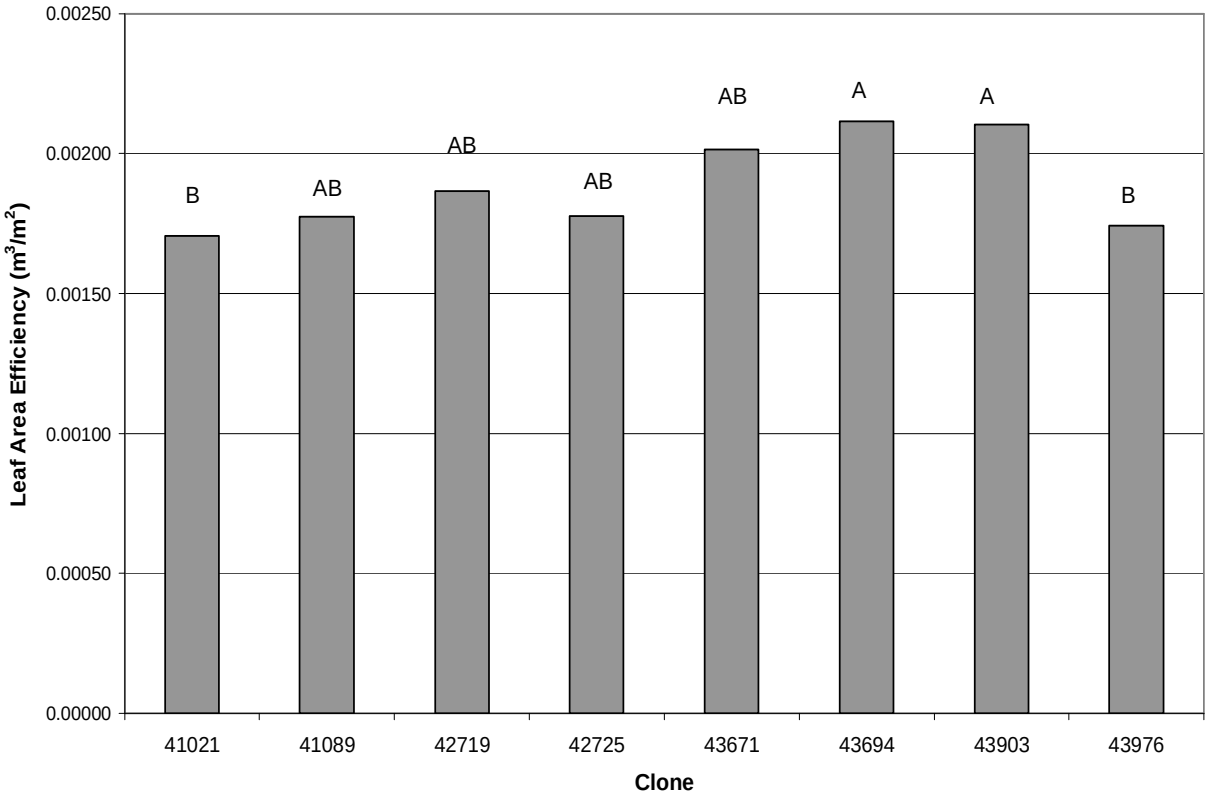


Figure 20. Mean clonal leaf area efficiencies at the end of the year (Nov. 15, 2004). Different letters denote significant differences at the 0.1 alpha level.

CHAPTER 5. DISCUSSION

Physiological Response

Photosynthesis

Although not always different, fertilization generally increased leaf specific photosynthesis on most dates sampled (Figure 1). Initially after fertilization, there was some variation in A_{sat} rates, but the fertilized seedlings had significantly higher rates ($p<0.05$) on the May 25 sampling date. Subsequent measurements in late July showed the fertilized seedling A_{sat} rates rose above the unfertilized rates and remained there through the final April 2005 sampling date. Light-saturated photosynthesis rates between the fertilized and unfertilized clones differed significantly ($p<0.1$) on 2 other sampling dates (Figure 1). These facts along with the significant fertilization x date interaction show that there was an increased P_n response in the fertilized seedlings at least over time.

These findings are in agreement with a greenhouse study of loblolly pine seedlings conducted by Gough et al. (2004b). They reported statistically greater light saturated photosynthesis (A_{sat}) rates in the N fertilized loblolly pines 6 days after the treatment application. A_{sat} levels in controls ranged from 1.5 µmol $m^{-2}s^{-1}$ to 4 µmol $m^{-2}s^{-1}$, while fertilized foliage levels ranged from 1.5 µmol $m^{-2}s^{-1}$ to 6 µmol $m^{-2}s^{-1}$ and were almost always higher than the control levels. A_{sat} levels remained high throughout most of the study but began to decrease towards control levels over the last 100 days. Despite these similarities, our study showed trends that were slightly muted in comparison to Gough's findings. One possible explanation for less pronounced differences in P_n after fertilization was due to the soil base fertility. The total soil N data collected showed that the fertilized soil N levels were mostly below the control levels with two dates being significantly below the controls ($p<0.05$). In fact, the only time the fertilized soil N levels were above the controls was for approximately 25 days immediately after fertilization. Hence, the addition of N fertilizer did not dramatically increased the N content of the soil as it would have in Gough's very sandy, infertile, Wakulla series soil type. This is evidenced by the fact that mean needle N concentration was 1.67% for the fertilized seedlings while the control mean was 1.49%. Gough et al. (2004b) noted a positive correlation following fertilization between foliar N and A_{sat} when both treatments (fertilized and control) were pooled into 24 observations ($R^2=0.47$). In comparison to this study, there was small, but significant positive correlation between foliar N and A_{sat} ($R^2=0.09$ $p<0.0001$). Examination of Figure 11

shows more seedlings with slightly higher fertilizer treatment P_n rates at higher foliar N levels than the control seedlings. Therefore, foliar N explained a small, but significant amount of the variation in P_n rates between treatments in this study. If there had been less saturating foliar N levels present in the control seedlings it is likely there would have been a greater positive correlation between P_n rates and foliar N, such as the one seen by Gough et al. (2004b).

Dark Respiration

The analysis of monthly mean R_d rates showed no significant differences due to the fertilization treatment or clonal types. Current literature seems divided about the effects of fertilization with some studies showing a positive correlation between R_d and foliar N (Strand 1997, Zhang et al. 1997, Kellomaki and Wang 1997) while other studies show no significant changes (Robernst and Stockfors 1998, Lavigne et al. 2001). Interestingly, Figure 3 shows a slight decreasing trend in mean R_d rates over the growing season for both treatments in this study. Samuelson 2000 found a significant decrease in R_d rates in the high N treatment during a high and low fertilization experiment using loblolly and slash pine seedlings. She hypothesized that the drop in R_d helped increase the amount of carbon available for use, which facilitated larger growth in the high N seedlings. However, Samuelson 2000 also notes this is in contrast with several other studies showing an increase in R_d.

Schaberg et al. (1997) noted a slight but statistically insignificant increase in R_d during a chronic low-level N addition study on montane red spruce. They found that foliar N was not significantly correlated to R_d levels and suggested that the influence of N on R_d was indirect (Schaberg et al. 1997). They concluded that there is no clear relationship between foliar N and R_d in N rich environments because these plants may put less N into proteins requiring maintenance respiration and more into unassociated amino acids (Van Dijk and Roelofs 1988, Shaberg et al.1997). Schaberg et al. (1997) also note there may be a negative correlation between R_d and other nutrient concentrations, such as Ca, Mg, or other base cations. Thus, checking other nutrient concentrations and how they change shortly after N fertilization may be beneficial to study in relation to R_d in the future.

Growth Response

Seedling heights, basal diameters, and volumes all increased above the controls as a result of fertilization. Although there were no significant fertilizer effects seen in the ANOVAs by date there were several significant complex interactions based on the time series analyses of

the variables. For example, volume had a highly significant fertilizer x clone x date interaction which made it difficult to determine the exact effects from the fertilizer treatment. Upon examining the fertilization x clone x date graphs, it became apparent that several clones, such as clones 43694, 42725, and 43903 tended to respond very favorably to fertilization. At the end of the year, the fertilized seedlings had 48%, 10%, and 14% more volume, height, and basal diameter growth than the controls. Gough et al. (2004b) noted significantly greater heights and basal diameters in the fertilized seedlings 32 days following treatment initiation. At the end of the study period he had mean fertilized seedling height and basal diameter increases of 82.4% and 79.6% while the controls only increased 44.3% and 46.4% (Gough et al. 2004b). Thus, the growth increases in this study support Gough's hypothesis although the increases in this study were less in comparison to his results. It is logical to assume the differences in base soil fertility between the two studies caused the growth discrepancies seen here. This hypothesis is supported by the needle N content data collected over the growing season. The control and fertilized seedlings all had tissue N concentrations at or above 1.2% dry weight, the critical concentration for foliar N where below this level less yield is generated (Colbert and Allen 1996). Hence, N was not as limiting or deficient in either treatment unlike in Gough et al. (2004b) where peak N amounts were 1.7% in the fertilized seedlings and 0.76% in the controls. Given this difference and the stated relationship between foliar N concentrations and yield, it is logical to see less pronounced effects due to fertilization in this particular study. It is also possible that some other nutrient, such as P, may have been deficient in the soil and was enhanced by the DAP fertilizer applied. Hence, the growth response seen here may have been a result of a P effect. However, foliar and soil P concentrations were not measured in this study.

In a greenhouse study similar to Gough et al. (2004b), Samuelson (2000) found comparable results in a low and high N fertilization study on loblolly and slash pine seedlings. At the end of the study, mean P_n rates for the high N treatment were significantly higher than the low N rates for both tree species. Furthermore, the loblolly pine seedling mean heights and diameters were 19% and 61% greater in the high N treatment than the low N treatment. A measure of mean seedling weight showed that on average the high N seedlings outweighed the low N seedlings by 128.8 grams. Hence, there were some large growth differences due to the fertilization treatment.

The results also show that the clones handled foliar N concentrations above the critical level in different ways, with some clones showing a large growth response (42725, 43903) and others showing none (41089, 43671). Hence, it seems likely that there are family differences in response to N concentrations above the critical level. Xiao et al. (2003) noted a similar trend when comparing fertilizer responses between genetically superior loblolly and slash pines versus unimproved slash pine. They found significant differences in foliar nutrient concentrations, crown structure, and growth between the genetically superior pines and the unimproved slash pines. Xiao et al. (2003) hypothesized that breeding may have modified some of the genetic or environmentally correlated traits like foliar nutrient levels and crown structure. They also noted differences in foliar nutrient levels between families based on changing nutrient demands during the growing season. Xiao et al. (2003) concluded that differences in nutrient uptake, utilization, and minimization of dilutions or deficiencies may be important growth strategies that set certain families apart.

In a research publication from the North Carolina State University-Industry Cooperative Tree Improvement Program (2001), thesis work by Vasquez (1994) noted there was some large variability in foliar N and P levels between 50 families representing several loblolly pine provenances in the Southeast. The results showed that critical foliar N and P levels for Florida provenances were lower than the Atlantic Coastal provenances upon which the current foliar N and P averages were based. This led him to conclude that the variability between families and provenances may indicate differences in critical foliar N levels (Vasquez 1994, cited in North Carolina State University-Industry Cooperative Tree Improvement Program 2001).

Physiological and Growth Response

Foliage Response

At the end of the growing season, the fertilized seedling clones had a larger mean index of leaf area than the control seedlings. The mean value for the fertilized seedlings was 30.5% greater than the controls, which was similar, but slightly less than the 36.5% difference in mean leaf areas found in the study by Gough et al. (2004b). The significant fertilization x date interaction proves that the mean fertilized ILA slope and the control slope were on different trajectories with the fertilized seedlings slope being greater. Thus, if the trend continues it would be logical to assume that the differences between treatment ILAs will be larger in the future. These facts correspond with the similar significant fertilizer x date interaction seen in the

treatment P_n rates leading to higher fertilized seedling P_n levels on most dates. The individual ANOVAs by date already showed the fertilized seedlings mean ILA was slightly significantly higher ($p=0.1$) than the control mean ILA during their peak measured ILAs in October. This evidence supports the final link of Gough's hypothesis that initial P_n increases lead to an extra pool of photoassimilate, which is used in creating larger leaf areas. Once the larger leaf areas have been established the fertilized seedling P_n rates may drop back, or down-regulate, to near the control seedling rates (Gough et al. 2004b). In fact, A_{sat} data collected this spring in the same study showed no differences between fertilized and non-fertilized seedlings. Then in July 2005 the fertilized seedling A_{sat} rates were actually significantly lower than the control seedling rates, which suggests the fertilized seedlings are down-regulating to near control levels (data not shown). However, the fertilized seedlings will now have extra photosynthetically active tissue, which will result in greater overall photosynthesis. The final result due to enhanced leaf areas and canopy photosynthesis will be increased loblolly pine growth (Gough et al. 2004b).

These results are supported in part by Samuelson (2000) as well as several collective studies from SETRES in the 1990's (Murthy et al. 1996, Albaugh et al 1998, King et al. 1999, Gough et al. 2004b). Samuelson (2000) found a significant increase in loblolly and slash pine P_n rates in a high N fertilization treatment over the course of 6 months. She also reported larger fractional dry weight allocations to the leaves and coarse roots of the seedlings in the high N treatment. The final mean biomass measurements showed significant height, diameter, and weight increases between the high and low N treatments for loblolly pine. The same was true for slash pine with the exception of height, which did not significantly increase due to the high N treatment. The seedling clones were in pots in a greenhouse environment and were between 7 and 13 months old during the course of the study (Samuelson 2000). Also, measurements were conducted with a Licor 6400 with comparable chamber conditions to this study and Gough's. Thus, there were many similarities between Samuelson's greenhouse experiment and Gough's greenhouse experiment.

The collective SETRES studies began on 8-year-old loblolly pine seedlings in the sandhills region of North Carolina in 1992. The treatments consisted of a fertilization regime meant to maintain foliar N at 1.3% and no fertilization. The fertilization treatment was initiated in 1992 and biomass measurements began in the same year. Murthy et al. (1996) found that the fertilizer treatment accounted for a 26% increase in A_{max} in the stand 1 year after treatment

initiation. Albaugh et al. (1998) tracked the biomass changed in the same stands under the same treatments. The fertilizer increased stem volume growth, biomass production, and peak LAI in 1993 by 109%, 81%, and 65% respectively. These variables continued to increase each year until they finished sampling in 1995. Besides those variables, heights, diameters, basal areas, and volumes also increased significantly in the fertilized plots by 1995. Gough et al. (2004a) sampled P_n rates in the same stands in 1999 when the pines were 14 years old. He noted an inconsistent trend in seasonal P_n rates, which were normally not significant between treatments. Also, there appeared to be no correlation between foliar N and P_n rates even though foliar N was typically higher in the fertilized pines. Hence, Gough et al. (2004a) concluded that there may not be a correlation between P_n rates and foliar N percents over the long-term.

These results and Gough's hypothesis are in conflict with several studies that show little or no evidence of P_n increases, but still have enhanced leaf areas after fertilization. Instead, biomass increases after fertilization are thought to be solely the result of increased leaf areas and their impacts on light interception, canopy level photosynthesis, and other physiological process (Teskey et al. 1994, Chang 2003). Typically, these higher leaf areas are well correlated to tree growth and yield (Vose and Allen 1988, Teskey et al. 1987). However, stand age and the timing of measurements after fertilization may be responsible for the difference in results (Teskey et al. 1994, Gough et al. 2004b).

Vose and Allen (1988) noted the significant relationship fertilization and LAI have in loblolly pine stands on nutrient deficient soils. After combining fertilization treatment and site data for three stands, they noted a positive, linear relationship ($r^2=0.75$, $p<0.01$) between LAI and stemwood growth. They concluded that their results supported the hypothesis that LAI as a determinant of radiation intercepted is well correlated to volume growth. Vose and Allen (1988) also concluded that no major changes in photosynthesis or stemwood biomass allocation must have occurred because growth efficiencies were not affected by fertilization in two out of the three stands. However, actual photosynthesis rates were not measured during the study.

Munger et al. (2003) conducted a N fertilization and weed control study on different age loblolly pine stands on two sites in Georgia. The sites received an annual fertilization regime applied in the spring with P_n and other measurements occurring from May until December. The stand ages ranged from 5 to 12 years old at each site. Fertilization had no effect on A_{sat} rates in the 5- or 12-year-old stands and negatively impacted A_{sat} rates in the 10-year-old stands. There

was an interaction between stand age and fertilization only at one site. Otherwise, stand age did not influence A_{sat} rates. Despite the insignificant impacts on stand A_{sat} rates, fertilization did positively impact foliar N at both locations. Munger et al. later noted that fertilization and competition control did increase stand growth. However, due to the lack of increased A_{sat} rates at both sites, they was left to conclude that increased stemwood growth was due to increased canopy size. They also stated that the relationship between A_{sat} and foliar N may depend on several factors including tree developmental stage, length of fertilization treatments, and site fertility (Munger et al. 2003).

Teskey et al. (1994) found similar results when working with 23-year-old field grown slash pine in Florida. The study was meant to determine the impact of fertilization and climate on P_n rates of slash pine. Fertilizer applications began a year before the study and continued at 3-month intervals during the measurement period. They found slight increases in P_n rates after fertilization, but they were not believed to be large enough to account for the growth increases seen over the study. They stated that the lack of P_n response to fertilization was similar to reports from other mature conifer stands. Teskey et al. (1994) concluded that the increase in dry matter was due to larger leaf areas. Chang (2003) met with the same conclusion while measuring sweetgum (*Liquidambar styricaflua* L.) half-sib clone responses to N and/or P fertilization over a 2 month period just after application. Although there was a significant P_n response to N and P fertilization by clone, there was no significant response to just N fertilization. Chang did find a significant increase in foliar nutrient concentrations due to fertilization. In addition, foliar N content explained 78% of the growth response in biomass production. However, Chang notes that there is no clear reason why P_n rates failed to respond to increased foliar N levels. Chang (2003) also brings up an interesting point that no one knows what or if there is a threshold in foliar N concentrations that must be surpassed before a change in P_n rate occurs. However, if the P_n response is subtle, it may require a long period of time which may be why Chang was unable to correlate increases in P_n to increased growth over the short 2-month sampling period.

Clonal Responses

In order to provide the most accurate representation of the complex interactions and mechanisms at work, several physiological and biomass variables must be further broken down to the clonal level. There were several measured characteristics that had significant clonal

effects, clone x time, clone x fertilization, or time x fertilization x clone interactions. For example, most of the characteristics sampled at the end of the growing season showed variable responses to fertilization when examined at the clonal level. Current literature on the subject also shows much variability in genotypic responses to fertilization or other treatments (Teskey et al. 1987, Li et al. 1991, McCrady and Jokela 1996, Samuelson 2000). However, more than half of the clones sampled appear to support Gough's hypothesis by varying degrees. Clones 41021, 42719, 42725, 43694, 43903, and 43976 generally showed a positive response to fertilization by increasing cumulative total carbon levels leading to enhanced indexes of leaf area and larger mean volumes. As mentioned previously, most of the clones did not show a statistically significant response to fertilization, but that was probably due in part to the clone and to the relatively high base soil fertility in the control plots.

Clones 43694 and 43903 appeared to be the more superior performing clones out of the eight clones measured in this study. These two clones typically had the highest mean A_{sat} rates of any clone during the study (Figure 2A). They also had relatively large responses to fertilization in terms of cumulative total carbon, final index of leaf area, and volume although none of these differences were significant at the end of the growing season (Figures 17, 14, and 7). Also, these clones were a large part of the significant relationship between A_{sat} rates and volumes by treatment as seen in the multiple linear regression analysis (Figure 12). Each clone had a dramatic positive response to fertilization in both mean A_{sat} rates and volumes. These clones appeared to have the highest leaf area efficiencies out of all the clones as well (Figure 20). However, the differences in leaf area efficiency were slight and may have been muted due to the high levels of foliar N in both the fertilized and control seedlings. Clone 42725 was the only clone to show a significant fertilizer response in cumulative total carbon, final index of leaf area, and volume at the end of the year. This clone typically had mediocre mean A_{sat} rates, but the basis for a fertilizer response depends on the difference between the mean control and mean fertilized seedling rates. On 12 out of 19 sampling dates between May and December 2004, clone 42725 had slightly higher fertilized seedling mean A_{sat} rates. This fact is further evident in Figure 12, where the fertilized seedlings mean A_{sat} slightly increased, but its volume dramatically increased. Clones 41021, 42719, 43976 showed the same general fertilizer response, but to a much smaller degree than the previous three clones. In comparison, these three clones had relatively lower mean A_{sat} rates and smaller indexes of leaf area as a result. Hence, their

cumulative total carbon amounts were slightly lower and this trend was compounded over time into their final volumes, which were much less than clones 42725, 43694, and 43903.

The last two clones (41089, 43671) showed no response or a slightly negative response to fertilization. Interestingly, clone 43671 had the second highest mean A_{sat} rates over the sampling period. However, larger leaf areas were not realized as a result of these higher A_{sat} rates. The mean cumulative total carbon amounts were nearly the same between the fertilized and control seedlings and were low compared to the more superior performing clones. As a result, clone 43671 had nearly identical volumes between treatments and the fertilized seedling mean volumes were much lower than clones 42725, 43903, and 43694. The same trend was present for clone 41089 although it had the lowest mean A_{sat} rates out of any clone measured over the growing season. This clone typically had lower fertilized mean A_{sat} rates than several control seedlings mean A_{sat} rates when examined over the entire sampling period. Although Figure 12 clearly shows a higher mean A_{sat} rate in the fertilized 41089 clones, leaf areas between treatments remained nearly the same at the end of the growing season. It is difficult to determine why larger ILAs were not achieved in the fertilized seedlings. Thus, the mean cumulative total carbon amount for the fertilized seedlings was slightly lower than the controls leading to no volume response over the controls at the end of the growing season.

Mean leaf area efficiencies did not significantly differ between the fertilizer treatment and the controls on individual dates although there was a significant fertilizer response over time based on the fertilizer x date interaction. Hence, mean leaf area efficiencies at the end of the year were only broken down by clone and showed only slightly significant differences between four of the clones. Clones 43694 and 43903 were significantly more efficient than clones 41021 and 43976 based on a Duncan's MRT at an alpha level of 0.1. Interestingly, clones 43903 and 43976 share the same parents. Thus, clone 43903 inherited a more efficient physiological mechanism for converting more carbon per leaf area into volume, at least when given nutrient amendments. This fact was readily apparent in Figure 7 where the fertilized seedlings mean volume response was over 1000 cm^3 larger than the control mean volume. Without further examination it is difficult to tell how much these mean leaf area efficiencies influenced the final volume totals for each clone and there are likely other physiological mechanisms at work that also contribute to the clonal differences seen here. Also, it appears that clonal responses will partially depend on the specific genes inherited by each seed during every pollination since

several full siblings showed relatively different responses to fertilization. For example, clone 42725 showed several significant physiological and growth responses to fertilization while its full sibling, clone 42719, showed no response to fertilization in most cases. This just furthers the evidence that inheriting genes from the same parents can still be highly variable. Based on the results, it seems apparent that some clones will perform better on nutrient rich sites or sites where fertilizer has been added while others will show no response. This is in agreement with other clonal studies showing certain genotypes can be selected for specific site conditions or silvicultural management techniques where they will show the best response (Li et al. 1991, Chang 2003).

CHAPTER 6. CONCLUSIONS

Early and intensive sampling of loblolly pine A_{sat} rates revealed that fertilization does increase mean P_n rates for at least one growing season. Mean fertilized seedling A_{sat} rates were significantly higher than the controls on three dates and generally higher than the controls throughout most of the sampling period. In contrast, fertilization does not appear to significantly alter mean R_d rates. This increase in the fertilized seedlings P_n rates lead to an enhanced pool of photoassimilates, or carbon, to be used by the seedlings. The larger pool of photoassimilates was invested in creating larger leaf areas for intercepting more photosynthetically active radiation. Hence, the fertilized seedlings had more tissue capable of carrying out photosynthesis, or greater canopy photosynthesis, despite there being no significant differences in mean leaf specific A_{sat} rates at the end of the growing season.

The final result for the fertilized seedlings was an increase in dry matter production leading to increased growth. In fact, all of the growth characteristics sampled increased above the controls as a result of the fertilization treatment. However, these responses to fertilization were typically only significant through time and were generally not significant when examining specific dates by themselves.

These findings support Gough's hypothesis that short-term increases in P_n led to an increase in photoassimilate to be used in creating larger leaf areas rather than fertilization resulting directly in larger leaf areas. The treatment differences in this study appeared to be slightly muted in comparison to Gough's greenhouse study findings. One reason why Gough may have seen more significant differences in photosynthetic response, leaf area growth, and biomass growth than in this study is due to the soil types used and nutrient deficiency levels present in the two studies. Gough et al. (2004b) used a sandy, infertile Wakulla series soil type while this study used Lloyd clay loam, Louisa loam, and Hiwassee loam series soil types, which typically have a clayey B horizon resulting in a larger nutrient holding capacity. Thus, the nutrient amendments did not have as pronounced of an effect on soil N levels. This fact is supported by the total soil N and foliar N data collected over the course of the study. Mean soil N levels were generally lower in the fertilized seedling plots than the controls and were significantly lower on at least three dates. Also, mean foliar N levels for the control seedlings were typically above 1.2%, which below that critical level, N is considered limiting to growth and yield for loblolly pine. Hence, both treatments had saturating foliar N levels throughout the

course of this study, which inevitably muted the overall physiological and growth responses. However, when looking at the clonal level the results imply there may be some variability in critical foliar N levels or differences in high foliar N usage between clones.

The clonal responses to fertilization were variable, but rarely significant different from the controls. Clones 42725, 43694 and 43903 appeared to be the most superior performers for this site and the conditions present. Furthermore, they showed large responses to fertilization although only clone 42725 showed a statistically significant response when comparing volumes, leaf areas, and cumulative total carbon at the end of the growing season. However, each of the clones showed desirable traits that could lead to increased loblolly pine growth and yield if the trends continue throughout the life of the clones. Clones 41021, 42719, and 43976 showed generally positive responses to fertilization although they were typically small. Clones 41089 and 43671 showed no response or a slightly negative response to fertilization and contributed to the most variability in the study. These clones rarely had any major differences in any variable sampled, which led to the almost identical mean volume totals seen at the end of the growing season. All of this information proves that clones show potential for greatly increasing loblolly pine productivity given the right breeding, site, and management. The different growth strategies that appeared during the study show the opportunity for clones to be tailored to fit certain environments based on those characteristics. Nevertheless, more in-depth studies need to be conducted to determine the mechanism behind the variable clonal responses seen in this project.

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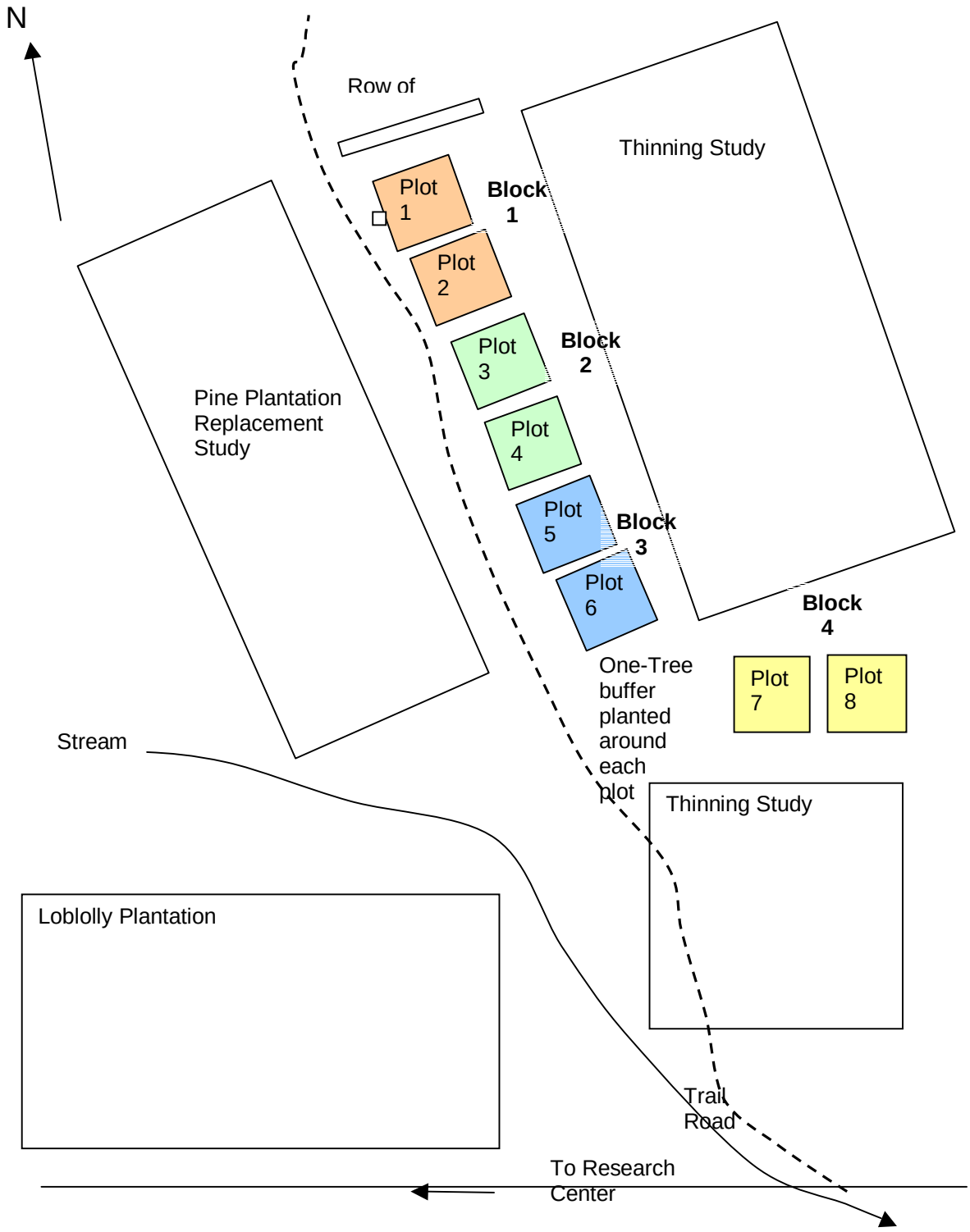
APPENDIX A

Clonal Fertilizer Trial

Reynolds Homestead Research Center



APPENDIX B

Leaf Area Determination Methodology

In order to do this, the lasso tool was used to circle the seedling and then the picture elements outside that circle were deleted to a bright red color. Then the magic wand tool set at a tolerance value of 30 was used to clean out the red background near the seedling canopy. The tolerance was adjusted as needed depending on the shading and/or canopy fuzziness in order to remove the same amounts of red around the needles. Hand scrubbing with the eraser tool was used to clean out any grass, weeds, or other non-uniform elements that the magic wand tool could not isolate and remove. Once the canopy and white square were isolated the pixel counts for subsequent area calculations could be determined using the magic wand tool. Since loblolly pine needles often had white colors from the sunlight shining on the needles, the white square was isolated at a magic wand tolerance of 50 with the "Contiguous" button checked to keep it from picking up the other white objects. Once the square was isolated it was deleted to a color that was not present in the seedling canopy in order to be easily identified on a histogram graph. While keeping the square selected with the magic wand, the pixel count of the square could be determined by going to "Image", "Histogram" and reading the pixel count number on the screen. Next the seedling canopy was isolated while keeping the colored square separate. This was done by removing the check mark in the "Contiguous" box then "shift-clicking" on the red area around the canopy. This was followed by "right-clicking" in order to select "Select inverse" from the pop-up window. The seedling canopy was then isolated from the red background and colored square so the pixel count could be determined for just the canopy. The same process as above was used to determine the pixel count of the canopy. These pixel count values were put into an Excel spreadsheet with the following formula:

$$\frac{\text{Pixel count of square}}{100\text{cm}^2} \times \frac{X}{\text{Pixel count of canopy}} = \text{Index of leaf area } (\text{cm}^2)$$

The resulting value is a 1-sided or projected leaf area estimate. However, since the stem and branches were present the value was considered an index of leaf area and was not meant as an exact leaf area measurement. Once the calculations were complete the resulting index of leaf area value was displayed on the photo using the text tool in Adobe Photoshop. Then the two layers were merged by clicking on "Layers" and "Flatten image". The finalized picture was resaved to the hard drive as a .jpeg image file using the "File", "Save as" command.

Furthermore, the picture quality was reduced to “7”, the highest medium quality photo option, in order to conserve hard drive space while still keeping a detailed picture.

VITA

Nathan Todd King was born in Roanoke, Virginia in 1981 and attended primary and secondary schools in Roanoke. He graduated with a B.S. in Forest Resource Management from Virginia Tech in 2003. While attending Virginia Tech, Nathan interned as a field forester for International Paper Company and Fountain Forestry during his summer breaks. After completing his undergraduate degree he was accepted to graduate school at Virginia Tech in Forest Biology. Nathan plans to work as a research associate at Purdue University upon completing his term at Virginia Tech.