1994

Physiological Variation in Loblolly Pine (Pinus Taeda L.) as Related to Crown Position and Stand Density.

Dennis Albert Gravatt
Louisiana State University and Agricultural & Mechanical College

PHYSIOLOGICAL VARIATION IN LOBLOLLY PINE (*Pinus taeda* L.) AS RELATED TO CROWN POSITION AND STAND DENSITY

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Forestry, Wildlife, and Fisheries

by
Dennis A. Gravatt
B.S., University of Kansas, 1988
M.A., University of Kansas, 1991
December 1994

ACKNOWLEDGMENTS

I wish to thank a number of people who contributed to this dissertation and played an important role in my completion of a doctoral degree.

Several people provided the muscles needed for the erection of the steel towers and walk-ways on the study site. Dr. Suresh Guddanti engineered, installed and maintained the access towers and data acquisition system with the help of Jim Scarborough and the Johnson Tract Crew. Mark Hebert was greatly appreciated for his field assistance and acting as an outstanding "go-fer", the least of which was to go for another Rolling Rock.

Consultations with Drs. Vernon Wright and David C. Blouin assisted me in the correct decisions regarding experimental design and analysis of the mountain of data from the field experiments.

I would like to thank my committee members for their efforts and support: Dr. Quang V. Cao, Dr. Sam E. Feagley, Dr. Thomas J. Dean, Dr. Reza S. Pezeshki and Dr. James P. Barnett. I am grateful to my major advisor, Dr. Jim L. Chambers, for accepting me as his student, and allowing me to pursue my research as an independent scientist.

A big thank you goes to my past and present office mates: Bill Patterson, Michelle Imbalzano, Jim Allen and Jian Sun. Thanks goes also to Karen Velupillai for her continuous laughter and harassment of Jim Chambers; she acted as a great diversionary tactic.

I am most grateful to Dr. Stanley B. Carpenter and the faculty/staff in the School of Forestry, Wildlife, and Fisheries at L.S.U. A special thank you to the Gilbert Foundation Fellowship for their financial support throughout my degree program.

I want to express my greatest thanks to my family and friends for their support during this often difficult time. Especially Mary, Steve and Sarah Bowen for a sanctuary in which to hide during the home-stretch of my dissertation. A big thank you to Dr. Craig E. Martin and Valerie S. Loeschen, University of Kansas (Go Jayhawks !), for the much needed comaraderie and vacation spot in Germany during the writing of my dissertation. A very special thank you goes to my best friend and kindred spirit, Patti Faulkner, who listened tirelessly to me complain. I will always be thankful for her immeasurable support and understanding.

A final thank you goes to my wife Patti. Without her support and understanding I would never have embarked on such a journey, nor would I have succeeded. Finally, a particular note of thanks to my parents, Albert and Helen Gravatt. My parents supported my educational pursuits, especially in biology, since I was old enough to remember. I will always remember, and keep the same sense of wonderment, about the world around me, that my parents first introduced me to while on childhood camping trips. I dedicate this dissertation to their memory.

"I am glad I did it. Partly because it was well worth it,
but chiefly because I shall never have to do it again"

Mark Twain

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ABSTRACT

Physiological parameters were measured under natural light conditions and needle orientation from towers and walkways erected in the crowns of loblolly pine (*Pinus taeda* L.) trees. Photosynthetic light response curves, chlorophyll content, nutrient content and specific leaf area were determined for current-year foliage on detached branches. Two silvicultural treatments were randomly assigned to the twelve plots in the fall of 1988. Plots were thinned to a density of 731 trees per hectare or left unthinned, at a density of 2990 trees per hectare. The plots were left unfertilized or fertilized with 744 kg per hectare of diammonium triple superphosphate was applied.

During the fifth growing season (1993) following thinning, light levels were greater in the thinned, fertilized plots than in the unthinned, fertilized plots. However, no effect of thinning on canopy light levels was found in the unfertilized plots. Needle level physiology was not different with respect to thinning treatment for fertilized or unfertilized plots. In contrast, upper crown levels within the fertilized and unfertilized plots, had significantly higher light levels and photosynthetic rates than lower crown foliage.

Fertilization significantly increased foliar phosphorous, calcium and magnesium levels, but nitrogen levels were reduced. Thinning significantly decreased foliar phosphorous and potassium levels within the fertilized plots. Total chlorophyll content and specific leaf area were greater in the foliage from the lower canopy than that of

the upper canopy due to lower light levels. The increase in nutrient availability accelerated leaf area development in the previously thinned plots.

Within crown variation in photosynthesis was strongly dependent on canopy light levels. A strong interaction of canopy level with thinning was apparent for net photosynthesis. Additionally, southern exposed foliage within the thinned treatments had higher net photosynthesis rates than that of the northern exposed foliage in both the *in situ* and light saturated studies.

Loblolly pine, being a shade intolerant species, showed rather small potential physiological differences between needles from different parts of the crown. Because of the variability found in physiological parameters in this study, more extensive sampling is needed to correctly describe the physiology of a forest canopy with adequate precision.

INTRODUCTION

An understanding of the variability of metabolic processes in natural stands and forest ecosystems is an essential requirement for ecosystem process models (Reynolds et al. 1993). Scaling physiological processes to the stand level is complicated and requires precise quantification of spatial and temporal variation. Models are important in developing and understanding potential forest ecosystem responses to changes in climate (Dahlman 1985).

Although there have been limited studies of forest tree species, most have focused on the physical differences using a small number of trees without replication. This study is important, in that it utilizes replication of experimental units (plots treated with fertilization and thinning) in a two-by-two factorial arrangement of large *Pinus taeda* L. trees. Previously, access to the crowns of larger trees was prohibitive because of economic restraints. However, steel towers and wooden walkways have allowed complete access to the crowns of a larger number of trees under managed conditions.

Climatic conditions, edaphic factors, genetics and biotic conditions within forest stands control physiological processes in forest stands. These controlling factors are all important in determining the growth and survival of a stand. The development of models to explain processes in a forest stand requires a thorough knowledge of these factors. Much of the past physiological research has focused on seedling or juvenile trees. However, work on more mature trees, *in situ*, is required in order to

develop a better understanding of how natural stands and forest ecosystems function in response to their environment. Research in field situations is necessary so that the knowledge gained can provide the basis for further work describing forest ecosystem functioning. Comparison of stands, with controlled density and nutrient status, will allow researchers to test the effects that environmental parameters have on tree physiology.

Since the dawning of the Industrial Revolution in the early 1800's, pollutants have increased in the Earth's atmosphere to levels that have alerted scientists about possible impending climatic changes. Increased CO_2, the primary contributor to the "Greenhouse Effect," is predicted to have direct effects on plant communities, as well as possible indirect effects (Mooney 1991). Direct impact may include a fertilizer effect, due to the increased atmospheric CO_2 concentrations, and diminished water stress from increased water-use efficiency of some C_3 plants. Indirectly, plants may be effected by relatively rapid, geologically speaking, climatic changes (Ryan 1991). The response of plants to these changes cannot be predicted without a solid understanding of the basic physiology of larger trees in a forest ecosystem. Physiological processes are related to the ecology of the species. A tree species in a forest ecosystem will therefore respond uniquely to the environment in which it is found.

Loblolly pine (*Pinus taeda* L.) occupies a wide range of sites across the Southern United States. Loblolly pine is an important component of a variety of forest types, and is one of the Nation's major commercial timber species (U.S.D.A. F.S. 1987). Loblolly pine is the primary species used for forest regeneration in the

southeastern United States. Within Louisiana, forest products are one of the primary economic resources. Some major concerns about southern forests include (1) evidence for growth decline in some areas, (2) demand for timber is expected to increase, and (3) changing climatic conditions will impact southern forests in unknown ways.

Research in forestry has already contributed much to the sustained growth and productivity of southern forests. However, if forest productivity is to be maintained, and if gains are to be made in the field of forest management, it is necessary to address the major concerns stated previously. Physiological processes, such as photosynthesis, transpiration, and carbon and nutrient allocation must be studied under stand conditions to understand how they interact with climatic and edaphic factors. A better understanding of the physiology of trees in a stand is necessary to produce the methodologies to measure and manage timber stands more effectively. Information from this study will yield results that will help explain some of the mechanisms involved in forest productivity. Additionally, the knowledge obtained will be useful in constructing accurate models of forest stands in a changing environment.

The physiological data collected in this study will be correlated with changes in the environment and will be useful in comparing different levels of the canopy. The null hypothesis, crown position has no impact on physiological responses, such as photosynthesis and water relations, will be tested. An alternative hypothesis could be that foliage in different parts of the forest canopy do not respond in identical ways to changes in micro-environmental conditions. That is, the forest canopy of a monoculture stand is a heterogeneous mass of foliage whose physiology is not

identical and differences observed are not just due to changes in the microenvironment.

These analyses may be helpful in verifying the need for submodels of crown processes. This type of analysis also is helpful for carbon allocation and phenology studies conducted as part of a cooperative agreement with the U.S.D.A. Forest Service. Predictions based on changes in the micro-environment (light, temperature, water status and nutrient allocation), seasonal, phenological, forest management and competition can be modeled with greater accuracy based on this work.

CHAPTER 1

LITERATURE REVIEW

PHYSIOLOGICAL PROCESSES

Net Photosynthesis

Photosynthesis provides the basis for all life since it supplies usable energy in the form of organic carbon compounds for higher trophic levels and the necessary oxygen for aerobic respiration. Joseph Priestley, an English chemist, in 1771 described the ability of plants to renew bad air made by the breathing of animals through release of what we now know as oxygen (Salisbury and Ross 1985). In 1779, Jan Ingen-Housz, a Dutch scientist, demonstrated that light was necessary for the renewal of air (Ting 1982).

Three different modes of photosynthetic carbon metabolism have been recognized in higher plants, C_3, C_4 and Crassulacean Acid Metabolism (CAM), based on the biochemical pathway by which CO_2 is fixed (Edwards and Walker 1983). This discussion will focus only on the predominate type, C_3 , which virtually all forest tree species utilize. Most of the individual reactions take place in specialized organelles called chloroplasts. Photosynthesis involves photochemical processes that can be separated into three components: 1) light reactions, in which radiant energy is absorbed and used to generate ATP and NADPH; 2) dark reactions, which include the

reduction of CO_2 to sugars using the products of the light reactions; and 3) the process of diffusion which results in the movement of CO_2 and oxygen between ambient air and the chloroplasts.

Light Reactions

The photochemical process is initiated when chloroplasts capture photosynthetically-usable radiation. Pigment systems are involved in the light-driven reactions and are embedded in the thylakoid membrane of the chloroplast. The main pigments, chlorophyll *a* and *b*, are most effective at absorbing in the red and blue while carotenoid and other pigments permit absorption of other wavelengths in the 400-700 nm range. The chlorophylls are embedded in three chlorophyll-protein complexes (Glazer and Melis 1987): the light harvesting complex (LHC); the photosystem I antenna complex (PS I); and the photosystem II antenna complex (PS II).

Absorption of radiation is often put in terms of individual units of light called quanta. Proper operation of the photosynthetic system requires balanced excitation of the two photosystems, and the energy transfer between them is regulated by the phosphorylation of LHC protein (Anderson 1986, Glazer and Melis 1987). Radiation absorption causes excitation of electrons in the pigment molecule with excitation being directed to one of the specialized reaction centers (P_{680} in PS II and P_{700} in PS I) by resonance transfer. Electrons are transported via a series of protein complexes imbedded in the thylakoid membrane often described as the Z-scheme. Eventually, the

electrons are passed to the final electron acceptor, ferredoxin NADP reductase, to produce NADPH. The transport pathway is asymmetrically arranged in the membrane, such that electron transport gives rise to a charge separation across the thylakoid membrane, and H+ moves from the stroma to the inside of the thylakoid. The return flow of H+ is then thought to drive the production of ATP by its linkage to an ATPase (Dilley et al. 1987). Products of the light reaction, ATP and NADPH, are then utilized in the photosynthetic carbon reduction cycle described below.

A measure of the rate and apparent efficiency (ϕ) of the light reactions can be estimated from the linear portion of the photosynthesis light response curve. As part of this dissertation, light response curves were estimated to predict the efficiency at which light energy was captured and used to fix carbon dioxide.

Dark Reactions

The use of $^{14}CO_2$ and paper chromatography were used to elucidate the dark reactions of photosynthesis by Melvin Calvin, Andrew A. Benson, James A. Bassham and others at the University of California-Berkley, from 1946 to 1953 (Salisbury and Ross 1985). Plants having the C_3 pathway use the enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco) for the primary fixation of CO_2. Rubisco is a light activated enzyme located in the stroma of the chloroplasts and accounts for approximately twenty-five percent of the plant's protein (Salisbury and Ross 1985). Rubisco also has an affinity for O_2 fixation, as the names implies. This oxygenase activity is temperature dependent and increases with an increase in temperature. This

pathway utilizing O_2 rather than CO_2 is called photorespiration because CO_2 is released in the process of regenerating ribulose bisphosphate (Ogren 1984).

Within the stroma of the chloroplast the energy captured in the form of ATP and NADPH in the light reaction is utilized to reduce CO_2 to sugars. The first product formed from ribulose bisphosphate (RuBP) and CO_2 is the compound 3-phosphoglyceric acid (PGA) which is then converted to triose phosphate using ATP and NADPH. Most of the triose phosphate enters the photosynthetic carbon reduction cycle (PCR) that requires additional ATP to regenerate the RuBP for use again in carboxylation. Some of the triose phosphate is shunted off to form sugar phosphate (fructose-1,6-bisphosphate) and sugars. This process requires additional ATP and NADPH derived from the light reactions.

Carbon fixation is limiting to plant productivity at high light levels as RuBP regeneration becomes the limiting factor (Salisbury and Ross 1985). Thus, at light saturated conditions an estimate of potential maximum carbon uptake (P_{max}) can be determined. In this research, P_{max} was determined for upper and lower crown foliage to study the impact fertilization and thinning had on potential carbon gain.

PHYSIOLOGICAL RESPONSES OF PHOTOSYNTHESIS

Light Response

It has been known for a long time that plants from sun and shade environments have different photosynthetic characteristics (Böhning and Burnside 1956, Björkman

1968, Lewandowska et al. 1976, Boardman 1977). Additionally, many species adapt to the light environment during growth. Thus, in many species the photosynthetic characteristics are modified in an individual plant during development. The adaptability of the photosynthetic system to changes in the light environment during growth is of interest when considering predictive models of tree canopy foliage photosynthesis.

In general, increased illumination of foliage leads to a concurrent increase in photosynthetic carbon uptake. The increase in CO_2 uptake is at first proportional to the increase in light intensity and then more slowly to a maximum value (Figure 1). There are several key characteristics found in such a light response curve. The light-dependent portion of the curve in dim light reflects the net loss of CO_2, since more CO_2 is lost through respiration than is fixed by photosynthesis. At the light compensation point (I_C), photosynthesis exactly fixes as much CO_2 as is released by respiration. Thus, plants that respire more rapidly require more light for compensation than do plants with lower respiration rates. The initial linear phase of the light response curve indicates the apparent quantum efficiency (ϕ) of the photosynthetic system. In this region of the light response curve, the speed of the light reactions is the limiting factor for the overall process. The term "apparent" is used because the actual quantity of light absorbed by the photosynthetic system is not known, and the observed incident radiation is used instead. The greater the slope, the higher the quantum efficiency, until at very high light intensity the yield of photosynthesis continues to increase only slightly or not all. At this point the reaction is light-

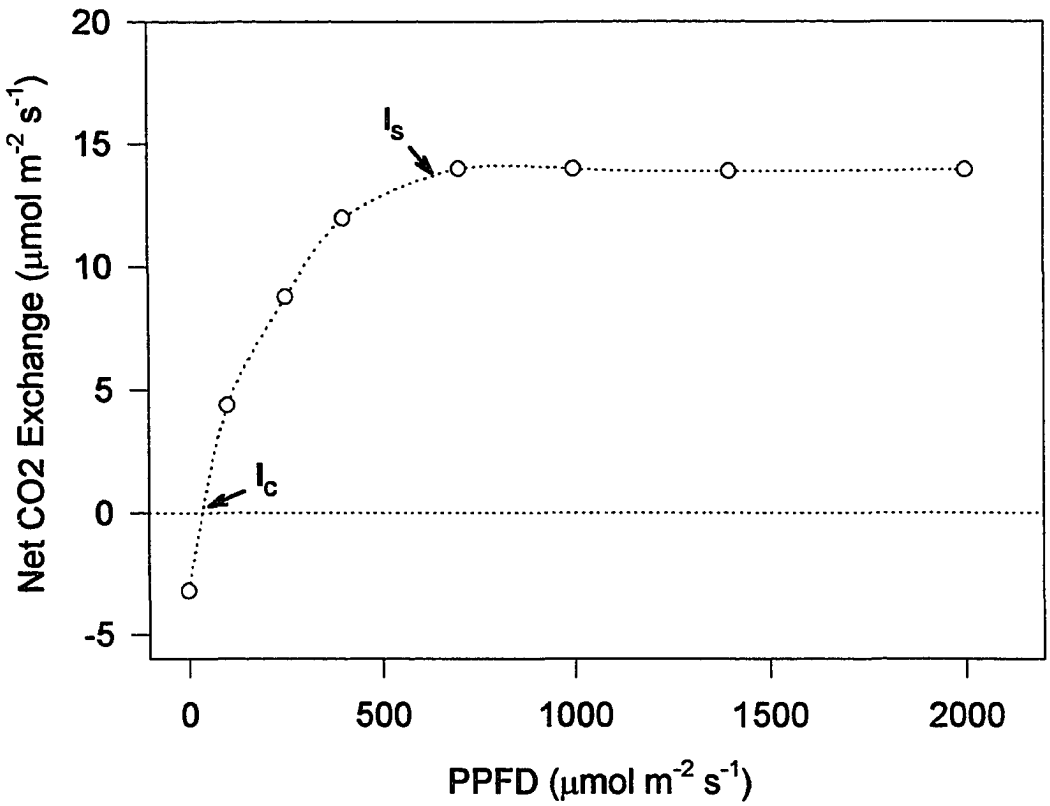


Figure 1-1. Idealized photosynthetic light response of loblolly pine fascicles. (I_C = light compensation point; I_S = light saturation point).

saturated (I_S) and the rate of CO_2 uptake is now limited by enzymatic rather than by photochemical processes, and by the supply of CO_2.

A comparison of light response curves and the cardinal points, I_C and I_S, reflect the light environment under which the foliage developed. Foliage adapted to lower light intensity (shade) respires less than high light adapted foliage and therefore has a lower light compensation (I_C) (Marshal and Biscoe 1980, Björkman 1981). Carbon dioxide uptake by sun adapted foliage is often saturated at a higher light intensity than that of shade adapted foliage (Boardman 1977). In a study of *Pinus taeda* L., Cregg (1990) investigated photosynthetic light response of foliage under three levels of shade treatment. In his study he found that I_S and maximum photosynthesis decreased with increasing levels of shade adaptations. Maximum photosynthesis decreased from 4.425 to 3.066 μmol m^{-2} s^{-1}, with 0% and 60% shade treatments, respectively. Additionally, from the data presented by Cregg (1990), the light saturation point (I_S) decreased from 1000 to 300 μmol m^{-2} s^{-1} PPFD. Similar result have been found in *Pinus radiata* D. by Warrington et al. (1988). They found that low light adaptation reduced maximum photosynthesis from 2.0 to 1.3 μmol m^{-2} s^{-1}. Ginn et al. (1991) found a reduction in light saturated photosynthesis, from 4.17 to 3.22 μmol m^{-2} s^{-1}, in lower canopy foliage (shade adapted) relative to that of the upper canopy foliage (sun adapted) in an unthinned stand of a nine-year-old *Pinus taeda* L. trees.

Theoretically, the quantum efficiency (ϕ) of foliage from different species should not vary using identical mechanisms (C_3 photosynthesis) of energy conversion. Björkman's (1981) review of the literature found that ϕ did not differ between species

with the same photosynthetic pathway. Furthermore, based on his earlier studies (Björkman et al. 1972a, Björkman et al. 1972b, Ehlringer and Björkman 1977) as well as work by Ludlow and Wilson (1971), he concludes that no differences exist between sun and shade leaves of the same species. Ehlringer and Pearcy (1983) using C_3 and C_4 monocots and dicots found ϕ did not differ between high light and low light grown plants. Similarly, Öquist et al. (1982) found ϕ did not differ between sun and shade leaves of *Betula verrucosa* and observed little difference in ϕ of *Betula pendula* seedlings grown at three levels of light intensity.

In loblolly pine, Cregg (1990) found widely varying values though none were significantly different for ϕ in response to shade treatments in July, 1989. In October 1989, the values observed were identical across all shade treatments. In another species of pine, *Pinus sylvestris*, Leverenz and Öquist (1987) found little variation in ϕ of seedlings during the course of a year. Similarly, no difference was found in values of ϕ for detached shoots of open- and forest-grown *Picea abies* L. shoots (Kull and Koppel 1987).

The theoretical minimum quantum requirement is 8 quanta of photosynthetically active radiation (PAR) per CO_2 fixed (Salisbury and Ross 1985) or a quantum efficiency (ϕ) of 0.125. The quantum efficiency is, however, lower because of photorespiratory loss of carbon dioxide. In practice, the typical quantum yield is around 19 (Jones 1992; $\phi = 0.053$). An important application of photosynthesis models is the prediction of productivity for different parts of the canopy using measured (Gay et al. 1971, Hutchison and Matt 1977, Sinclair and Knoerr 1982) or

modeled (Norman and Jarvis 1974 & 1975, Smolander 1984, Jordan and Smith 1993) light levels. Thus, as a practical matter, it is important to know whether or not silvicultural practices and/or canopy position influence the efficiency at which carbon is fixed.

Temperature

In general, temperature affects metabolic processes by way of its influence on reaction kinetics of chemical reactions. Seasonally, net photosynthesis is strongly limited by high and low temperatures (Kozlowski et al. 1991). As is the case with the light environment, plants are able to adapt to changes in ambient temperature. The degree to which a species is able to adapt defines their geographic range, and the range of habitats that they can occupy. Strain et al. (1976) found an increase in the optimum temperature for net photosynthesis for *Pinus taeda* L. seedlings with an increase in air temperature during growth. Furthermore, they were successful at using their phytotron results to predict seasonal trends in maximum photosynthesis. In a study of the seasonal patterns of photosynthesis, Drew and Ledig (1981) found that the optimum temperature for net photosynthesis shifted with seasonal temperatures from a high of 25°C, in the summer, to a low of 10°C, during the winter, for loblolly pine seedlings.

The competitive inhibition of rubisco by oxygen, photorespiration, generally increases with temperature (Ogren 1984). Samuelson and Teskey (1991) found that photorespiration, as indicated by the enhancement of photosynthesis in 2% O_2,

increased from 10% to 30% as temperature increased from 15 to 30°C in loblolly pine seedlings. In other conifers, photorespiration also has been found to increase with increases in temperature for *Picea sitchensis* (Bong.) Carr. (Ludlow and Jarvis 1971) and *Pseudotsuga menziesii* (Mirb.) Franco (Doehlert and Walker 1981).

PHYSIOLOGY OF FOREST CANOPIES

Recent ecophysiological studies suggest that the forest canopy is a heterogeneous structure (Ford 1992, Ginn et al. 1991, Leverenz and Hinckley 1990, Nowak et al. 1990). These studies point out the potential important differences in the foliage in the upper and lower canopy and how management practices may modify their morphology and physiology. Photosynthesis is an important metabolic process in the growth of plants. During photosynthesis, light energy is captured and converted into glucose to form structural carbohydrates. The quantity and efficiency at which this occurs, in part, determines the rate of carbohydrate accumulation (i.e., growth).

Atmospheric water vapor pressure is an important factor controlling tree growth. The potential for leaves to lose water is measured as the difference in vapor pressure of water inside the leaf versus that of the atmosphere. As the difference in vapor pressure gradient (VPG) increases so does the loss of water from the plant. Increased loss of water due to an increase in the VPG is in part counteracted by the closing of the stomata (Larcher 1983). The closing of the stomatal pores, however, at some point restricts the uptake of CO_2 needed in photosynthesis. Increased temperatures lowers the free energy of water and result in steeper gradient for water

loss from the leaf (Larcher 1983). Also, any increase in exposure to wind due to thinning, gap formation or other disturbances will increase the potential of stands to lose water (Teskey et al. 1987). Thus, a trade-off is attained between water loss and CO_2 capture, often limiting the growth of a plant.

Respiration rates of trees also may be affected by changes in the microclimate. A balance is maintained between the allocation of carbon for maintenance and growth. Imbalances may occur because photosynthesis and respiration respond differently to the environment (e.g., increases in temperature). Changes in climatic conditions may alter the balance between them and result in a net loss of carbon from an ecosystem. Imbalances between respiration and photosynthesis are likely to occur in older forest ecosystems first (Waring & Schlersinger 1985). With increased temperature and atmospheric CO_2 concentration, forest ecosystems may grow faster and die at a younger age (Ryan 1991).

Temperature is predicted to limit productivity by modifying the length of the growing season (Teskey et al. 1987) and to some degree by increases in metabolism as mentioned above. Growth of woody plants is sensitive to temperature. For example, increasing the day temperature during the growing season from 23 to 30°C increased shoot growth of loblolly pine seedlings approximately 1.2 cm per degree (Kramer 1957). In contrast, even larger increases in temperature may injure plant tissues.

Carbon and nutrient allocation patterns are especially important. Nutrients limit many metabolic pathways and, in part, determine the potential rate of most reactions (Salisbury & Ross 1985). Carbon allocation, on the other hand, is determined by

source and sink relations of individual trees. The quantity of carbon directed toward roots, stems, and new foliage is particularly important in predicting forest productivity. In coniferous forests, mostly in the northern latitudes, the climate change that may be associated with increased CO_2 concentration may have a stimulatory effect on productivity (Long & Hutchin 1991). Nutrient and water limitations may, however, offset or limit forest productivity. Direct correlation of nutrients to photosynthetic rate and other metabolic pathways has proven to be complex due to the labile nature of most nutrients (Switzer and Nelson 1963, Switzer et al. 1966, Adams et al. 1986).

Physiological studies of larger loblolly pine trees in response to cultural practices are generally lacking. Ginn et al. (1988) measured the response of lower and upper crown branches of loblolly pine from thinned and unthinned treatments. They used detached branches collected at midday and exposed to full sunlight to evaluate photosynthetic responses. Whole tree photosynthesis was predicted from the branch data by estimating total tree needle surface area and using light extinction values in the stands. Using this crude estimate of whole tree photosynthesis they were unable to find significant differences in photosynthesis between the thinning treatments.

In an examination of the effects of shading on loblolly pine branches, Cregg (1990) demonstrated that the responses of branches exposed to decreasing levels of sunlight are very similar to whole plants grown and adapted to low light. Dark respiration and maximum photosynthesis were lowest for foliage grown under the 60% shade treatment. Concomitant, leaf weight ratio and specific leaf area increased in response to shading. In addition, chlorophyll content increased under shaded

conditions. This is in contrast to another study (Higginbotham 1974) that found no differences in photosynthesis rates between the canopy levels for loblolly pine. Cregg concludes that physiologically and morphologically, individual loblolly pine branches adapt much the same way to shade as do individual sun and shade grown plants. Furthermore, these morphological adaptations allow for the optimization of leaf surface area per unit of carbon fixed.

Thinning has noticeable effects on environmental factors within the stand, such as increased soil moisture and nutrient availability. In conjunction, thinning results in a number of changes in microclimatic factors, e.g., increased light to lower branches. Nowak et al. (1990) examined the relationship between thinning and several physiological parameters. Nowak et al. (1990) used scaffolding to access the crowns measuring photosynthesis, needle chlorophyll content, dark respiration, and water potential of thinned and unthinned stands of loblolly pine. Sampling design prevented interpretation of diurnal physiological patterns. These researchers were only able to obtain data on one tree per treatment. The means of two samples for each measurement period were then lumped over the season for statistical analysis. Nowak et al. (1990) reported photosynthesis was lower on a seasonal basis for the lower crown position of the unthinned treatment than in the upper crown position. No difference was apparent in the thinned treatment. They did not detect differences among the thinning treatments in midday water potential, which never dropped below -1.4 MPa. Photosynthesis was higher for the upper crown position of the unthinned treatment than in the lower crown position on a seasonal basis. Differences among

crown positions were not found in the thinned treatment. They concluded that photosynthetic rates paralleled light levels within the crown and that water potential and chlorophyll content had no effect on photosynthesis. The absence of a water potential effect on photosynthesis was probably a result of the high soil moisture levels found throughout the study.

Later, Ginn et al. (1991), reporting results on the same stand, demonstrated that photosynthetic rate of needles of loblolly pine at different vertical levels in the canopy vary with management practices. They used detached branches collected at midday, placed in bags, and transported to a field station where they were exposed to full sunlight to evaluate the potential maximum photosynthetic responses. In thinned stands, lower and upper crown photosynthesis did not differ significantly. However, they found significantly higher mid-day stomatal conductances and greater net photosynthesis on a dry weight basis in the lower crowns of the thinned versus the unthinned treatment. This is in contrast to the unthinned stands where light saturated photosynthesis was decreased in the lower crown. Branch water potential was significantly lower in the lower crown of the thinned treatment during the second year of their study than that of the unthinned. Soil moisture was not measured in the study; therefore, differences associated with soil moisture could not be assessed between treatment plots.

In a study involving different branch types of *Pinus contorta* Nutt., significantly higher needle photosynthetic rates were measured on branches bearing male cones in the autumn (Dick et al. 1991). There were no significant differences in

assimilation of needles on vegetative shoots and branches bearing female cones. Included in these results were significantly higher respiration rates of 2-year-old female cone bearing branches in the spring and summer. It is suggested that the differences observed may have been the result of (1) increased shoot elongation as well as the development of female cones, (2) male cones in the developing bud creating a sink demand for assimilates, and (3) the current year's male cones reducing the photosynthetic area. Together these three types of studies (Dick et al. 1991, Ginn et al. 1991 and Nowak et al. 1990) suggest that the canopy cannot be treated as a homogenous volume of foliage. These studies also suggest more intensive studies could produce insight into physiological responses leading to increased growth in thinned stands.

The majority of past studies have used point measurements of light and photosynthesis of needles held horizontally. Photosynthesis estimated with portable gas exchange systems often measure only a small number of needles flattened into a single plane and held horizontally. An assumption is made that all needles normally receive identical amounts of light and that PPFD is accurately measured in the horizontal plane. Oker-Blum et al. (1983) demonstrated that there existed a functional relationship between light and photosynthetic response that was different for branches than for needles. Light response curves differed significantly relative to direction of radiation on the needles. Therefore, studies of trees within stands, may also need to account for PPFD relative to branch orientation rather than those measured only at the horizontal position.

NUTRIENT RESEARCH

The mineral nutrient status of a forest stand plays an important role in determining stand productivity. Fertilizer application can significantly enhance the productivity of forest stands where nutrient deficiencies occur (Adams and Allen 1985, Wells and Allen 1985). Thinning can effect how nutrients are distributed within the forest canopy (Vose 1988) and affect increased volume growth (Allen 1987). Allen (1987) suggested that thinning with fertilization may shorten or eliminate the period of time that a stand requires to regain maximum production of volume. Within species, there are strong positive correlations between nitrogen and both chlorophyll and RuBP carboxylase (Evans 1989, Field and Mooney 1986). In *Prunus persica*, DeJong and Doyle (1985), collected data to support the hypothesis that whole-tree photosynthesis is optimized by portioning photosynthetic capacity with respect to natural light levels. This may imply that a predictor of photosynthetic capacity, maximum assimilation, may be foliar nutrient content.

When and how to sample for foliar nutrient content have been difficult questions to answer. Much of the difficulty in adequately sampling for foliar nutrients is due to the mobile nature of nitrogen, phosphorus and potassium in plants. Shifts in concentrations of nutrients are due to changes in source and sink locations throughout the year as older tissues age and new tissues develop. One possible solution is to sample for nutrients during periods of reduced nutrient translocation. That is sample during the fall and late winter when nutrient content of foliage is more stable. However, some studies have not found a stable period at which time nutrients could be

reliably sampled (Miller 1966, Rathfon et al. 1993, Wells and Metz 1963). In fact, nitrogen and potassium have been found to increase during the winter months (Lassoie and Hinckley 1991, Miller 1966, Wells and Metz 1963). The accumulation of nitrogen during the winter months following the first growing season could be accounted for by the loss of nitrogen from older needles in the fall just before abscission (Wells and Metz 1963). A further problem encountered in the southern U.S. is the lack of a true dormant season. Extended periods of low metabolic activity of above and below ground tissues is not found in the warmer latitudes of the southern U.S.

The importance of nutrient limitations during periods of rapid growth cannot be totally ignored and can be missed if sampling is restricted to a specific season. Thus, some degree of sampling during the growing season should be done and relationships between nutrient content and needle physiology should be investigated. A sampling scheme which included peak nutrient demand periods has been suggested as a way to measure the nutrient status of the tree (Rathfon et al. 1993, Wells and Metz 1963). However, nutrient concentrations change too rapidly during these periods to sample over large areas of forests to provide reliable diagnosis for fertilizer recommendations, and thus, dormant season sampling has been suggested (Rathfon et al. 1993). This may apply only to more northern latitudes where a distinct and predictable dormant season is found.

A third suggestion is the use of nutrient ratios in determining nutrient status of forest stands (Comerford and Fisher 1984). The idea is that plant nutrient status may

be ascertained by the relative amounts of the mineral nutrients. Ideally, this works when any one of two nutrients may be limiting growth. The ratio of the mineral nutrients in question would give an indication which nutrient is limiting the other's potential. Absolute quantities of the nutrients could then be examined and proper amounts applied, through fertilization, to maximize site conditions. Thus, any deficiency will be detected by the imbalance of specific nutrient ratios.

Variation of element content within crown position and leaf age in needles of *Pinus banksiana* was studied by Morrison (1972). In his study, no trends in nitrogen were found between the different levels of the canopy. Higher amounts of phosphorus and potassium are associated with young needles and the upper crown. However, both of these elements decrease with increasing age and depth downward into the canopy. In contrast, Wells and Metz (1963) found a lower nitrogen, calcium and magnesium concentration in foliage from the upper crown position than that of the middle, which itself had less than the lower crown foliage. However, concentrations of phosphorous and potassium were greater in the upper canopy foliage.

In peach trees (*Prunus persica*), a seasonal relationship between leaf nitrogen with both canopy light levels and maximum assimilation was found (DeJong & Doyle 1985). DeJong and Doyle found a tradeoff of nutrient-use efficiency (NUE) and water-use efficiency (WUE) in sun versus shade foliage. In sun-lit foliage, conservation of water, and an increase in WUE, lead to a reduction in CO_2 uptake and thus, a decrease in NUE.

Within species, there are strong positive correlations between nitrogen and both chlorophyll and RuBP carboxylase (Evans 1989). In *Prunus persica*, DeJong and Doyle (1985) collected data that supported the hypothesis that whole-tree photosynthesis is optimized by portioning photosynthetic capacity with respect to natural light levels. This may imply that a predictor of photosynthetic capacity, maximum assimilation, may be foliar nutrient content.

The concentration of mobile nutrients, nitrogen, phosphorous and potassium, are usually highest in younger foliage because of the stronger sink created by their high metabolic activity (Mengel and Kirkby 1982, Smith et al. 1971). Presumably nitrogen and phosphorous are translocated from the older to the younger foliage (Smith et al. 1971, Vitousek 1982). On the other hand, Smith et al. (1971) did not find a depletion in nitrogen content of previous flushes during the development of subsequent flushes in mature *Pinus taeda* L.. They suggested that nitrogen is probably being received from other sources: older foliage prior to senescence, stems, branches and roots.

Fertilization with nitrogen and phosphorous are generally believed to increase the foliar concentration of each of these minerals (Allen 1987, Switzer and Nelson 1963). However, some studies have found a reduction in foliar phosphorous and potassium levels in Douglas-fir after fertilization (Heilman and Gessel 1963). Switzer et al. (1966) demonstrated loblolly pine stands accumulate nitrogen at the mean rates of 6.3 and 5.0 kg ha^{-1} $year^{-1}$, for good and poor sites, respectively, during the initial thirty years of stand establishment.

No single nutrient can act alone in producing physiological changes leading to growth responses. Studies on fertilization in loblolly pine have shown that nitrogen and phosphorous produce a greater response when used together than when applied alone (Allen 1987, Vose and Allen 1988, Wells and Allen 1985). Plant growth is usually restricted by the most limiting availability of an individual nutrient. Thus, the balance between foliar nutrients is an important diagnostic tool in determining tree growth response to fertilization. Comerford and Fisher (1984) reported that the proportion of nitrogen to phosphorous was a more reliable and accurate method for identifying nitrogen deficient soils. Adams and Allen (1985) later reported critical phosphorous to nitrogen proportions that were convenient for determining which foliar nutrient was most limiting. These were extremely useful in determining which mineral nutrient, nitrogen or phosphorous, to apply to produce a growth response.

The proposed optimum ratio of P:N is 0.095 to 0.105 (Adams and Allen 1985) for loblolly pine. At ratios above this range, nitrogen is most limiting to growth, and a strong nitrogen response would be expected. For ratios below this range, a strong phosphorous response is expected.

Stand density effects on nutrient dynamics are usually restricted to arguments concerning increased nutrient supply and growing space following thinning. In theory, thinning should provide more nutrients for the remaining trees in a forest. However, thinning also results in increased leaf area in the mid- and lower crown positions (Vose 1988). The photosynthetic rate of newly exposed foliage also is affected, because of the increased light, resulting in greater carbon gain used to supply

carbohydrates for new foliage growth. This increase in foliar biomass may impose further demands on an already limited nutrient supply.

The productivity of a forest is determined by a combination of factors and interactions including water, nutrients, light, temperature, pathogens, and competition (Ford 1992, Teskey et al. 1987). These and many other environmental factors emphasize the need to elucidate the impact changes in climate and management practices may have on loblolly pine forests. Plant physiological ecology has made advances in the ability to predict plant responses to environment (Mooney et al. 1987). Changes in physiology are in response to a changing environment and involve qualitative changes in anatomy and physiology, as well as reallocation of nutrients. This active acclimation to changes in environment can be seen within crowns in response to competition and shaded conditions (Cregg 1990, Cregg et al. 1992, Mitchell and Hinckley 1993, Vose 1988).

An increased understanding of how silvicultural practices and their interaction with climate change and soils affect physiological responses is a key to increasing productivity and identifying the techniques necessary to make such changes in future productivity. Whitehead and Hinckley (1991) suggest the need for more mechanistic models to explain stomatal responses in the forest canopy. Therefore, development of additional models, for other physiological processes, would seem to be appropriate if forest productivity is to be predicted.

MODELING

Models of forest ecosystems are based on space and time and recognize compartments and transfers within the system. The compartments are state variables, and they represent the storage of materials and energy in the system (Waring & Shlesinger 1985). The rate at which materials and energy move between the compartments or between the compartments and environment is a function of environmental, edaphic and biotic factors. In order to predict consequences of climatic change at the regional or climatic scale, scientists are faced with the job of scaling up from knowledge of the leaf's response to the microenvironment, to the tree, and finally the stand or ecosystem level. Models are important for two reasons, (1) they produce the information necessary to generate hypotheses and (2) they allow for more efficient and focused research.

Models can be of much more practical value to foresters, research scientists and politicians. Models can be used to estimate stand productivity and have been used by foresters for many years. Of recent interest, and under development, are models useful in predicting the effects of management techniques on forest productivity. Finally, models are being developed to predict the effects of climate change on forests. The current models being developed are of overlapping levels of different resolution. The models start at the physiological level and progress through populations and ecosystems to regional and global scales (Ågren et al. 1991). At the physiological level, plant processes are characterized in terms of their biochemistry and physiology. The population scale integrates the physiology and biochemistry to

the whole plant and adds the environmental feedback interactions to deal with plants at the level of population dynamics. At the lowest resolution are the regional and global models used to explain the distribution of different biomes.

The objective of physiologically based models of canopy processes is to integrate up from the micro-environmental scale and explain and predict the functioning of a community of plants. This type of model attempts to interrelate the various environmental variables and processes over space and time. For some processes this has been done quite successfully. Examples are the model of leaf gas exchange of Farquhar and Sharkey (1982) and the equations of canopy energy balance by Jarvis (1985). These two studies were able to identify the key elements of responses to environmental change.

Several forest models have been developed in an attempt to predict ecosystem response to climate change. The following models, BACROS (de Wit et al. 1988), BIOMASS (McMurtrie et al. 1989, 1990), FORGO (Mohren et al. 1984), MAESTRO (Wang & Jarvis 1990 a, b), and FOREST-BGC (Running & Coughlan 1988) are but a few of the many models developed recently. The BACROS model is for use on any crop plant. The FORGO model describes forest growth and water balance. Running and Gower (1991) developed the model FOREST-BGC to predict carbon allocation and nitrogen budgets. They were able to show that the dynamics of annual carbon partitioning were controlled by water and nitrogen limitations in managed stands.

BIOMASS and MAESTRO estimate carbon uptake by trees and provide a means of integrating over space and time. The BIOMASS model is structured to

predict growth and water balance of a forest stand. The MAESTRO model is designed to explain canopy assimilation and transpiration. The model MAESTRO needs the differences in the spatial position of the tree defined. However, the spatial heterogeneity must be taken into account when considering the measurements made and when testing the model. Therefore, the variability must be known and adequate steps taken to sample the variation found. Where the aim is to estimate stand productivity the influence of the variation on the parameters determining growth must be studied and modeled (Jarvis et al. 1985, Caldwell et al. 1993).

Population (stand) models have been developed to predict tree growth as affected by competition from other individuals and population dynamics. These models either apply to single- or multispecies stands (Ågren et al. 1991). Generally, these models greatly simplify competition and measure only a single point, or not greater than two positions in the canopy. Examples of these are STEMS (Belcher et al. 1982) and LINKAGES (Pastor & Post 1985). Most of these models also assume that the canopy is a homogeneous mass that responds the same way to climate change. One exception to the above statement is the JABOWA/FORET class of models. This class of model starts with some *a priori* assumptions of how climate effects tree growth and recruitment (Botkin et al. 1972, Shugart 1984). Therefore, no assumption of homogeneity is made.

Problems with Scaling Up

From the literature, briefly outlined above, various problems exist in scaling up from an individual tree in a stand to a population model. Among those problems mentioned is the nonuniform nature of a forest stand. Variation in physiological response to microenvironmental changes exist among trees, as well as within trees. The question then is how to deal with this heterogeneity when constructing models. Additionally, it is important to estimate how much variation exists. It is not enough to know that differences in rate of a metabolic process exist. Instead, it must be determined if the physiological processes between canopy layers respond in a similar fashion to changes in environment, i.e., comparison of response curves should be made. Foliage from different age classes, vertical and horizontal position, and exposure must be characterized in order to predict how trees in a stand responds to climatic changes.

Solving the Problems

First, scientists must identify the variability as it exists in stands. The canopy must be stratified into recognizable and ecologically meaningful modules. These separate modules then can be experimentally tested and analyzed with respect to their relative physiological importance. Those parts of the canopy that are determined not to be physiologically different may then be combined into a simpler strata. The difference in the various components of the canopy may thus be discriminated based on the environmental, and more easily measured, differences within the canopy.

Ford (1992) proposed that foliage and branch structure, including physiology, does not remain constant during tree growth. The need exists to study changing characteristics (physiology in this case) throughout growth. Changes in physiology are in response to a changing environment and involve qualitative changes in anatomy and physiology. This active acclimation to changes in environment is seen within crowns in response to competition and shaded conditions. The foliage photosynthetic system and foliage leaf thickness are two of the possible modifications due to environmental pressure. Changes in crown structure are also accompanied by shifts in nutrient dynamics and water relations. Both of these characteristics interact with physiological processes to determine growth.

Information from seedling studies, both in the greenhouse and field, only provide the basic understanding of physiological response to the environment. What is needed are more large scale experiments involving more mature trees under natural environmental conditions (Mooney 1991). This concept however, will require extensive sampling and modification or development of new techniques to measure older tree canopies. Based on the amount of variability found within the forest canopy, sampling schemes can be developed to accurately represent this variation. With data of microenvironmental parameters, the relationships between physiological processes and the variation found can be established and modeled.

CHAPTER 2

PHYSIOLOGICAL RESPONSE OF TWELVE-YEAR OLD LOBLOLLY PINE STANDS FIVE YEARS AFTER THINNING

INTRODUCTION

The overall objectives of this project were to develop a better idea about the variability of physiological responses in a managed loblolly pine stand and provide base line data for future global climate change research. The specific objectives were to contrast physiological responses between upper crown and lower crown foliage in thinned and unthinned treatments within unfertilized plots. Secondly, to contrast physiological responses between upper crown and lower crown foliage in thinned and unthinned treatments within fertilized plots.

Climatic conditions, edaphic factors and biotic conditions within forest stands control physiological processes in forest stands. The physiological processes in turn control growth and tolerance to environmental stress. The controlling factors are all important in determining the survival, growth, and development of forest stands. The development of working models to explain processes in a forest stand requires a precise knowledge of the variation in these factors and in the physiological responses. Research information from field situations is necessary so that the knowledge gained can be the basis for describing ecosystem functioning. Comparison of responses in stands, with controlled density and nutrient status, allows researchers to test the effects

that different environmental parameters have on a tree's physiological responses and growth. Solar radiation (Leverenz et al. 1982, Johansson 1989) and ambient air temperature (Bargain 1974) vary temporally and spatially within the forest canopy. Foliar physiological responses to these change in response to environmental conditions (Leverenz and Jarvis 1979, 1980, Leverenz et al. 1982, Perry 1971, Waring et al. 1963, Woodman 1971) are modified by silvicultural practices such as thinning and fertilization (Ginn et al. 1991, Leverenz and Jarvis 1979, Nowak et al. 1990, Waring et al. 1963).

Pollutants have increased in the Earth's atmosphere to levels that have alerted scientists about possible impending climatic changes. Increased CO_2, the primary contributor to the "Greenhouse Effect," is predicted to have both direct and indirect effects on plant communities (Mooney 1991). Direct impacts may include a fertilizer effect due to the increased atmospheric CO_2 concentrations and diminished water stress caused by increased water-use efficiency of some C_3 plants. Indirectly, plants may be effected by relatively, geologically speaking, quick climatic changes (Ryan 1991). The response of plants to these changes cannot be predicted without a precise understanding of the basic physiology of trees in a forest ecosystem.

Research in forestry has already contributed much to the continual growth and productivity of southern forests. However, if forest productivity is to be maintained, and if gains are to be made in the field of forest management, it is necessary to address the major concerns stated previously. Physiological processes, such as photosynthesis, transpiration, and carbon allocation must be studied in order to

understand how they interact with climatic and edaphic factors. A better understanding of the physiology of trees in a stand is necessary to produce the methodologies to measure and manage timber stands more effectively. This study provides information on the physiological responses related to forest productivity and evaluates the efficiency and desirability of cultural practices.

Loblolly pine (*Pinus taeda L.*) occupies a wide geographic range across the southern United States. It is an important component of a variety of forest types, and is one of the nation's major commercial timber species (U.S.D.A. F.S. 1987). Loblolly pine is the primary species used for forest regeneration in the southeastern United States. Within Louisiana, forest products are one of the primary economic resources. Some major concerns about southern forests include (1) evidence for growth decline in some areas, (2) demand for timber is expected to increase, and (3) changing climatic conditions will impact southern forests in an unknown way. Much of the past research has focused on seedling and juvenile tree physiology. However, work on more mature trees, *in situ*, is required in order to develop a better understanding of how natural stands and ecosystems function in response to their environment.

Recent ecophysiological studies suggest that the forest canopy is a heterogeneous structure (Ford 1992, Ginn et al. 1991, Leverenz 1990, Nowak et al. 1990). Thinning has noticeable effects on environmental factors within a forest stand, such as increased soil moisture (Cregg et al. 1990) and increased nutrient availability. Thinning also results in a number of changes in microclimatic factors, e.g., increased light to lower branches. Nowak et al. (1990) examined the relationship between

thinning and several physiological parameters. They used scaffolding to access the crowns for measuring photosynthesis, needle chlorophyll content, dark respiration and water potential in thinned and unthinned stands of loblolly pine. These researchers were only able to obtain data on one tree per treatment. The means of two samples for each measurement period were then lumped over the season for statistical purposes. Nowak et al. (1990) reported photosynthesis was lower on a seasonal basis for the lower crown level of the unthinned treatment than for the upper crown level. No difference in photosynthesis between crown levels was apparent in the thinned treatment. They did not detect differences among the thinning treatments in midday water potential by crown level. They concluded that photosynthetic rates paralleled light levels within the crown and that water potential and chlorophyll content had no effect on photosynthesis.

Later, Ginn et al. (1991) reporting results on the same site, demonstrated that light saturated photosynthetic rate of needles of loblolly pine at different vertical levels in the canopy varied with management practices. However, they used detached branches collected at mid-day and exposed them to full sunlight to evaluate the potential maximum photosynthetic responses. In thinned stands, they found lower and upper crown photosynthesis did not differ significantly. However, they found significantly higher midday stomatal conductances in the lower crowns of the thinned compared to the unthinned treatment. This is in contrast to the unthinned stands where light saturated photosynthesis was lower in the lower crown. Since light levels in the lower canopy of unthinned stands are unlikely to ever reach saturation, this may

have little meaning in terms of actual photosynthesis rates. It also is unclear as to the rate of change in the light response once stands are thinned.

The majority of past studies estimated photosynthesis with portable gas exchange systems that commonly measure only a small number of needles flattened into a single plane and held horizontally. An assumption is made that all needles normally receive identical amounts of light and that PPFD is accurately measured in the horizontal plane. Oker-Blum et al. (1983) demonstrated that there exists a functional relationship between light and photosynthetic response that was different for branches than for needles. Light response curves significantly differed relative to direction of radiation on the needles. Therefore, studies of trees within stands need to account for PPFD relative to branch orientation rather than those measured only at the horizontal position.

Together the previously discussed studies (Ginn et al. 1991, Nowak et al. 1990, Oker-Blum et al. 1983) suggest that the canopy of a loblolly pine stand cannot be treated as a homogenous volume of foliage. The previous studies also suggest more intensive studies could produce insight into physiological responses leading to increased growth in thinned stands. In contrast to previous studies (Ginn et al. 1991, Nowak et al. 1990), the design of this study is such that the measurement periods over a growing season are kept separate in the statistical analysis by including measurement period in the model as a repeated measure. This study also involves sampling of attached branches under ambient environmental conditions of a larger number of trees than previously reported.

MATERIALS AND METHODS

Study Site

Loblolly pine (*Pinus taeda* L.) trees for this study are located in the West Pasture, Johnson Tract, Palustris Experimental Forest, Rapides Parish, Louisiana, in North 1/4 of section 4, T.2N., R.3W. adjacent to the South Road. The site has a Beauregard silt loam soil (fine-silty, siliceous thermic plinthaquic paleudults). Soil drainage and slope is sufficient that water does not stand on the site.

The 0.93 ha study area was originally planted in May 1981 (at 1.82 meter by 1.82 meter spacing) with 14-week old loblolly pine seedlings that were grown in styrofoam blocks. The understory hardwood trees, shrubs and *Rubus sp.* were cut from between the rows of pine trees with a mower. In 1988, twelve research plots were established for study.

Experimental Treatments--For growth studies, the U.S.D.A. F.S. Timber Management research Unit, Pineville, LA, installed two cultural treatments in the fall of 1988. The treatments described below were randomly assigned to the twelve plots in a two-by-two factorial design with three replicates. Each plot is 23.77 meters by 23.77 meters (0.056 ha). The treatments were as follows:

(1) Pine density. The plots were either thinned to a density of 731 trees per hectare or left unthinned at a density of 2990 trees per hectare. Thinned plots were obtained by removing every other row of trees and every other tree in the remaining rows. This thinning resulted in a 3.66 by 3.66 meter spacing between trees.

(2) Fertilization. The plots were either left unfertilized or fertilized with diammonium triple superphosphate was applied at 744 kg per hectare (150 kg phosphorus and 134 kg nitrogen per hectare).

Study Plots--In April 1990, four plots representing the treatments were chosen from the twelve plots available. The plots chosen are located adjacent to one-another, around a central point (Replicate 1). This design was necessary in order to accommodate the construction of a central data acquisition system. The high cost and maintenance problems associated with long sensor leads and crown platforms necessitated this design. The central data acquisition system collects environmental and edaphic information on the four study plots in replicate one. Measurements of light and temperature acquired by the data acquisition system are sent to an on-site computer.

In 1991, a series of steel towers and wooden walk-ways were constructed to gain access to the upper and lower half of each tree crown accessible from the tower system. The tower system completely surrounds at least two trees and borders on one side of at least eight more trees, depending on the treatment, thus allowing at least partial access to a minimum of ten tree crowns per treatment.

Replicates of each treatment combination (replicate 2) were established using sixteen sets of steel towers, i.e., four towers per treatment combination. These access towers were permanently erected in plots adjacent to the main plots discussed previously. Each of the towers in the second replicate allow access to portions of the

south side of two trees and the north side of two additional trees for a total of eight trees per treatment combination.

Microenvironmental Data

Four data acquisition systems (Keithely, Model 576) continually recorded average ambient branch air temperature and average branch photosynthetic photon flux density (PPFD) levels at two different levels in the canopy. Average branch PPFD and ambient air temperature within canopy were measured on three sample branches for branch exposures (north and south) within each of two crown levels (upper and lower) and for each treatment combination on the first replicate only. This scheme provides estimates of average branch air temperature and average branch PPFD levels for a total of twelve branches per treatment and forty-eight overall. All data acquisition systems were periodically polled, and data was uploaded to a personal computer via a fiber optic interface.

Sample branches were selected to represent branches from three trees within each treatment combination. Sample branches were selected at random from the south side of available branches within the upper one-third and lower one-third of each sample tree crown. Microenvironmental data was polled, via the data acquisition system, at fifteen minute intervals during the day and night.

Average branch PPFD along sample branches was measured by attaching a series of four photodiodes to the foliated part of the branch. Two sensors were placed in a vertical position, 180° from each other, facing toward the tip and base of the branch. The other two were in a horizontal position with one sensor facing upward

and the other down towards the ground. The PPFD sensors were matched to LI-COR, LI192 quantum sensors. Average branch temperature was measured by sensors mounted on the same housing as the light sensors and consisted of two solid state temperature sensors wired in parallel.

Experimental Approach

Sampling--For each of the four treatment combinations (thinned/unfertilized, thinned/fertilized, unthinned/unfertilized and unthinned/fertilized) a standard sampling scheme for physiological measurements was established. The trees in these plots were of one social structure, i.e., no obvious dominate and codominate individuals, owing to the even-age planting and relative young stand age, but they were changing as of the spring of 1994. Current mature foliage from branches on the south side of the tree crowns was randomly sampled at two vertical levels (upper and lower canopy). Branches of the upper and lower one-third of the canopy delineated the two canopy levels. For the purpose of comparison between canopy levels and treatments, only terminal shoots or adjacent lateral shoot foliage was sampled. The physiological measurements were conducted on two to three fascicles (6-9 needles) for each sample branch. All measurements were taken on each of the four treatments, replicated twice, and repeated four times during the growing season. Physiological measurements were recorded for each set of needles as described in the following sections.

Gas Exchange Measurements--Photosynthetic rate, transpiration, stomatal conductance, PPFD, and water-use efficiency(WUE) were measured or calculated on

the mid-section of needles of two to three fascicles per branch using a LI-COR, LI6200 Portable Photosynthesis System under ambient light and temperature conditions. The needles were then removed and the xylem water potential determined with a pressure chamber (PMS Instrument Corp., Corvallis, OR) using the precautions of Ritchie and Hinckley (1975). The needles were then sealed in plastic bags, placed on ice, and later analyzed for fresh and dry weights as described in the appropriate section. Projected needle area was calculated and recorded as described in the following section. Needle dry weight also was used as a basis for photosynthetic CO_2 uptake since the projected area of a needle does not take into account differences in needle thickness. Differences in leaf thickness in sun and shade leaves of woody plants is well documented (Larcher 1983).

Three branches, from three different trees, were chosen from the upper and lower canopy levels for each of the treatment combinations. The sampling procedure was such that on the first day of a sample period a single fertilizer treatment (e.g., unfertilized) was measured to compare thinning treatments. In the morning, one thinning treatment (e.g., thinned) was tested followed by the other treatment (e.g., unthinned) on replicate one. Immediately following this set of measurements the sampling scheme was repeated for the second replicate treatment blocks. In the afternoon, the sampling scheme was repeated as described for the morning except the sampling order was reversed. On the second day of each sample period the above procedure was performed for both thinning treatments on the other fertilizer (e.g.,

unfertilized) treatment. Time constraints and sampling procedures prevented sampling all four treatments on the same day.

Methods to Determine Needle Properties

After returning to the laboratory, projected leaf area was measured with a leaf area meter (LI-COR model LI-3000, Lincoln, NE) and the tissue oven-dried at 65° C for 48-hours. Projected leaf area and dry weights were recorded and specific leaf weights calculated on the portion of the fascicle enclosed in the LI-COR cuvette.

Branch Carbon Exchange Index (BCEI)

Predicted branch carbon uptake was calculated for the current flush of foliage used in the physiological measurements. This index was used as an estimate of the net amount of carbon being assimilated by the most recent fully expanded cohort (mature foliage of the same age) of a branch. On the same site, a study assessing the impact of silvicultural practices on tree phenology was being conducted. Information regarding mean fascicle length and number from the phenology study was used to calculate projected branch needle surface area, for a cohort. Linear regression equations for the fertilizer by thinning by canopy level combination were found to have significantly different slopes and levels for each combination in predicting projected leaf area from fascicle length (PROC GLM, SAS Institute, Inc. Cary, NC). Therefore, separate equations were used to determine total needle projected surface area for a cohort, within a given canopy level/treatment combination. Branch carbon

exchange indices were calculated by multiplying the total number of fascicles by the mean projected leaf area per fascicle, and multiplying the previous result by the mean photosynthesis rate per unit needle surface area. Regression equations were used to predict projected leaf area as follows:

Unfertilized-Unthinned:	Lower LA = -0.9884 + 0.33350 x Length;	$R^2 = 0.72$
	Upper LA = -0.6452 + 0.33447 x Length;	$R^2 = 0.71$
Unfertilized-Thinned:	Lower LA = -0.3817 + 0.28994 x Length;	$R^2 = 0.57$
	Upper LA = -0.4797 + 0.32838 x Length;	$R^2 = 0.75$
Fertilized-Unthinned:	Lower LA = -0.7793 + 0.28698 x Length;	$R^2 = 0.72$
	Upper LA = -1.1797 + 0.37033 x Length;	$R^2 = 0.65$
Fertilized-Thinned:	Lower LA = -1.0410 + 0.33806 x Length;	$R^2 = 0.75$
	Upper LA = -0.9401 + 0.35846 x Length;	$R^2 = 0.57$

where LA = projected leaf area (cm^2) per fascicle, Length = length of fascicle (cm). Number of needles per fascicle rarely deviated from three.

Statistical Analyses

In general, analysis of variance was used to test the significance of differences between treatments within fertility levels for xylem water potential, photosynthetic rate, transpiration, stomatal conductance, and water-use efficiency using PC-SAS statistical software (SAS Institute, Inc.). Plots of ground were the experimental unit for physiological and environmental comparisons. The experimental design is a repeated measure with a split-split-plot. The whole plot consists of the thinning treatment, and subplot treatments of canopy level (upper and lower) and time-of-day (AM and PM), with the measurement periods (8/92, 4/93, 5/93, 6/93 and 9/93) as the repeated measure. A separate ANOVA was used for each of the fertilization treatments. This

was necessary because sampling time constraints did not allow us to sample both the unfertilized and fertilized treatments on the same measurement day.

Null hypotheses tested in this study included:

H_0: Crown position has no impact on physiological responses, such as photosynthesis, water relations, and transpiration in fertilized plots.

H_0: Crown position has no impact on physiological responses, such as photosynthesis, water relations, and transpiration in unfertilized plots.

H_0: Thinning has no impact on physiological responses, such as water relations, photosynthesis, and transpiration in unfertilized plots.

H_0: Thinning has no impact on physiological responses, such as water relations, photosynthesis, and transpiration in fertilized plots.

RESULTS

Thinning resulted in a significant (Table 2-1; $p= 0.001$) increase in PPFD levels within the canopy of the thinned, fertilized plots (Figure 2-1, A&B). Although not significant (Table 2-2; $p= 0.061$), the thinned, unfertilized treatment also had higher within canopy PPFD levels higher than the unthinned, unfertilized plots (Figure 2-2, A&B). A significant time-of-day effect was found within both the fertilized and unfertilized treatments. PPFD levels in the lower crown of all treatments were higher in the PM (1-3 p.m.) than in the AM (9-11 a.m.). Additionally, PPFD levels were significantly different for canopy level, with the upper canopy level having higher values.

Thinning did not have a significant impact on any of the needle physiological characteristics studied in either the unfertilized or fertilized treatments (Figures 2-1 & 2-2, C-F; Figures 2-3&2-4, A-H). There was however, a tendency for the thinned, lower crown foliage to have greater mean photosynthesis, stomatal conductance and transpiration than that of the unthinned, lower crown.

Within treatments, photosynthesis was significantly different with respect to canopy level photosynthesis rates. The upper canopy foliage generally had higher physiological activity than did the lower crown foliage. For example, photosynthesis per unit needle surface area was found to be significantly higher in the upper canopy foliage within both the fertilized (Figure 2-1, C-D) and unfertilized (Figure 2-2, C-D) treatments ($p= 0.001$). Photosynthesis in unfertilized treatment showed a significant canopy level by time-of-day interaction (Figure 2-2, C-D; $p= 0.01$). Photosynthesis in lower canopy foliage was significantly higher in the afternoon than in the morning sampling period within the unfertilized plots (Figure 2-1, C versus D). The photosynthesis pattern, on a per unit dry weight basis, was similar except a significant interaction of level with thinning ($p= 0.04$) was detected for the fertilized treatment plots (Figure 2-1, E&F). Lower canopy net photosynthesis was generally greater for the thinned versus unthinned treatments.

Needle conductance (Figure 2-3, A&B) also differed significantly by canopy level and an interaction was found between canopy level and measurement period ($p= 0.005$). A nearly significant level by time-of-day interaction ($p= 0.06$), was also

Table 2-1. ANOVA table and probability values for photosynthetic photon flux density (PPFD), net carbon exchange on a leaf area (A_{la}) and dry weight basis (A_{dw}), needle conductance (g_C), transpiration (E), water-use efficiency (WUE) and needle xylem water potential (Y) within the fertilized treatment.

		p-value						
SOURCE	DF	PPFD	A_{la}	A_{dw}	g_C	E	WUE	Y_{xylem}
Thinning (Th)	1	0.0013	0.4082	0.4993	0.5584	0.8643	0.5245	0.6781
Error A	2							
Measurement Period (MP)	4	0.0168	0.0001	0.0001	0.0076	0.0028	0.0001	0.0001
Th x MP	4	0.4414	0.1997	0.0767	0.0718	0.2571	0.7834	0.7468
Error B	8							
Time-of-Day (Td)	1	0.0067	0.7325	0.6100	0.0360	0.0122	0.0714	0.0043
Th x Td	1	0.2233	0.3983	0.7471	0.5693	0.8393	0.6647	0.6449
Error C	2							
MP x Td	4	0.2622	0.4593	0.1914	0.2106	0.2450	0.1600	0.3321
Th x MP x Td	4	0.3681	0.3153	0.0998	0.9441	0.9589	0.6886	0.9159
Error D	8							
Level (Lev)	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0196	0.0001
Th x Lev	1	0.1756	0.0689	0.0398	0.1056	0.0714	0.4111	0.4291
Error E	2							
MP x Lev	4	0.4039	0.1425	0.0125	0.0292	0.0198	0.1914	0.6524
Th x MP x Lev	4	0.6329	0.4626	0.1609	0.2814	0.2599	0.8685	0.4237
Error F	8							
Td x Lev	1	0.5575	0.0992	0.1163	0.0616	0.0034	0.0113	0.2418
Th x Td x Lev	1	0.8413	0.7155	0.5921	0.6837	0.0664	0.0743	0.7171
Error G	2							
MP x Td x Lev	4	0.3582	0.7436	0.3203	0.8393	0.0545	0.0038	0.5142
Th x MP x Td x Lev	4	0.9495	0.7930	0.6256	0.6873	0.5166	0.0849	0.8872
Error H	8							
TOTAL	79							

Table 2-2. ANOVA table and probability values for photosynthetic photon flux density (PPFD), net carbon exchange on a leaf area (A_{la}) and dry weight basis (A_{dw}), needle conductance (g_C), transpiration (E), water-use efficiency (WUE) and needle xylem water potential (Y) within the unfertilized treatment.

		p-value						
SOURCE	df	PPFD	A_{la}	A_{dw}	g_C	E	WUE	Y_{xylem}
Thinning (Th)	1	0.0631	0.2586	0.5237	0.6444	0.5075	0.8617	0.1076
Error A	2							
Measurement Period (MP)	4	0.0061	0.0009	0.0028	0.0010	0.0170	0.0110	0.0233
Th x MP	4	0.6993	0.6455	0.4562	0.9937	0.9387	0.1778	0.9956
Error B	8							
Time-of-Day (Td)	1	0.0199	0.8737	0.9634	0.0764	0.0239	0.1108	0.0034
Th x Td	1	0.3491	0.1072	0.6533	0.3900	0.6051	0.9617	0.4181
Error C	2							
MP x Td	4	0.7470	0.0062	0.0003	0.4483	0.0138	0.0376	0.2111
Th x MP x Td	4	0.6813	0.2236	0.0293	0.3186	0.0377	0.2111	0.2516
Error D	8							
Level (Lev)	1	0.0001	0.0001	0.0002	0.0001	0.0001	0.0062	0.0055
Th x Lev	1	0.2208	0.1370	0.4001	0.0408	0.0677	0.2192	0.9864
Error E	2							
MP x Lev	4	0.2469	0.0699	0.0098	0.3019	0.0768	0.1412	0.7465
Th x MP x Lev	4	0.1446	0.3610	0.6718	0.2638	0.3082	0.4202	0.7750
Error F	8							
Td x Lev	1	0.0745	0.0135	0.0099	0.0054	0.7995	0.1562	0.3115
Th x Td x Lev	1	0.4324	0.1362	0.9531	0.0032	0.0813	0.3017	0.8656
Error G	2							
MP x Td x Lev	4	0.6134	0.0776	0.0067	0.0186	0.2505	0.1200	0.0042
Th x MP x Td x Lev	4	0.3288	0.9382	0.1737	0.1558	0.3962	0.6267	0.8602
Error H	8							
TOTAL	79							

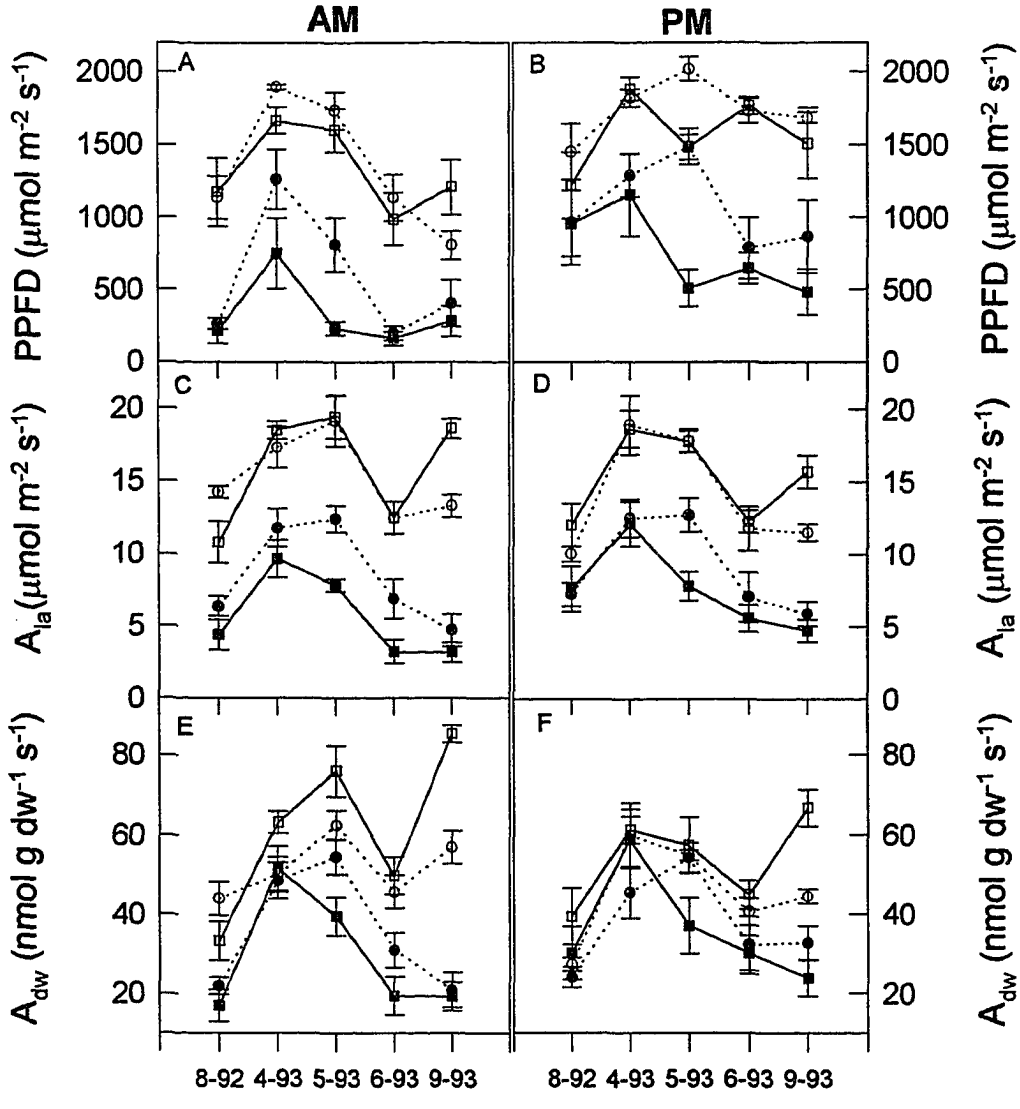


Figure 2-1. Fertilized treatment photosynthetic photon flux density (PPFD) and net CO_2 exchange on a projected leaf area (A_{la}) and dry weight (A_{dw}) basis for fertilized treatment during the AM and PM sampling period. Bars indicate plus or minus one standard error of the mean. (n=6; symbols: ■ = unthinned-lower, □ = unthinned-upper, ● = thinned-lower, ○ = thinned-upper).

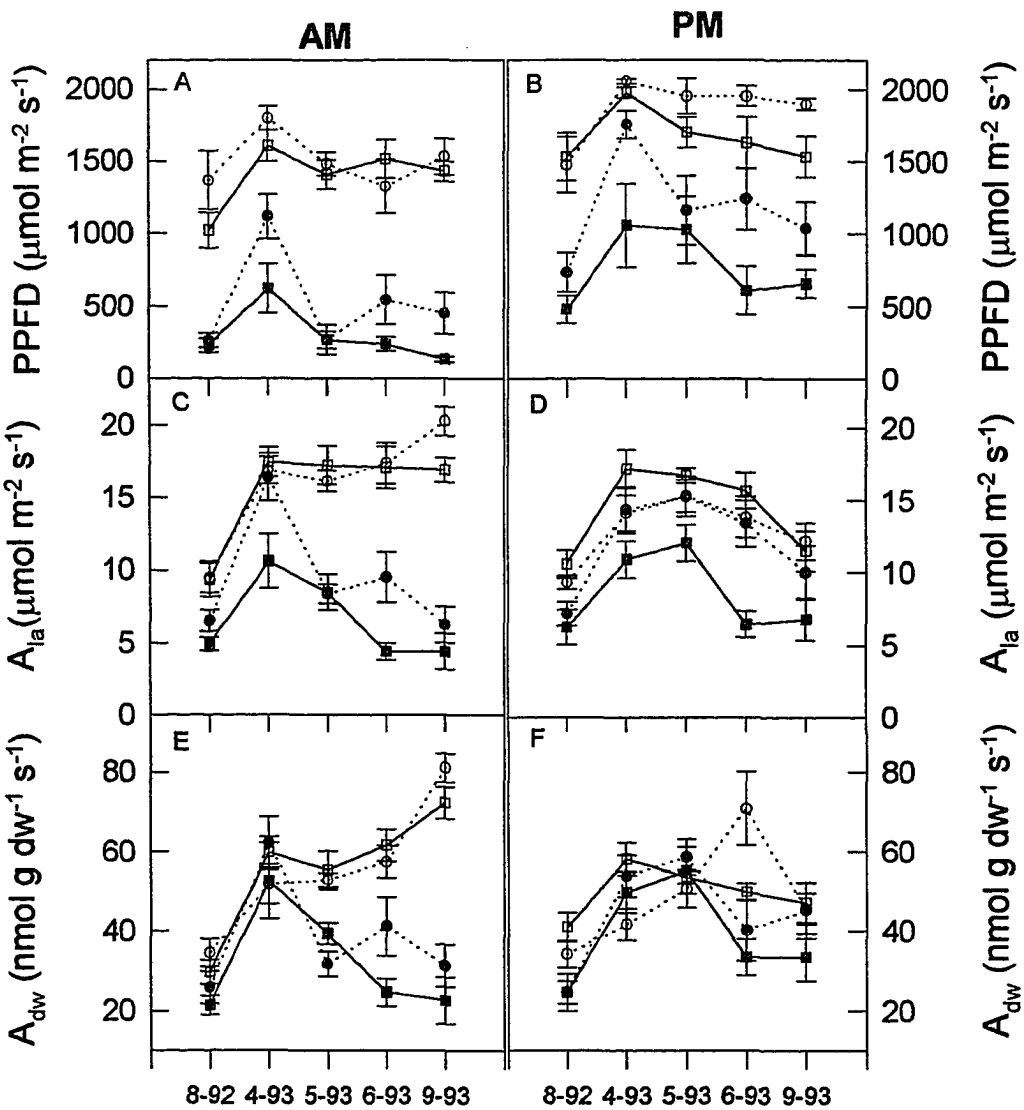


Figure 2-2. Unfertilized treatment photosynthetic photon flux density (PPFD) and net CO_2 exchange on a projected leaf area (A_{la}) and dry weight (A_{dw}) basis for fertilized treatment during the AM and PM sampling period. Bars indicate plus or minus one standard error of the mean. (n=6; symbols: ■ = unthinned-lower, □ = unthinned-upper, ● = thinned-lower, ○ = thinned-upper).

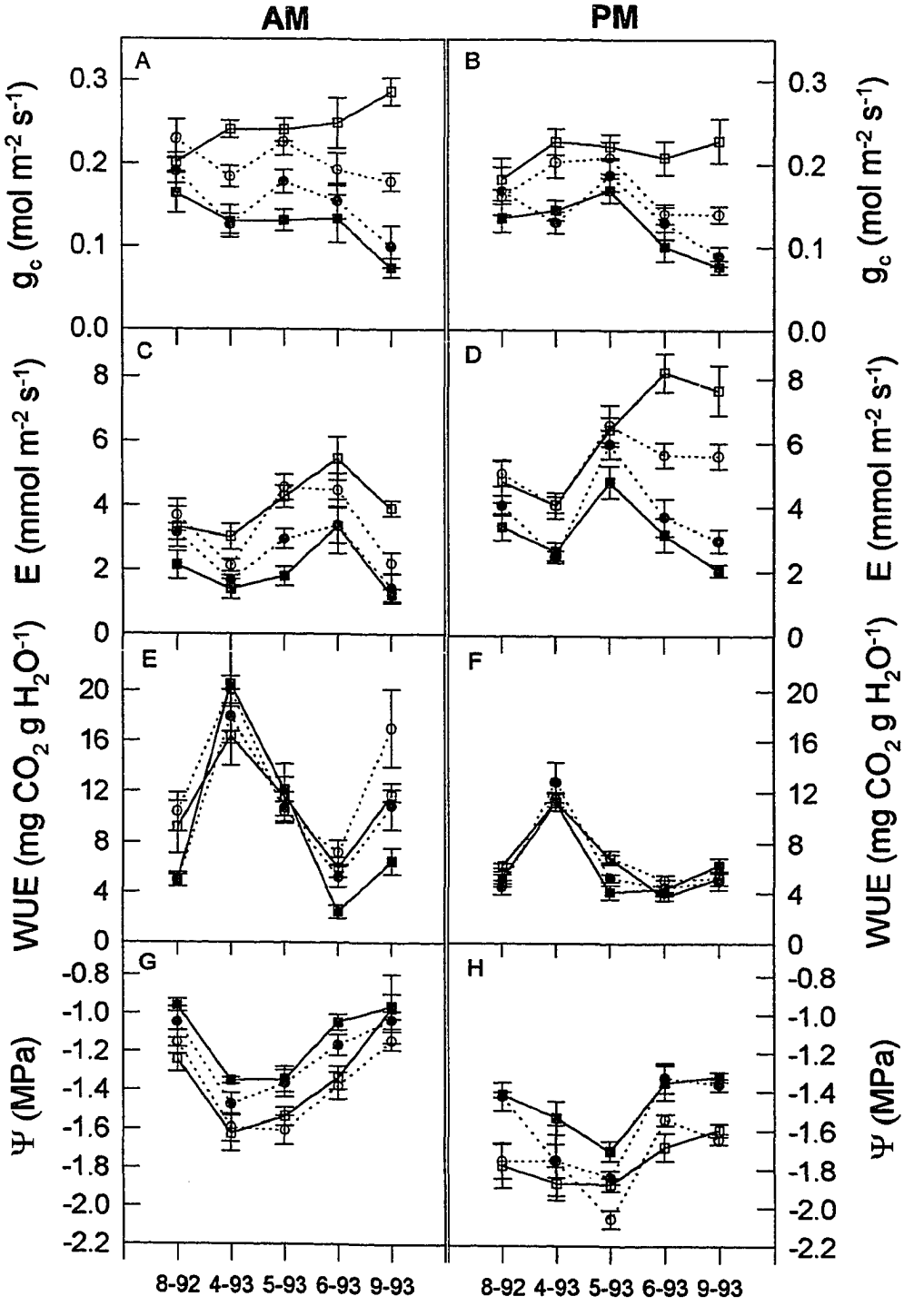


Figure 2-3. Fertilized treatment needle conductance (g_c), transpiration (E), water-use efficiency (WUE) and needle xylem water potential (Ψ) during the AM and PM sampling period. Bars indicate plus or minus one standard error of the mean. (n=6; symbols: ■ = unthinned-lower, □ = unthinned-upper, ● = thinned-lower, ○ = thinned-upper).

detected within the fertilized treatment. Generally, upper canopy foliage had higher needle conductances and concurrently higher transpiration rates than the lower canopy foliage. The unthinned upper canopy foliage tended to have slightly higher values for conductance and transpiration than the thinned plots.

Needle conductance in the unfertilized treatment (Figure 2-4, A&B) followed the same pattern as in the fertilized treatment. A significant thinning by time-of-day by canopy level interaction ($p= 0.003$) was apparent. Needle conductance in the lower crown of the unfertilized, thinned plots tended to have higher needle conductance and transpiration values in the afternoon, while the lower crown decreased in the unfertilized, unthinned plots. Needle conductance values were greater at the upper canopy level of both the thinned and unthinned plots. Transpiration rates for thinned and unthinned stands within both fertilizer treatments mirrored those trends seen in needle conductance (Figures 2-3 & 2-4, C&D).

Water-use efficiency within the unfertilized treatment (Figure 2-4, E&F) differed significantly by canopy level in the morning. A significant interaction between time-of-day and measurement period existed within the unfertilized treatment. The fertilized treatment (Figure 2-3, E&F) had a significant canopy level by time-of-day by measurement period interaction. Water-use efficiency was generally greater for the upper canopy level than for the lower canopy level in both the fertilized and unfertilized treatments during the morning (Figure 2-3 & 2-4, E). Afternoon values for WUE were lower and not significantly different for any of the treatment combinations (Figure 2-3 & 2-4, F).

Xylem water potential in the fertilized treatment (Figure 2-3, G&H) was not significant for any of the interactions, only the main effects of measurement period, time-of-day and canopy level were significant. Fertilized treatment xylem water potentials were higher in the lower canopy than in the upper canopy, during the morning sampling period. Xylem water potential in the unfertilized treatment (Figure 2-4, G&H) was also significant for a canopy level by time-of-day by measurement period interaction. Upper canopy level foliage had lower water potentials than the lower canopy level, and the afternoon water potentials were lower than in the morning.

Mean predawn xylem water potentials were higher (-0.55 MPa) for the thinned versus the unthinned stands (-0.61 MPa) within the fertilized treatment (Figure 2-5A), and the differences were significant for eleven of the thirty days measured. Although consistent thinning effects were not evident within the unfertilized plots (Figure 2-5B), the unthinned stands generally had greater (less negative) mean predawn water potentials than that of the thinned stands.

Branch carbon exchange index values were significantly different with respect to canopy level within both the fertilized and unfertilized treatments (Figures 2-6 & 2-7), respectively. However, a significant interaction of thinning by canopy level by time-of-day was found within both fertilizer treatments. The potential amount of carbon taken up by branches in the upper canopy was significantly greater than that of the lower branches. Thinned plot, lower branches had greater carbon uptake than lower branches in the unthinned treatments. Branch carbon exchange index in the fertilized plots was significantly higher for the upper crown branches of the thinned versus

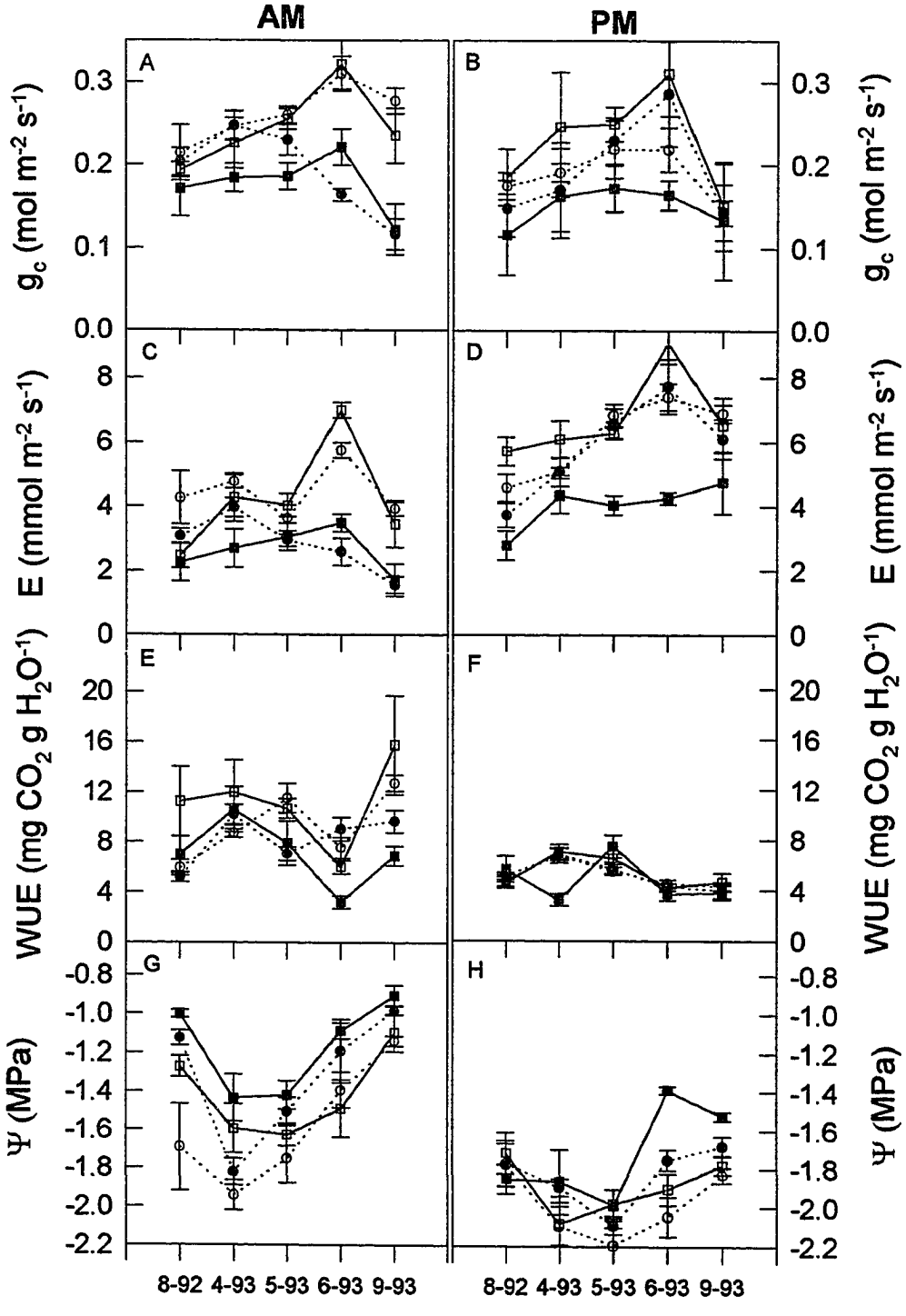


Figure 2-4. Unfertilized treatment needle conductance (g_c), transpiration (E), water-use efficiency (WUE) and needle xylem water potential (Ψ) during the AM and PM sampling period. Bars indicate plus or minus one standard error of the mean. (n=6; symbols: ■ = unthinned-lower, □ = unthinned-upper, ● = thinned-lower, ○ = thinned-upper).

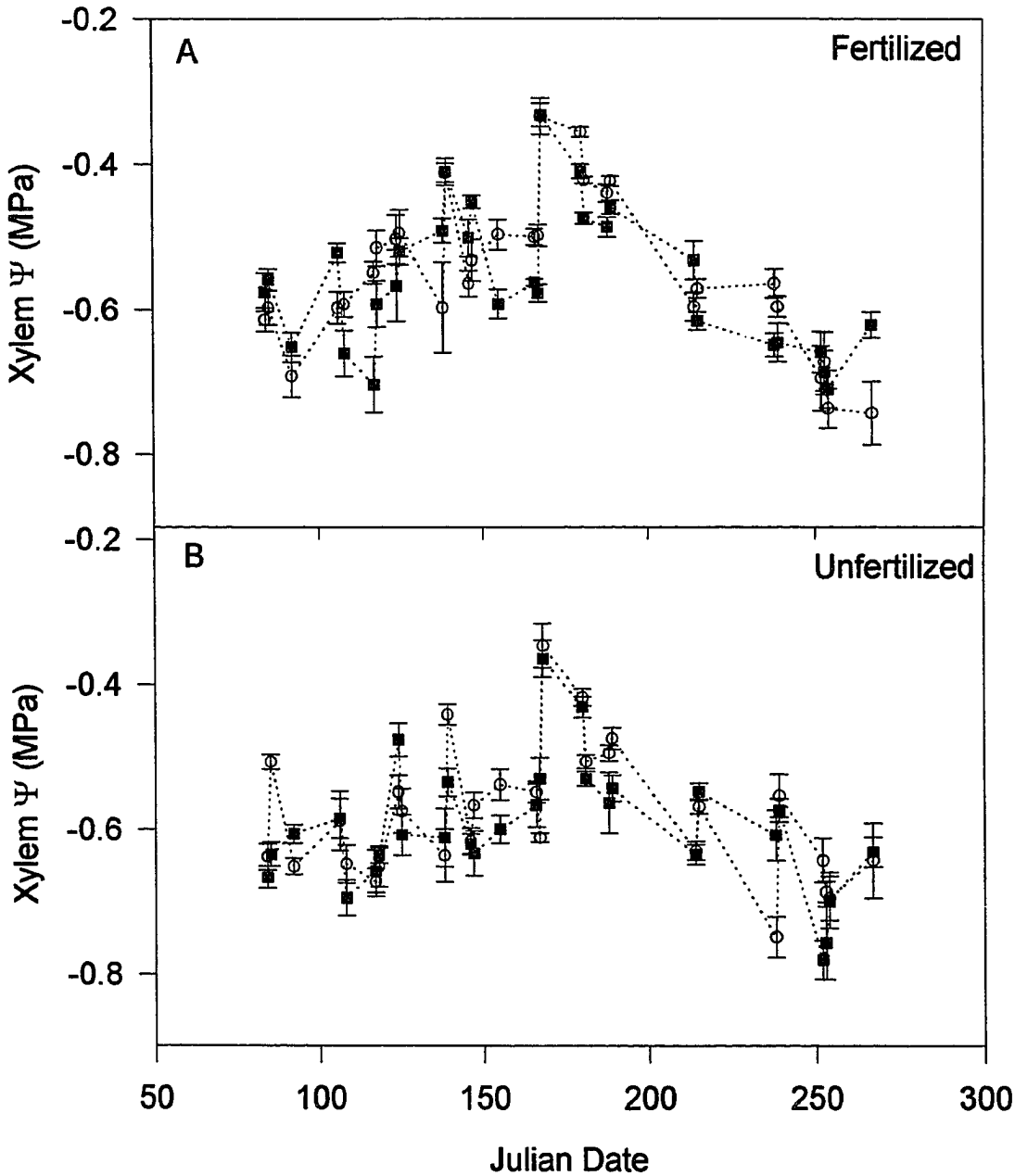


Figure 2-5. Seasonal trends in predawn needle xylem water potential (Ψ) for fertilized (panel A) and unfertilized (panel B) treatments. (n=6, symbols: ■ = unthinned, ○ = thinned)

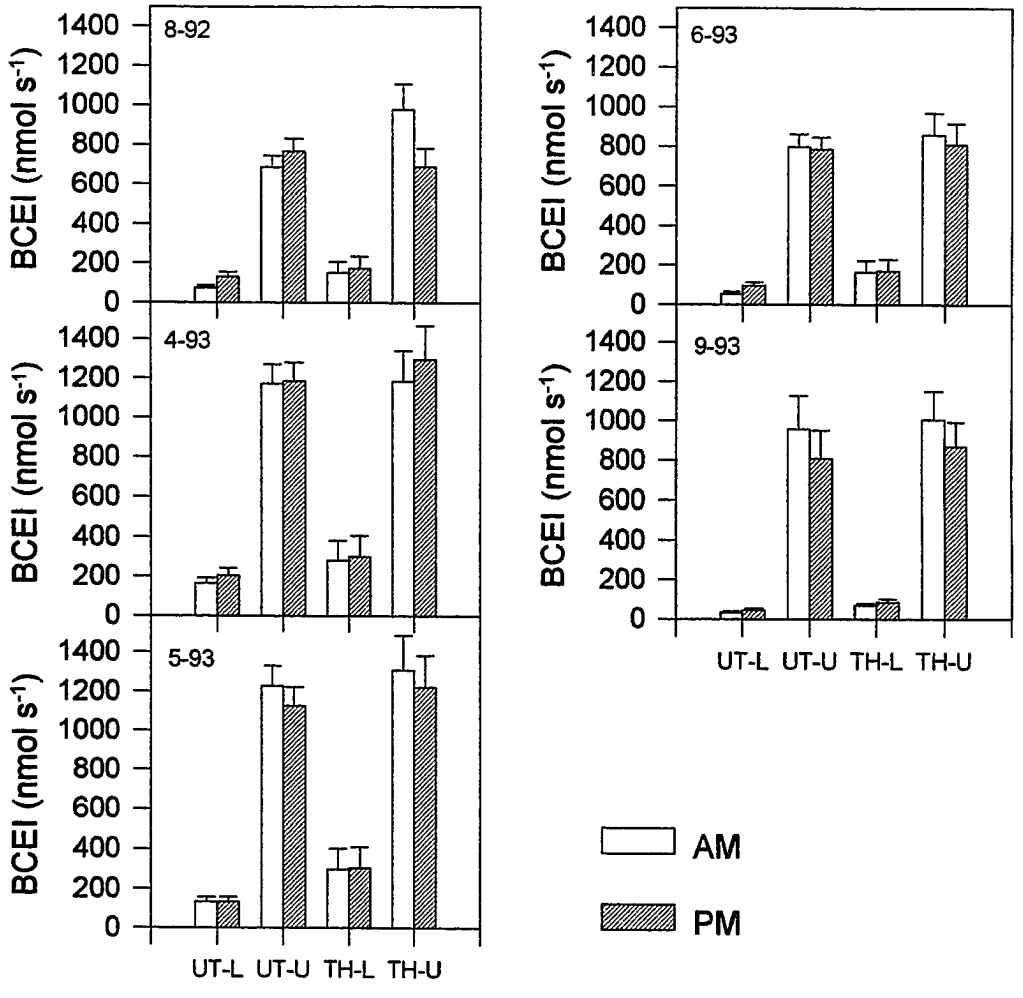


Figure 2-6. Fertilized treatment mean Branch Carbon Exchange Index (BCEI). Error bars represent plus one standard error of the mean. (UT = unthinned, TH = thinned, L = Lower, U = Upper; n=4)

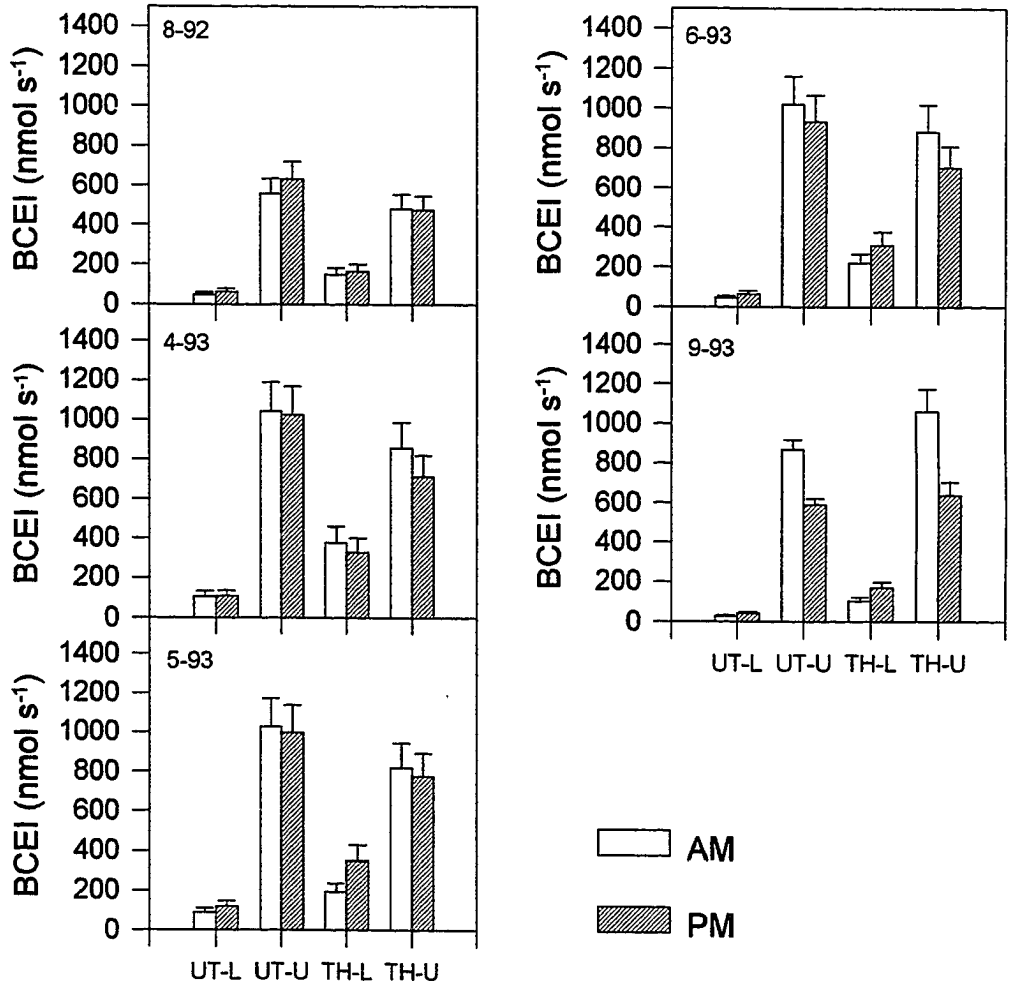


Figure 2-7. Unfertilized treatment mean Branch Carbon Exchange Index (BCEI). Error bars represent plus one standard error of the mean. (UT = unthinned, TH = thinned, L = lower, U = upper; n=4).

unthinned treatments. The reverse situation occurred in the unfertilized plots with upper branches of the unthinned plots higher than the thinned plots.

DISCUSSION

Physiological activity was generally greatest during the April and May, 1993 (Figures 2-1 to 2-4) measurement periods. This physiological activity coincided with the peak needle and shoot development and the branch elongation period during the spring (unpublished data). In addition, spring environmental conditions were more favorable for photosynthesis with lower air temperatures, lower fascicle water stress, and somewhat greater PPFD levels, relative to the other measurement periods. Lower temperatures are especially conducive to higher net photosynthesis because of the impact higher temperatures have on increasing photorespiratory carbon loss.

The effect of thinning on canopy PPFD levels was still evident in the fertilized plots five years after the thinning treatment was applied. Average PPFD levels for measured branches were higher in the lower canopy of the thinned stand, than in the lower canopy level of the unthinned plots. While not significant ($p= 0.07$), PPFD patterns were similar in the unfertilized treatment. Unlike the results found by Nowak et al. (1990), measured three years after thinning, lower canopy needle photosynthesis did not differ between the lower canopy level of the thinned and the lower canopy level of the unthinned plots within either fertilizer treatment. Also, few interactions of thinning and canopy level were found for any of the physiological measurements in this study. This indicates that the lower canopy foliage was not responding differently

to thinning with respect to within canopy PPFD or needle physiology, i.e., the lower crown foliage of both the thinned and unthinned stands are similar in their physiology. This could be explained by the results of crown growth during the four years following thinning. Shoot growth during the four years after thinning was probably sufficient to reduce the environmental differences between the lower crowns of the thinned and unthinned plots. Density for the thinned and unthinned stands, within both the fertilizer treatments, are at 40% and 90% of the maximum Reinke Stand Density Index , respectively, (personal communication, Baldwin 1993) based on data from Dean and Baldwin (1994) for loblolly pine. This lends support to the theory that a high SDI leads to the mutual shading of tree crowns. Furthermore, physiological differences between lower and upper crown foliage becomes re-established in the previously thinned stand as lower crown foliage becomes adapted to shade conditions.

It appears some residual effects of thinning may remain in the lower crown of the thinned stands (e.g., increased light availability). However, on a per unit leaf area or per unit weight basis, the foliage in the lower crown of the thinned plots may not be able to make use of the increased light, or the inherent variability in the physiological measurements did not allow us to detect differences, i.e., the variability in environmental factors is increasing in the interim between just after thinning and crown closure. The later may be the case because the foliage of branches from the lower, thinned canopy level did tend to have higher physiological activity than that of the lower, unthinned canopy. Thus, the quick utilization of the increased light seen in previous studies (Nowak et al. 1990, Ginn et al. 1991) by lower canopy foliage

following thinning tends to disappear as the crowns close. This appears to be true for this stand five years after the thinning event occurred. It is interesting to note that the traditional practice of thinning and returning in ten years may be questioned. Crown closure on a physiological basis may be much faster than the commonly used ten year cycle followed by many foresters. Of course a compromise between maximum individual tree productivity and higher stand productivity may be desirable. Thinning guidelines, using the SDI, would call for another thinning when the unthinned stands reached the current level.

Significant differences were found between the lower and upper canopy level mean PPFD measurements and all physiological measurements within both fertilizer treatments. Lower canopy PPFD levels for thinned and unthinned stands were lower than upper canopy PPFD levels by 48% and 64%, respectively, within both fertilizer treatments. In the unfertilized plots lower canopy net photosynthesis was 20% lower in the thinned and 32% lower in the unthinned treatments than that of the upper canopy level. In contrast, fertilized treatment net photosynthesis on a dry weight basis, was lowered by 23% and 45% for thinned and unthinned stands, respectively.

In addition, morning (9-11 AM) xylem water potentials were lower for the lower crown positions and generally lower in the thinned plots and no apparent relationship between predawn water potentials and photosynthesis rates were apparent. This is in line with what Cregg et al. (1990) found for stomatal conductance and the lack of a good correlation with predawn xylem potentials and absolute humidity deficits. Thus, it appears that light and or CO_2 levels may be the main controlling

factors for determining net carbon uptake in upper versus lower canopy levels, as others have found (Nowak et al. 1990, Ginn et al. 1991).

Time-of-day was significant for those physiological measurements most influenced by environmental conditions, especially incident PPFD solar radiation. The heat load on foliage from solar radiation has a direct and immediate impact on leaf vapor pressure gradients (VPG). The significant increase in radiation load on upper foliage in the AM, and all foliage during the PM sampling time resulted in reduced relative humidity, higher air temperatures and increased transpiration. Those variables most influenced included needle conductance, transpiration, water-use efficiency (WUE) and xylem water potential. A significant interaction of time-of-day by canopy level for transpiration, and a nearly significant ($p= 0.06$) one for stomatal conductance, was found for the fertilized treatment. In these stands, presumably less shortwave radiation was able to penetrate to lower foliage during afternoon in the unthinned, fertilized plots as occurred in the thinned, fertilized plots. Therefore, the lower foliage of the thinned plots received a greater radiation load and concomitant greater VPG than unthinned plots. Thus, the foliage in the lower canopy of the thinned, fertilized plots had a higher transpiration rate than the unthinned plots. The same was not true in the unfertilized plots. In this case no interaction of canopy level by time-of-day was evident. It is hypothesized that the more open nature of the crowns of the unthinned, unfertilized plots allowed more light to reach the lower canopy foliage, relative to that in the unthinned, fertilized plots (more dense upper canopy foliage). This would result in greater needle transpiration rates in the lower canopy level of the

unfertilized, unthinned stand and thus, it is physiologically similar to that of foliage in exposed crown positions. These results on transpiration patterns support the findings of Cregg et al. (1990), where a strong relationship between transpiration and the observed differences in light interception and crown exposure were found.

Water-use efficiency was primarily significant for the canopy-level effect. The unfertilized and fertilized treatments both responded in a similar manner. Water-use efficiency also is affected by changes in the vapor pressure gradient. This seems to be the case during the morning sampling period, and in the upper canopy where the WUE was higher. Transpiration was increased proportionally more in the afternoon, than the gains in photosynthesis brought about by the increased light availability, because of increased stomatal conductance and subsequent water loss. In large part, this is because of the less responsive nature of stomata to changes in the water content of the surrounding air and more directly linked to light and photosynthesis rates (Fites and Teskey 1988). Fites and Teskey (1988) using loblolly pine seedlings and trees demonstrated that the sensitivity of transpiration (E) to variations in stomatal conductance (g, $\partial E/\partial g$) increased, while the sensitivity of net photosynthesis (A) to variations in stomatal conductance(g; $\partial A/\partial g$) remained constant with increasing light. They concluded stomata do not optimize their function, as Cowan and Farquhar (1977) hypothesized, to minimize water loss for a given amount of carbon uptake.

With the intent to scale-up to the branch level a Branch Carbon Exchange Index (BCEI) was created. Branch carbon exchange index calculations allows for the convenient comparison of the potential carbon uptake by a given branch cohort. The

BCEI took into account the number and morphological differences (leaf area) of the needles on a given branch. The BCEI reflects the greater physiological activity of the upper canopy foliage. The upper canopy branches were potentially able to take up three to ten times more carbon than the lower canopy branches. This clearly points out the need to collect phenological data, in conjunction with physiological data, if we wish to understand the exchange rate and efficiency of the whole canopy. In addition, phenological studies will be required in order to scale up to the whole stand and ecosystem levels. The significant interaction of thinning by level by time-of-day is not clearly understood or easily explained. It appears that the BCEI of the upper and lower canopy levels, as well as the effects of thinning on environmental factors, function differently depending on the time-of-day. The BCEI of upper canopy branches remains similar regardless of mid-morning or mid-afternoon time period. However, in the lower canopy level the BCEI is higher in the afternoon than in the morning. This change in relative branch carbon uptake is likely to occur when afternoon water deficits restrict photosynthesis in the upper crown, while the lower crown foliage has not yet reached critical water levels, and the additional light can stimulate increased photosynthesis.

Finally, upper canopy, unthinned treatment foliage tended to have higher physiological activity than the upper canopy, thinned treatment foliage similar to the pattern reported by Nowak et al. 1990. In their study, Nowak et al. suggested that the increased activity was because the upper canopy foliage was compensating for the lower, shaded foliage. However, an alternative explanation based on current

developments and understanding of branch autonomy can be hypothesized. Sprugel et al. (1991), in a review on branch autonomy, concluded that photosynthesis rates were determined primarily by the atmosphere surrounding each branch, with the possibility that the atmosphere around a tree may have some effect. They further state that most branches are autonomous during most of the growing season. In studies using labeled carbon (Cregg 1990, Cregg et al. 1993, Loach & Little 1963, Rangnekar 1969) it has been shown that even rapidly growing shoots draw only small amounts of carbon from outside the branch. A logical conclusion is that rapidly photosynthesizing leaves may be providing photosynthates to a nearby sink. The strong local sink is hypothesized to be the rapidly growing shoots in the upper, unthinned canopy. This could result in the increased height growth often seen in dense stands under competition for light (Shugart 1984).

Franco (1986) proposed that phytochromes may be involved in triggering the increased demand by terminal buds. In addition , the role of nutrient supply also may be of importance. First of all, most of the growth in unthinned, fully-stocked stands is occurring in the upper canopy in the form of increased height and branch growth. Secondly, nutrient uptake is directed towards the upper canopy in an unthinned stand. This would in effect provide more nutrients, per unit foliage mass, for the upper canopy of the unthinned stand and would allow for the increased height growth. Thus, the increased demand by rapidly growing shoots for photosynthates, and relative abundance of nutrients to these shoots, may be responsible for the increased physiological activity of upper canopy foliage in unthinned stands.

In this study, no direct comparisons of the fertilizer treatments were done because of time constraints (because sampling of fertilized and unfertilized plots on the same days was not possible). Therefore, this discussion will be limited to the possible effects fertilization may have on thinning, time-of-day or canopy level. However, since all data were made on back-to-back days under similar conditions the possible impact that fertilization may have had on needle physiology could be hypothesized. The data generally indicate that fertilizer acts as a modifying factor by accelerating canopy closure in thinned stands leading to shade conditions, more rapidly than in unfertilized plots.

CONCLUSIONS

In assessing the cultural practice of thinning, it is clear that light levels still were significantly increased in the lower canopy of the thinned stands five years after the thinning treatment. Significant physiological differences between the foliage of thinned and unthinned stands within the upper or lower canopy as reported during the second growing season after thinning (Ginn et al. 1991) were no longer present, however.

Temporal and spatial dimensions play an important part in the variation of the physiological parameters we measured. The main effect of time-of-day for photosynthesis measurements was not significant for individual needles in the upper canopy. However, a significant interaction of time-of-day with canopy position confirmed that the increased light penetration in the afternoon led to increased

photosynthesis, stomatal conductance and transpiration of foliage on branches in the lower canopy. As found in previous studies (Ginn et al. 1991, Nowak et al. 1990), higher light levels measured for upper canopy branches resulted in greater metabolic rates for foliage in both the thinned and unthinned treatments.

Past studies (Nowak et a. 1990, Ginn et al. 1991) have indicated the need to sample immediately after a thinning treatment and continue monitoring foliar physiology at least three years. This study presents evidence that indicates some effects on foliar physiology last until the fifth growing season following the thinning treatment. Although, it does appear the differences attributed to thinning are diminishing by the fifth year. Physiological similarity of foliage between the lower canopy branches of thinned and unthinned, fertilized stands indicates that fertilization leads to quicker canopy closure, although, the BCEI data shows some potential differences continue to exist.

Of equal importance is the need to sample all canopy positions at a greater frequency during the day. Photosynthesis values of upper canopy foliage were three times those of the lower canopy, on a per unit leaf area basis (two times on a per unit dry weight basis). Nevertheless, lower canopy foliage still contributes a significant amount to the carbon uptake of a tree, particularly in the afternoon when light levels were twice that found in the morning and resulted in a significant increase (~35%) in lower canopy photosynthesis.

Diurnal response curves should be constructed to predict photosynthetic carbon gain on a daily basis by canopy level (at least upper and lower) and repeated

frequently throughout the growing season for each treatment combination. Relatively little information is known about dormant season physiology and whether spatial and temporal differences continue during the winter. Some studies, using loblolly pine seedlings, indicate continued activity in the winter (McGregor and Kramer 1963, Perry 1971).

Global changes in climate patterns, more variable rainfall, and extremes in air temperature may put greater stress on the upper canopy of forest stands. This study, as well as others, emphasize the importance of the upper canopy foliage in the overall carbon budget of the stand. Changes in environmental conditions, especially global warming and decreased rainfall, may lead to greater stress in the upper canopy. More frequent and earlier daytime water deficits could occur in the upper canopy leading to an earlier depression in mid-day stomatal conductance and a reduction in afternoon photosynthesis.

Based on this study, the consistently higher physiological activity of upper crown foliage may be important in determining overall canopy activity. This is especially true when viewed using a branch carbon exchange index with which has demonstrated appreciable carbon gain by the upper branches to the overall carbon budget. This study was conducted on a site thinned five years ago and has afforded the opportunity to look at the physiological changes that may occur as the crowns re-close. Finally, the physiological similarity of lower crown foliage of thinned versus unthinned plots is indicative of thinned stands beginning to experience crown closure. Based on this study, the increased light and growing space afforded to residual stands

after thinning have begun to decline or are at least some aspects are lost at least by the fifth growing season, especially in the fertilized stands. This study suggests that the fertilized treatment may have accelerated crown closure following the thinning treatment as might be expected. Studies of the time for the physiological responses and recovery from thinning and fertilization are needed.

CHAPTER 3

PHYSIOLOGY AND MORPHOLOGY OF BRANCHES WITH A NORTHERN OR SOUTHERN EXPOSURE IN LOBLOLLY PINE STANDS

INTRODUCTION

The primary productivity of a forest depends on the spatial distribution of foliage as well as its interaction with biotic and abiotic factors associated with photosynthesis. The distribution of foliage in forest canopies has been studied extensively for many tree species (Assman 1970, Ford et al. 1990, Ford 1992, Hallé et al. 1978, Kinerson et al. 1974). Likewise, numerous studies describing the characteristics of horizontal canopy layers in terms of foliage physiology (Ginn et al. 1991, Nowak et al. 1990, Cregg et al. 1993) and microenvironmental conditions (Bergen 1971, 1974) have been done. However, little work has been reported on the comparison of physiology and morphology within different vertical sections in the tree canopy. The purpose of this study was to compare the physiology and morphology of branches on the northern and southern sides of the tree crowns (exposure). Other work on this same site was constrained to sampling for photosynthesis and other physiological parameters from a fixed position on the tree (i.e., branches with southern exposure). Consequently, we had to assume that the southern oriented branches, occurring in the southern half of the crown, represented a

similar pattern of physiological responses and environmental conditions found in the entire tree canopy. However, one can not ignore the fact that the northern oriented branches and their associated foliage may be exposed to a different microenvironment and that they may have different physiological response levels or traits. Therefore, the purpose of this study was to test whether northern aspect foliage was physiologically and morphologically similar to the southern aspect foliage for thinned and unthinned stands.

MATERIAL AND METHODS

Study Site

Loblolly pine (*Pinus taeda* L.) trees used for this study are located in the West Pasture, Johnson Tract, Palustris Experimental Forest, Rapides Parish, Louisiana. The site has a Beauregard silt loam soil. Soil drainage and slope (1-3%) is sufficient that water does not stand on the site. The 0.93 ha study area was originally planted in May 1981 at 1.82 meter by 1.82 meter spacing with 14-week old loblolly pine seedlings grown in styrofoam containers.

In the fall of 1988, two cultural treatments were randomly assigned to the twelve plots in a two-by-two factorial design with three replications. Each plot is 23.77 meters by 23.77 meters. The treatments were as follows: (1) pine density- the plots were either thinned to a density of 731 trees per hectare or left unthinned, at a density of 2990 trees per hectare; (2) fertilization -the plots were either left unfertilized

or diammonium triple superphosphate was applied at 744 kg per hectare (150 kg phosphorus and 134 kg nitrogen per hectare)

In April 1990, four plots representing these treatments were chosen from the twelve plots available. The plots chosen are located adjacent to one-another, around a central point. Towers, with scaffolding, extending up beyond the canopy of the trees were erected in the summer of 1991. This series of steel towers and wooden walkways was constructed to gain access to the upper and lower half of each tree crown accessible from the tower system. The tower system completely surrounds at least two trees and borders on one side of at least eight additional trees, depending on the thinning treatment. This arrangement provides at least partial access to a minimum of ten tree crowns per treatment.

The experimental design was setup without regard to fertilizer effects. This was done so that the thinning treatments were replicated on plots which we had access to both north and south aspects of the crown for at least two individual trees. The experimental design was a split-split plot with thinning as a whole plot and canopy position (upper versus lower) and canopy exposure (northern versus southern crown aspects) as subplots.

In situ Study

The *in situ* physiological evaluation followed a standardized sampling scheme and was repeated for each of the canopy levels by thinning treatment in a randomly assigned order. Two branches from two different trees were chosen from both the

northern and southern side of each tree crown from both the upper and lower canopy levels for each of the treatment combinations (a total of eight samples). On each measurement day, a particular canopy level was randomly chosen, and the northern and southern sides of the crowns were sampled for physiological response characteristics. This measurement scheme was repeated for both replicates until all of the thinning treatments by canopy levels were sampled. Measurement periods were restricted to the hours of 09:30 to 12:30 (local apparent time) to minimize time related environmental differences and concentrate on spatial differences in environmental and physiological responses. Photosynthetic photon flux density (PPFD), net photosynthesis (A), stomatal conductance (g_c), transpiration (E), leaf vapor pressure gradient (VPG), water-use efficiency (WUE), and needle xylem water potential (Y_{xylem}) were measured or calculated on the mid-section of needles attached to measurement branches using a LI-COR, LI6200 Portable Photosynthesis System. Net carbon exchange rates were based on a per unit needle area and on a per unit needle dry weight basis. The needles were then removed and the xylem water potential on one fascicle per branch determined with a pressure chamber (PMS Instrument Corp., Corvallis, OR) using the precautions of Ritchie and Hinckley (1975). The needles were then sealed in plastic bags, placed on ice and later analyzed for fresh and dry weights. In addition, projected needle area was measured and recorded.

This experiment consisted of sampling twice during the 1993 growing season. The first period was in April 1993, and needles sampled were from the branch cohort with recently fully expanded needles. This flush of needles was produced in 1992 and

was the second flush for that year. The experiment was then repeated in the early fall, during September 1993, on the most currently expanded needles produced in 1993 and was the first flush for 1993.

Detached Shoot Study

Evaluation of physiological potential of northern and southern exposed foliage was accomplished by measuring photosynthesis under light saturated conditions and similar temperatures. Thus, physiological differences would represent effects not caused by crown induced PPFD or temperature differences. Light saturated physiological response measurements also were performed on two detached shoots chosen from each treatment combination in a completely randomized order. Two sample branches were chosen, the terminal or lateral shoot was clipped off, sealed in a plastic bag, and used in the physiological measurements. Immediately after returning to the light table, a shoot was recut under water and a twenty-centimeter section of vinyl tubing filled with water was attached over the cut end of the shoot using a hose clamp. This precaution assured an adequate supply of water to the detached shoot while performing physiological measurements. Preliminary experiments showed no change in maximum photosynthetic rates after forty-five minutes under light saturated conditions using this technique.

Light response of photosynthesis was measured under artificial light using a 400 watt metal halide lamp (Sylvania) positioned over a cuvette to maintain a PPFD level of 1200 $\mu mol\ m^{-2}\ s^{-1}$. A hot mirror was used between the cuvette and light

source to reflect infrared radiation and reduce chamber heating. Net carbon exchange rates expressed on a per unit needle area and on a per unit needle dry weight basis. Respiration rates were determined following the photosynthesis measurements by covering the cuvette with a dark cloth and allowing the foliage to stabilize for five minutes. Dark respiration rates were based on a per unit needle area and on a per unit needle dry weight basis. The entire set of physiological measurements was done within ten to twelve minutes after branch removal from the tree.

Needle Properties

After returning to the laboratory, needle material was measured for projected leaf area using a leaf area meter (LI-COR model LI-3000, Lincoln, NE). The tissue were then oven-dried at 65° C for 48-hours. Projected leaf area before drying and dry weights were recorded, and specific leaf areas were calculated on the portion of each fascicle enclosed in the gas-exchange cuvette.

Specific leaf area of needles from branches sampled for physiology were pooled from the *in situ* and detached shoot foliage, for chlorophyll analysis. For each measurement period, the samples collected from each experiment, during each two week period, were pooled and analyzed with analyses of variance.

A subset of loblolly pine needles sampled from the branches used for gas exchange work in April and September were analyzed for chlorophyll *a* and *b* by methods described by Moran (1982) and Inskeep and Bloom (1985) using dimethyl-formamide. Samples that could not be immediately analyzed were stored at -70° C

until processing. A sample of the extract was placed in a spectrophotometric tube, and the absorbance at 647 nm and 665 nm was recorded. The concentrations of chlorophyll *a* and *b* were calculated as

$$\text{chlorophyll a} = 12.70\ A_{665} - 2.79\ A_{647}$$

$$\text{chlorophyll b} = 20.70\ A_{647} - 4.62\ A_{665}$$

The chlorophyll concentration in $\mu g\ ml^{-1}$ was multiplied by the total amount of chlorophyll solution to determine the total amount of chlorophyll in the sample. Chlorophyll *a:b* ratios were used to detect sun-shade differences in the forest canopy foliage. Thus, the relationship between canopy level, photosynthesis, and chlorophyll content could be investigated.

Statistical Analyses

Analysis of variance was used to test the significance of differences between treatments for xylem water potential, photosynthetic rate, transpiration, stomatal conductance, and water-use efficiency using PC-SAS statistical software (SAS Institute, Inc.). Plots of ground were the experimental unit for physiological and environmental comparisons. The experimental design is a repeated measure with a split-split plot. The main plot consists of the thinning treatment, and subplot treatments of canopy level (upper and lower) and exposure (northern and southern crown aspects), with the measurement periods (4/93 and 9/93) as the repeated measure.

RESULTS

In situ

Analysis of variance using measurement period as a repeated measure showed no significant time, or interaction with time, effects for any of the variables measured in the *in situ* experiment. The pattern and levels in the two measurement periods were not different. Therefore, the periods were combined for analysis. Figure 3-1 shows the mean physiological response and morphological measurement values over both measurement periods. Analysis of *in situ* needle PPFD levels showed significant effects of branch exposure and canopy level (Table 3-1, Figure 3-1A). Light levels in the lower level of the south exposed canopy were significantly greater than that of the lower level, northern exposed branches in the thinned stands. Light levels also were significantly greater in the upper canopy than lower canopy for both the northern and southern exposed branches, regardless of stand density.

Net CO_2 uptake per unit needle dry weight did not show any significant effects of thinning, exposure, or canopy level trend (Table 3-1, Figure 3-1C). Needle stomatal conductance showed a similar pattern to that of assimilation and transpiration (Figure 3-1D). Net CO_2 uptake on a per unit needle area basis was significant for the thinning treatment (Table 3-1, Figure 3-1B). Canopy exposure and level produced similar responses in net CO_2 uptake with both showing marginal significance ($p=0.056$, Table 3-1).

Table 3-1. ANOVA table (p-value) for photosynthetic photon flux density (PPFD), net CO_2 exchange on a needle area (A_{la}) and dry weight (A_{dw}) basis, needle conductance (g_c), transpiration (E), water-use efficiency (WUE), vapor pressure gradient (VPG) and needle xylem water potential (Ψ) measured during the *in situ* experiments.

Source	df	PPFD	A_{la}	A_{dw}	g_c	E	WUE	VPG	Ψ_{xylem}
Thinning (Th)	1	0.384	0.042*	0.417	0.806	0.378	0.092[1]	0.809	0.590
Error A	2								
Measurement Period (MP)	1	0.593	0.129	0.319	0.170	0.409	0.175	0.270	0.532
MP x Th	1	0.659	0.973	0.583	0.956	0.500	0.576	0.796	0.268
Error B	2								
Exposure (Exp)	1	0.026*	0.056	0.096	0.887	0.275	0.680	0.180	0.904
Th x Exp	1	0.309	0.158	0.194	0.872	0.657	0.669	0.407	0.228
Error C	2								
MP x Exp	1	0.277	0.837	0.656	0.077	0.175	0.911	0.279	0.466
MP x Th x Exp	1	0.387	0.226	0.984	0.684	0.202	0.606	0.720	0.083
Error D	2								
Level (Lev)	1	0.044*	0.056	0.095	0.084	0.001*	0.197	0.131	0.074
Lev x Th	1	0.240	0.295	0.201	0.362	0.006*	0.480	0.346	0.677
Error E	2								
MP x Lev	1	0.810	0.675	0.093	0.550	0.446	0.443	0.333	0.275
MP x Th x Lev	1	0.229	0.085	0.047*	0.117	0.297	0.778	0.919	0.605
Error F	2								
Lev x Exp	1	0.673	0.764	0.473	0.569	0.678	0.783	0.518	0.887
Th x Lev x Exp	1	0.673	0.928	0.817	0.935	0.178	0.559	0.199	0.625
Error G	2								
MP x Lev x Exp	1	0.736	0.507	0.683	0.129	0.294	0.895	0.951	0.993
MP x Th x Lev x Exp	1	0.412	0.774	0.539	0.734	0.141	0.953	0.118	0.292
Total	31								

* Significant $p \leq 0.05$.

[1] Underline indicates significant response at 0.10 level for discussion of potential differences.

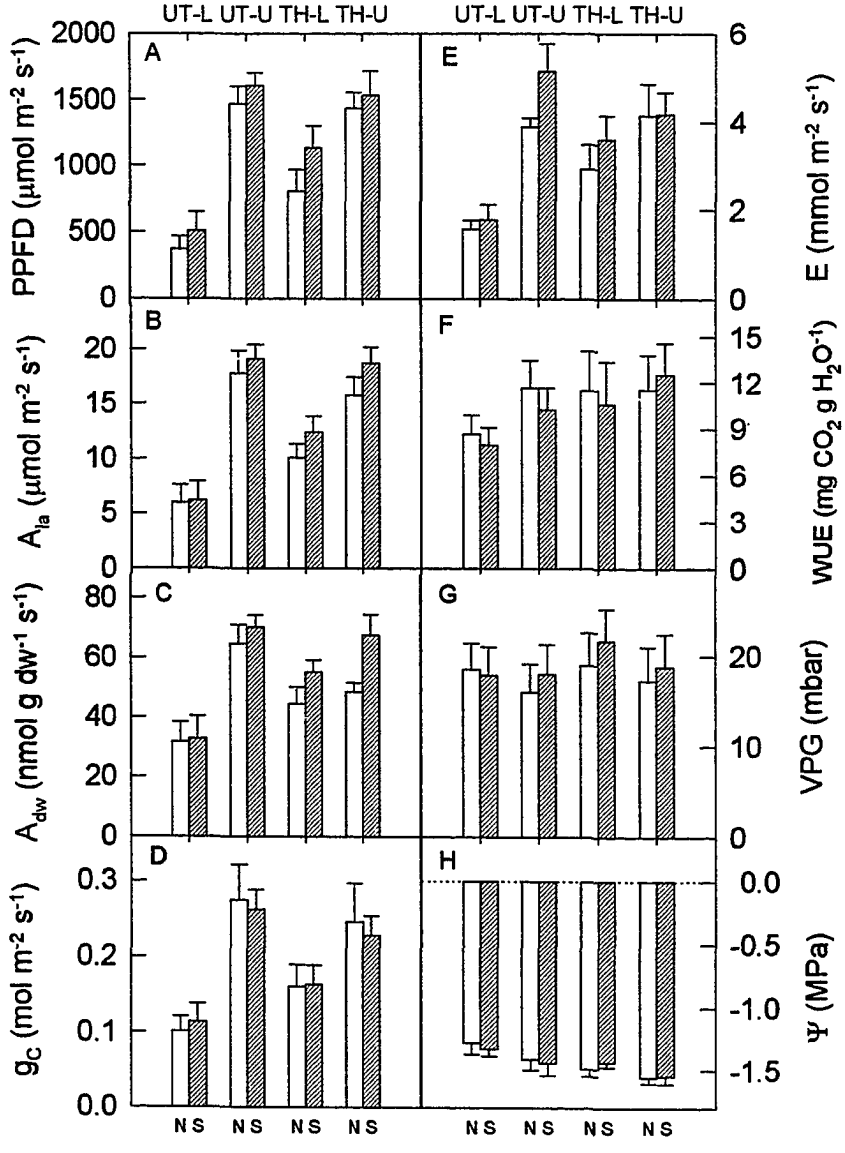


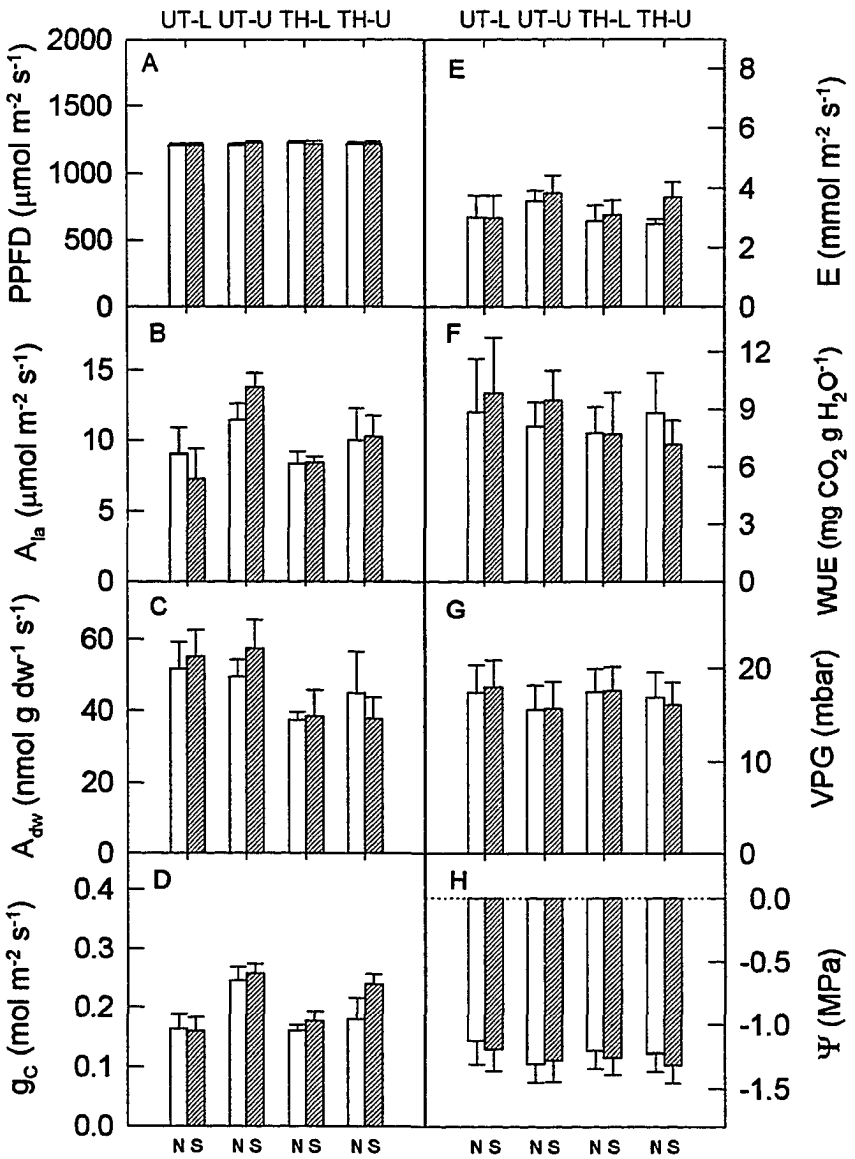
Figure 3-1. A) Mean photosynthetic photon flux density (PPFD), net CO_2 exchange on a B) projected needle area (A_{la}) and C) dry weight (A_{dw}) basis, D) needle conductance (g_C), E) transpiration (E), F) water-use efficiency (WUE), G) vapor pressure gradient (VPG) and H) needle water potential (Ψ) for the *in situ* experiment for the April and September 1993 measurement periods combined. Bars indicate plus one standard error of the mean. (n=6; open bars=branches from northern crown aspect, hatched bars=branches from southern crown aspect; UT=unthinned, TH=thinned, L=lower canopy position, U=upper canopy position).

Assimilation in the lower canopy of the thinned stands was significantly greater than that of the lower canopy of unthinned stands. A significant interaction of canopy level and thinning was found for the transpiration rate (Table 3-1, Figure 3-1E). Transpiration was greater in the lower, canopy of the thinned stand than in the unthinned stand. However, upper canopy foliage tended to have greater conductance than the lower canopy. Water-use efficiency, vapor pressure gradient and xylem water potential were not significant for any of the factors in the analysis (Figure 3-1H).

Although not statistically significant at the usual $p \leq 0.05$ level, a strong trend existed ($p \leq 0.10$) for foliage from the northern versus southern canopy aspects, as well as, thinned versus unthinned, upper foliage that tended to differ in physiology and microenvironment. Needles from branches with a southern exposure, in the lower crown generally had higher PPFD levels, net carbon exchange (A_{la} and A_{dw}), needle conductance and transpiration than that of needles from branches with a northern exposure, in the lower crown (Figure 3-1 A-E).

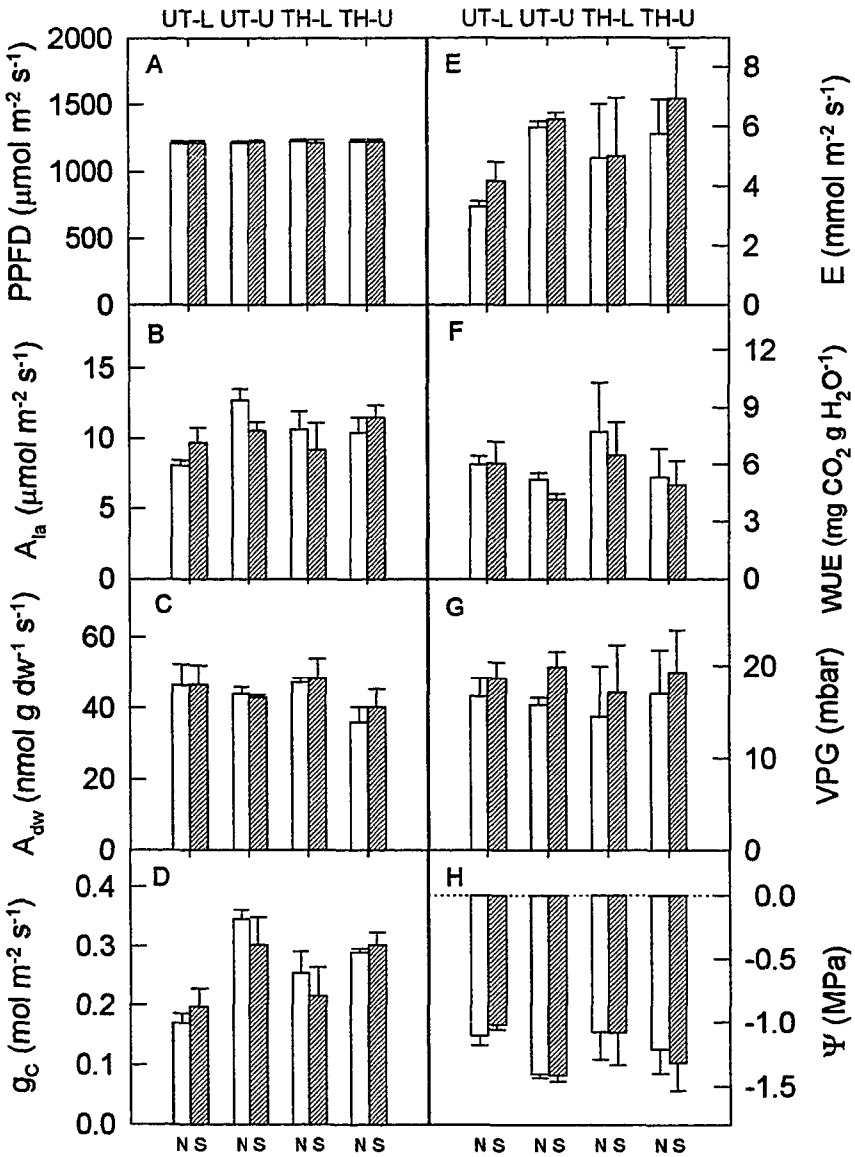
Detached Needles

To evaluate the physiological potential of canopy foliage a series of detached branch experiments was done. Photosynthetic photon flux density was held at light saturating conditions (Figures 3-2A & 3-3A), and physiological responses were measured under ambient temperature and atmospheric moisture conditions.



April 1993

Figure 3-2. A) Mean photosynthetic photon flux density (PPFD), potential net CO_2 exchange on a B) projected needle area (A_{la}) and C) dry weight (A_{dw}) basis, D) needle conductance (g_C), E) transpiration (E), F) water-use efficiency (WUE), G) vapor pressure gradient (VPG) and H) needle water potential (Ψ) for the detached shoot experiment, under light saturated conditions, for the April 1993 measurement period. Bars indicate plus one standard error of the mean.(n=3;UT=unthinned, TH=thinned, L=lower canopy position, U=upper canopy position).



September 1993

Figure 3-3. A) Mean photosynthetic photon flux density (PPFD), potential net CO_2 exchange on a B) projected needle area (A_{la}) and C) dry weight (A_{dw}) basis, D) needle conductance (g_C), E) transpiration (E), F) water-use efficiency (WUE), G) vapor pressure gradient (VPG) and H) needle water potential (Ψ) for the detached shoot experiment, under light saturated conditions, for the September 1993 measurement period. Bars indicate plus one standard error of the mean.(n=3;UT=unthinned, TH=thinned, L=lower canopy position, U=upper canopy position).

Measurement period and some interaction with other factors were significant for some responses thus, data are presented separately for each period (Figures 3-2 & 3-3). Thinning had a significant impact on light saturated assimilation per unit leaf area (Table 3-2; Figures 3-2B & 3-3B), but not on a dry weight basis (Table 3-2; Figures 3-2C & 3-3C). A significant interaction of measurement period by thinning by canopy level and measurement period by canopy level was found for A_{la} and A_{dw}, respectively. Assimilation on a per unit leaf area (Figure 3-3B) was greater for the lower, thinned canopy in September than in the April measurement period (Figure 3-2B), whereas the two periods were not different for the unthinned treatment. Assimilation on a dry weight basis was significantly greater for the upper canopy foliage in April than in September (Figures 3-2C & 3-3C). In contrast, no difference in assimilation rate was seen for lower canopy foliage between the two periods. However, needles from branches in the upper canopy of unthinned stands generally had higher incident PPFD and photosynthesis rates than needles from branches in the upper canopy of thinned stands in the *in situ* branch study.

Needle conductance and transpiration was significant overall for canopy level effect (Table 3-2; Figures 3-2D & 3-3D). Needle conductance was greater in the foliage from the upper canopy in both thinning treatments and for both canopy exposures. Needle conductance of southern exposed foliage in the upper canopy, thinned stand was greater than that of the northern exposed branches. This was not true for the other northern versus southern exposed foliage within any other canopy level or thinning treatment.

Table 3-2. ANOVA table (p-value) for net CO_2 exchange on a needle area (A_{la}) and dry weight (A_{dw}) basis, needle conductance (g_c), transpiration (E), water-use efficiency (WUE), vapor pressure gradient (VPG) and needle xylem water potential (Ψ) measured during the detached shoot experiment.

Source	df	A_{la}	A_{dw}	g_c	E	WUE	VPG	Ψ_{xylem}
Thinning (Th)	1	0.050*	0.073[1]	0.910	0.876	0.905	0.951	0.918
Error A	2							
Measurement Period (MP)	1	0.583	0.545	0.077	0.296	0.175	0.921	0.894
MP x Th	1	0.495	0.236	0.509	0.771	0.528	0.919	0.868
Error B	2							
Exposure (Exp)	1	1.000	0.829	0.818	0.174	0.632	0.038*	0.246
Th x Exp	1	0.992	0.799	0.751	0.620	0.620	0.401	0.165
Error C	2							
MP x Exp	1	0.804	0.967	0.357	0.652	0.408	0.011*	0.570
MP x Th x Exp	1	0.960	0.305	0.541	0.737	0.378	0.740	0.749
Error D	2							
Level (Lev)	1	0.244	0.742	0.037*	0.041*	0.221	0.732	0.021*
Lev x Th	1	0.544	0.906	0.187	0.280	0.932	0.225	0.132
Error E	2							
MP x Lev	1	0.008*	0.043*	0.381	0.152	0.562	0.181	0.067
MP x Th x Lev	1	0.040*	0.100	0.688	0.701	0.764	0.760	0.537
Error F	2							
Lev x Exp	1	0.412	0.945	0.604	0.018*	0.344	0.170	0.364
Th x Lev x Exp	1	0.509	0.755	0.150	0.009*	0.954	0.090	0.364
Error G	2							
MP x Lev x Exp	1	0.230	0.736	0.165	0.702	0.694	0.257	0.002*
MP x Th x Lev x Exp	1	0.087	0.358	0.124	0.306	0.189	0.436	0.010*
Total	31							

* Significant at $p \leq 0.05$.

[1] Underline indicates significant response at 0.10 level for discussion of potential differences.

Transpiration, however, had a significant thinning by canopy level by exposure interaction (Table 3-2; Figures 3-2E & 3-3E). In this interaction, the primary pattern seen can be attributed to a canopy level by exposure interaction. Xylem water potential was significantly different for canopy level (Table 3-2), with the lower level having higher water potentials (Figures 3-2H & 3-3H). Predawn water potentials were lower (more negative) in the unthinned stands and were significantly different from thinned treatments two of the four physiological measurement periods (Table 3-3). Significant effects of exposure, and measurement period by exposure were found for needle vapor pressure gradients despite attempts at minimizing environmental differences (Table 3-2; Figures 3-2G & 3-3G). Chamber air temperatures ranged from 22.0 to 23.1°C in April , and 33.0 to 34.1°C in September.

Dark respiration rates, measured in September only, did not differ statistically with respect to thinning, exposure, or canopy level (Figure 3-4). A general pattern of higher respiration per unit of needle area in the upper foliage was found. However, when expressed on a dry weight basis, no such pattern was apparent.

Needle specific leaf area was used as a morphological measure of leaf thickness. Needle specific leaf area was found to be significantly different for measurement period and canopy level by thinning interaction (Table 3-4). Needles in the lower, unthinned canopy had significantly greater specific leaf area than the other canopy positions within either the northern or southern exposures in April (Table 3-5). In September, needles in the lower canopy of the unthinned stand had greater specific leaf area than the upper, with no difference found in the thinned stands (Table 3-5).

Table 3-3. Mean (s.e.) Predawn fascicle xylem water potentials for thinned and unthinned stands. Values within a row are significantly different ($P \leq 0.05$) if followed by a different letter. (n=12).

	Predawn Ψ (MPa)	
Date	Thinned	Unthinned
17 Apr 93	-0.55 (0.032) a	-0.59 (0.024) a
18 Apr 93	-0.62 (0.024) a	-0.68 (0.028) b
08 Jul 93	-0.45 (0.016) a	-0.50 (0.022) b
23 Sep 93	-0.63 (0.039) a	-0.69 (0.013) a

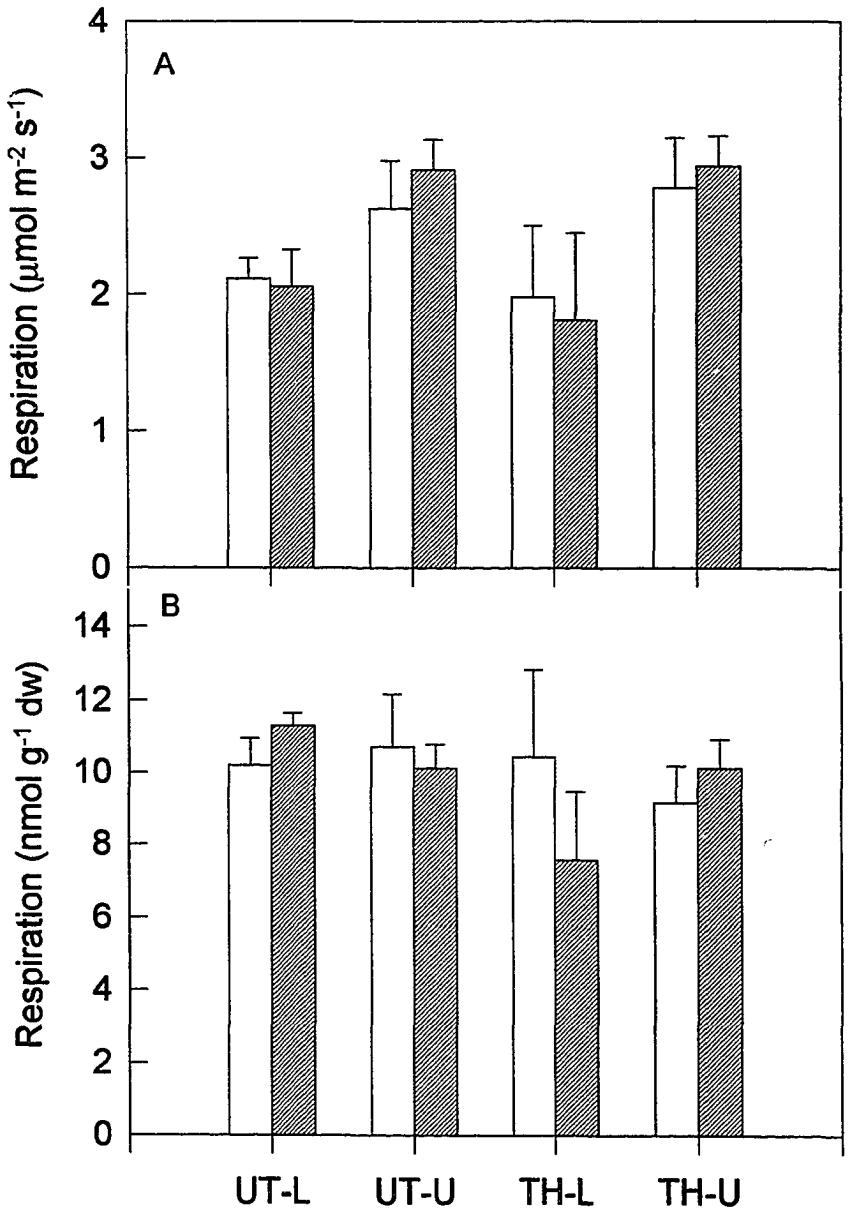


Figure 3-4. Mean dark respiration rates, on a A) unit area and B) unit dry weight basis for needle from detached branch experiment for the September 1993 sampling period. Bars indicate plus one standard error of the mean. (n=3; open bars=branches from northern crown aspect, hatched bars=branches from southern crown aspect; UT=unthinned, TH=thinned, L=lower canopy position, U=upper canopy position).

Table 3-4. ANOVA table (p-value) for needle specific leaf area sampled in April and September 1993. (April n=26, September n=4).

Source	df	p-value
Thinning (Th)	1	0.062
Measurement Period (MP)	1	0.001*
MP x Th	1	0.682
Canopy Exposure (Exp)	1	0.143
Th x Exp	1	0.170
MP x Exp	1	0.357
MP x Th x Exp	1	0.550
Canopy Level (Lev)	1	0.001*
Th x Lev	1	0.021*
MP x Lev	1	0.400
MP x Th x Lev	1	0.900
Exp x Lev	1	0.566
Exp x Th x Lev	1	0.521
MP x Exp x Lev	1	0.689
MP x Th x Exp x Lev	1	0.319

* Significant $p \leq 0.05$.
[1] Underline indicates significant response at 0.10 level for discussion of potential differences.

Table 3-5. Mean (s.e.) specific leaf area of needles for foliage sampled during the April and September measurement periods for northern and southern aspect foliage. Means followed by a different small letter are significantly different ($P \leq 0.05$) for upper versus lower canopy level within thinning, and those followed by a different capital letter are significantly different for north versus south canopy exposure within a canopy level and thinning treatment. (April n=26; September n=4).

Spring Sampling Period (April 1993)			
		Specific Leaf Area ($cm^2\ g^{-1}$ dw)	
Thinning	Level	North	South
Unthinned	Lower	60.95 (2.06) aA	56.94 (2.16) aA
Unthinned	Upper	46.28 (3.22) bA	42.95 (3.17) bA
Thinned	Lower	48.33 (2.27) aA	51.20 (2.41) aA
Thinned	Upper	43.80 (2.84) aA	42.23 (2.78) aA

Fall Sampling Period (September 1993)			
		Specific Leaf Area ($cm^2\ g^{-1}$ dw)	
Thinning	Level	North	South
Unthinned	Lower	59.92 (1.70) aA	59.04 (1.47) aA
Unthinned	Upper	43.97 (3.41) bA	39.54 (0.71) bA
Thinned	Lower	53.09 (4.57) aA	37.71 (2.12) aA
Thinned	Upper	51.45 (2.96) aA	39.96 (2.88) aA

Chlorophyll content of needles was investigated to survey differences in the photosynthetic apparatus of foliage from northern and southern exposed branches. No significant effect of canopy exposure nor measurement period was found for chlorophyll content (Table 3-6). A significant interaction of thinning by canopy level was found for chlorophyll *a*, chlorophyll *b* and total chlorophyll per unit dry weight (Table 3-6). Within the thinned stands the lower and upper canopy positions did not differ with respect to chlorophyll content (*a*, *b* or *a+b*). In contrast, the lower canopy foliage of the unthinned stand had greater chlorophyll concentrations (*a*,*b* and *a+b*) than did the upper canopy of the unthinned stands (Table 3-7). The foliage of the unthinned stands had significantly higher chlorophyll concentrations than did the foliage of the thinned stands. Additionally, chlorophyll *a:b* ratios were greater in the upper canopy than in the lower canopy in thinned and unthinned stands (Table 3-7).

In general, a similarity in the physiological patterns was found between the *in situ* and detached shoot experiments. Net carbon exchange (A_{la}), needle conductance, transpiration and dark respiration rates were greater in the upper canopy foliage, than in the lower canopy foliage in both thinning treatments. Likewise, lower canopy foliage, thinned treatment generally had greater values for the physiological measurements than that of the lower canopy, unthinned foliage in both experiments.

Table 3-6. ANOVA table (p-value) for needle chlorophyll content of foliage collected in 1993. (n=12).

Source	df	Chl *a*	Chl *b*	Total	*a/b* Ratio
Thinning (Th)	1	0.318	0.342	0.325	0.407
Exposure (Exp)	1	0.860	0.878	0.931	0.088
Th x Exp	1	0.664	0.789	0.696	0.228
Level (Lev)	1	0.004*	0.007*	0.001*	0.030*
Lev x Th	1	0.002*	0.004*	0.002*	0.892
Lev x Exp	1	0.229	0.396	0.253	0.654
Th x Lev x Exp	1	0.719	0.832	0.835	0.969

* Significant $p \leq 0.05$.
[1] Underline indicates significant response at 0.10 level for discussion of potential differences.

Table 3-7. Mean (s.e.) chlorophyll content of needles for foliage sampled during the April and September 1993 combined measurement periods for north and south exposed foliage (n=12). Different letters following means within a column represent statistical significance ($p \leq 0.05$).

Chlorophyll *a* (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	2.77 (0.16) a	2.73 (0.24) a
Unthinned	Upper	2.36 (0.17) b	2.16 (0.28) b
Thinned	Lower	2.08 (0.12) c	2.18 (0.21) b
Thinned	Upper	1.98 (0.21) c	1.98 (0.23) c
Chlorophyll *b* (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	1.02 (0.07) a	1.02 (0.10) a
Unthinned	Upper	0.83 (0.07) b	0.81 (0.12) b
Thinned	Lower	0.73 (0.05) c	0.78 (0.09) b
Thinned	Upper	0.67 (0.08) c	0.68 (0.09) b
Total Chlorophyll (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	3.79 (0.23) a	3.75 (0.34) a
Unthinned	Upper	3.19 (0.23) b	2.96 (0.40) b
Thinned	Lower	2.81 (0.17) c	2.96 (0.30) b
Thinned	Upper	2.65 (0.29) c	2.66 (0.33) b
Chlorophyll *a*:*b* Ratio			
Thinning	Level	North	South
Unthinned	Lower	2.75 (0.03) a	2.69 (0.03) a
Unthinned	Upper	2.87 (0.04) b	2.84 (0.12) b
Thinned	Lower	2.87 (0.04) b	2.85 (0.07) a
Thinned	Upper	3.01 (0.07) b	3.01 (0.08) b

DISCUSSION

The thinning treatments in this study preceded the physiological measurements by four years. Thus, this discussion is based on physiological parameters measured during the fifth growing season following the thinning treatments. *In situ* canopy light levels were significantly higher for foliage on southern aspect branches (range: 3-7% higher for unthinned and 16-18% higher for thinned) than that of the northern aspect branches. The foliage surface area on the southern aspect branches was significantly greater than that of the northern branches in the lower canopy of the thinned stand. In contrast, no difference in the mean light level was found for the north versus south exposed foliage of the unthinned stands. This difference is explained by shading of the southern sides of tree crowns by closer spaced neighboring trees in the unthinned stands. Shading by neighbor trees is possible given that the calculated solar angle (using equations in Jones 1992) was 63° and 68°, for April and September, respectively.

Additional information is needed on how east and west exposed foliage may differ with respect to their physiological responses, how this changes diurnally, and what physiological responses may occur because of changes in the microenvironment. This is especially true in light of the dramatic differences in water potential, temperature, and VPG of east illuminated foliage in the morning versus those illuminated from the west in the afternoon. The east-west pattern are not likely to be mirror images in time.

No thinning effects on light levels were detected in this study contrary to what Nowak et al. (1990) found in their stands three years after thinning. This can be explained by the results of four years following the thinning treatment. Shoot growth during this time has been sufficient to reduce the environmental differences between the thinned and unthinned plots. However, as expected a significant effect of canopy level was found. Not surprisingly, needles in the upper canopy had a higher mean PPFD level than the lower crown for both thinned and unthinned stands for both canopy exposures.

Thinning had a significant impact on net carbon exchange (NCE) per unit needle area (A_{la}) of the lower foliage for both the northern and southern exposed crown exposures despite not finding statistical differences in PPFD levels. This supports the studies of Nowak et al. (1991) and Ginn et al. (1990) which had similar results for lower crown foliage of thinned and unthinned stands. Marginal differences ($p= 0.056$) in canopy exposure and level were found for A_{la} concomitant with trends seen in PPFD. Similar patterns were seen for NCE per unit dry weight (A_{dw}), though not significant ($p= 0.10$). *In situ* results suggest that NCE rates of foliage from different canopy exposures and levels may be related more to differences in incident PPFD than to inherent physiological potential. Similar studies (Ginn et al. 1991) have shown no difference in NCE rates under light saturated conditions.

The second experiment in this study was done to examine the potential physiological differences between canopy positions, under light saturated conditions. Preliminary work, as well as light response curves, indicated that needles saturated at

PPFD levels of approximately 1000 μmol m^{-2} s^{-1}, a light level not commonly read in the lower canopy. In this study, consistent light levels were achieved throughout the day and measurement periods (1200 μmol m^{-2} s^{-1}) but some differences in VPG (Figures 3-2 & 3-3) and small differences in temperatures (temperature range: April 1993, 22.0 to 23.1°C and September 1993, 33.0 and 34.4°C) did exist on foliage from detached shoots. At constant PPFD levels, no indication of a significant difference in physiological potential for northern versus southern exposed foliage was found in this experiment (Table 3-2).

The significant impact of measurement period by thinning by canopy level interaction on A_{la} was primarily due to a measurement period by level interaction. The A_{la} of lower foliage tended to increase and the upper to decrease in September, relative to A_{la} in April. A possibility for the increase in A_{la} of the lower branch foliage is that the April foliage was being shaded to some extent by the presence of newer (not yet mature) needles above it. Subsequently, the September measurements were taken on recently mature needles located near the tips of the shoots and therefore not shaded by a previous flush. This may have resulted in a greater A_{la} rate for September. Net CO_2 uptake per unit dry weight (A_{dw}) followed a comparable trend.

Thinning significantly affected light saturated A_{la} and is likely to be the result of chlorophyll concentrations and specific leaf area differences of shade versus sun adapted needles. Specific leaf area and chlorophyll content per unit dry weight were greater for lower crown, and unthinned treatment foliage, characteristic of shade adapted needles (Boardman 1977). These findings are in contrast to what

Higginbotham (1974) and Nowak et al. (1990) found. In their studies, no difference in chlorophyll concentration in needles from different crown positions was found. In this study, no differences in available light were found but morphological and biochemical evidence points to sun shade differences between lower and upper crown positions. The lower canopy foliage in the unthinned treatment was found to have greater potential to capture and utilize light than the lower canopy foliage in the thinned treatment, i.e., higher assimilation rates seen in lower level, unthinned treatment was the result of greater chlorophyll content and thinner needles. As indicated by the *in situ* study net carbon exchange is limited by light reaching the lower canopy.

General trends in the dark respiration rates of foliage from the detached shoots were similar to Nowak et al. (1990) but not significantly different in this study. Dark respiration rates of foliage from the upper canopy were greater, than foliage from the lower canopy level, in thinned and unthinned treatments on per unit area basis. The general trend for higher respiration rates in the upper canopy needles is consistent with the foliage having a lower specific leaf area and higher construction maintenance costs relative to the lower canopy foliage. Furthermore, because of thicker leaves, the evidence to support higher rates on a leaf area basis is seen by the lack of a similar pattern on a per unit dry weight basis.

No significant differences in light saturated photosynthesis were found for lower versus upper crown foliage. However, differences in chlorophyll content and needle specific leaf area were found and may help explain the physiology of the lower and upper crown needles. Needles from the lower canopy branches were thinner than

those of the upper canopy branches. Net carbon exchange (NCE) on a per unit needle area basis was then greater for the upper canopy foliage because of the greater amount of tissue per unit needle area. However, the greater chlorophyll concentration in the lower canopy foliage allowed these needles to capture and utilize light more efficiently, under light saturated conditions, compared to the upper canopy foliage. Thus, these two factors contributed to the equivalent NCE rates of the lower and upper canopy foliage.

The absence of north versus south light saturated physiological differences was at first, surprising. Similar needle morphology and chlorophyll concentrations indicate that there is little variation in a horizontal direction (north and south) for these variables within the canopy. Thus, it appears that something other than biochemical or morphological characteristics plays a role in needle photosynthetic rates for northern and southern exposed needles. We conclude from this study that the major force behind physiological differences between northern and southern exposed foliage is light availability, as was found to be significant in the *in situ* study.

One of the primary goals of this study was to detect levels of variation and sources of variation as a basis for future modeling efforts. Further study of the potential importance of within canopy positions may be indicated if we consider, for discussion only, the relaxation of the traditional p-value from 0.05 to 0.10. A relaxed p-value would then indicate potentially important responses for which future studies may be based. The use of the 0.10 p-level may be justified for use in uncontrolled environmental studies where responses can be greatly influenced by inherent variation

in the system. *In situ* work in this study did not detect a significant difference ($P \geq 0.05$) between the upper and lower crown position physiology, except for transpiration. However, a strong trend ($P \leq 0.10$) exists for foliage in the upper canopy position to have higher NCE (A_{la} and A_{dw}) and needle conductance values than foliage from the lower crown level. Similar results have been found before for loblolly and other conifers (Ginn et al. 1991, Nowak et al. 1991, Woodman 1971). In addition, *in situ* PPFD levels and needle physiology of branches from southern crown aspect, in both the upper and lower crown levels, were generally greater than that of the northern aspect within the thinned stand. This view is supported by Woodman (1971), who found significantly higher net photosynthetic rates for foliage on the south side of the crown of a large Douglas-fir (*Pseudotsuga menziesii* (Mirb.) FRANCO) tree for all whorl positions of the crown. Given these facts, there is strong evidence for increased physiological activity of foliage of branches with a southern aspect and foliage in the upper crown position. Studies focusing on these differences will lead to a better understanding of the dynamics of tree crown structure and its effects on foliar physiology. With changes in global climate anticipated the foliage from these parts of the canopy may be under additional stress with possible increases in temperature and water deficits.

Finally, because light is not uniformly distributed within a canopy level it is important to consider the exposure (e.g., north and south) of branches to solar radiation when considering whole canopy carbon gain or modeling within crown response. Physiological differences between north and south foliage were greater in the thinned

stands even though the responses were measured four years, during the fifth growing season, after thinning and some crown reclosure was already apparent. The removal of individual trees through thinning increased the light to branches on the southern crown aspect to a greater extent than the northern crown aspects. Thus, northern and southern exposure differences are important in modelling physiological responses in thinned stands, particularly in the lower crown after thinning and continues until crown closure. The possibility exists that east-west aspect differences may exceed those of the north-south aspect.

Data from this study stresses the importance of recognizing the variation when modeling forest stands and forest ecosystems. Additional studies are needed to gain information on the within crown variations in PPFD, vapor pressure gradient and air temperature because of the physiological differences found in this study.

CHAPTER 4

EFFECTS OF CROWN POSITION, STAND DENSITY AND FERTILIZATION ON TEMPORAL AND SPATIAL VARIATION IN FOLIAR NUTRIENT CONCENTRATIONS AND CHLOROPHYLL LEVELS

INTRODUCTION

The mineral nutrient status of a forest stand plays an important role in determining stand productivity. Fertilizer application can significantly enhance the productivity of forest stands where nutrient deficiencies occur (Adams and Allen 1985, Wells and Allen 1987). Thinning can affect how nutrients are distributed within the forest canopy (Vose 1988) and increase volume growth (Allen 1987) in loblolly pine. Allen (1987) suggested that fertilization at the time of thinning may shorten or eliminate the period of time that a stand requires to regain maximum volume production. Within species, there are often strong positive correlations between nitrogen and both chlorophyll and RuBP carboxylase (Evans 1989, Field and Mooney 1986). In *Prunus persica*, DeJong and Doyle (1985) collected data to support the hypothesis that whole-tree photosynthesis is optimized by proportioning photosynthetic capacity with respect to natural light levels. Past studies have found a strong relationship between total nitrogen content and maximum rates of assimilation (Field and Mooeny 1986, Tissue et al. 1993). Therefore, foliar nitrogen content may be a good predictor of photosynthetic capacity (i.e., maximum assimilation).

Nitrogen, phosphorous and potassium are important nutrients which are highly mobile in plants (Salisbury and Ross 1985). Translocation of these nutrients occur in response to changes in sink locations, and nutrients are drawn from older tissue and senescent foliage. Much of the difficulty in sampling for foliar nutrients is due to the mobile nature of nitrogen, phosphorus and potassium in plants. Thus, sampling for nutrients during periods of reduced nutrient translocation may be possible, as a potential means to reduce the sampling variation in tissue nutrient content. That is, sampling restricted to the fall and late winter when the nutrient content of foliage is more stable. However, some studies have not found a stable period at which time nutrients could be reliably sampled (Miller 1966, Rathfon et al. 1993, Wells and Metz 1963). In fact, nitrogen, as well as, potassium have been found to increase during the winter months (Lassoie and Hinckley 1991, Miller 1966, Wells and Metz 1963). The accumulation of nitrogen during the winter months following the first growing season could be accounted for by the loss of nitrogen from older needles to newer needles in the fall just before abscission (Wells and Metz 1963). A further problem encountered in the southern U.S. is the lack of a true dormant season. Extended periods of low metabolic activity of above and below ground tissues is not found in the warmer latitudes of the southern U.S.

The importance of nutrient limitations during periods of rapid growth cannot be totally ignored and can be missed if sampling is restricted to a specific season. Thus, some degree of sampling during the growing season should be done, and relationships between nutrient content and needle physiology should be investigated. A sampling

scheme which included peak nutrient demand periods has been suggested as a way to measure the nutrient status of the tree (Rathfon et al. 1993, Wells and Metz 1963). However, nutrient concentrations change too rapidly during these periods to provide reliable diagnosis and fertilizer recommendations large areas of forests. Thus, dormant season sampling has been suggested (Rathfon et al. 1993). However, this method may apply only to more northern latitudes where a distinct and predictable dormant season is found.

A third suggestion is the use of nutrient ratios for determining nutrient status of forest stands (Comerford and Fisher 1984). Ideally, this works when any one of any two nutrients may be limiting growth. The ratio of the mineral nutrients in question would indicate which nutrient is limiting the other's potential role. Absolute quantities of the nutrients could then be examined and proper amounts applied through fertilization to maximize site conditions. Thus, any deficiency will be detected by the imbalance of specific nutrient ratios.

Variation of nutrient content among crown positions and with leaf age in needles of *Pinus banksiana* was studied by Morrison (1972). In his study, no trends in nitrogen are found among different levels of the canopy. Higher amounts of phosphorus and potassium were associated with young needles in the upper crown. However, both phosphorous and potassium decreased with increasing age and depth downward into the canopy. Wells and Metz (1963) also observed that concentrations of phosphorous and potassium were greater in the upper canopy foliage. In contrast, Wells and Metz (1963) found increasing nitrogen, calcium and magnesium

concentrations in foliage progressing from the upper crown position to the middle and lower crown foliage.

Stand density effects on nutrient dynamics are usually restricted to arguments concerning increased nutrient supply and growing space following thinning. In theory, thinning should provide more nutrients for the remaining trees in a forest. However, thinning also may result in increased leaf area in the mid- and lower crown positions (Vose 1988). Increased foliage biomass can then lead to a dilution effect which results in reduced nutrient content per unit leaf area and/or weight. The photosynthetic rate of newly exposed foliage also is affected because of the increased light, resulting in greater carbon gain used to supply carbohydrates to new foliage growth. The increase in foliar biomass may impose further demands on an already limited nutrient supply.

The productivity of a forest is determined by a combination of factors and interactions including genotype, water, nutrients, light, temperature, pathogens, and competition (Ford 1992, Teskey et al. 1987). These effects, and those of many other environmental factors emphasize the need to elucidate the impacts that changes in climate and management practices may have on loblolly pine forests. Plant physiological ecology has made advances in the capacity to predict plant responses to environment (Mooney et al. 1987). The need exists to study changing characteristics of foliar nutrient content as related to growth. Changes in physiology are in response to a changing environment, age, and involve qualitative changes in physiology, as well as reallocation of nutrients. The active acclimation to changes in environment can be

seen within crowns in response to competition and shaded conditions (Cregg 1990, Cregg et al. 1992, Mitchell and Hinckley 1993, Vose 1988).

An increased understanding of how silvicultural practices and their interaction with climate change and soils affect physiological responses is crucial to increasing productivity and identifying the techniques necessary to adjust for changes in future productivity. The results of this research play an important role in linking together several other studies and provides new information on how environment and management induced changes are linked to changes in foliar nutrient status and physiological responses. This paper presents the pattern of nutrient dynamics in foliage of managed stands of loblolly pine five years after thinning and fertilization treatments.

MATERIALS AND METHODS

Plant Materials

This study is located on the Johnson Tract, of the Palustris Experimental Forest, in Rapides Parish, Louisiana. The site has a Beauregard silt loam soil (fine-silty, siliceous thermic plinthaquic paleudults). Soil drainage and slope is sufficient that water does not stand on the site. The 0.93 ha study area was originally planted with 14-week old loblolly pine seedlings in May 1981, at 1.82 meter by 1.82 meter spacing. In 1988, twelve research plots (0.056 ha) were established for study. The understory hardwood trees, shrubs and *Rubus* sp. were removed from between the rows of pine trees with a mower.

For growth studies, the U.S.D.A. Forest Service installed two cultural treatments which were randomly assigned to the twelve plots in a two-by-two factorial design with three replicates in the fall of 1988. The thinning treatment plots were either thinned to a density of 731 trees per hectare or left unthinned, at a density of 2990 trees per hectare. Thinned plots were obtained by removing every other row of trees and every other tree in the remaining rows. This thinning resulted in a 3.66 by 3.66 meter spacing between trees. The fertilization treatments were plots either left unfertilized or were treated with diammonium triple superphosphate applied at a level of, 744 kg per hectare (150 kg phosphorus and 134 kg nitrogen per hectare). Mean tree height, diameter at breast height (dbh) and basal area for the study plots during the 1993 growing season are given in Table 4-1.

In April 1990, four plots representing the treatments were chosen from the twelve plots available (replicate 1). A series of steel towers and wooden walk-ways were constructed (in the summer of 1991) to gain access to the upper and lower half of each tree crown. This system allowed access to a minimum of eight trees per plot.

Replicates of each treatment combination were established using sixteen sets of steel towers (four towers per replicate) on separate treatment plots (replicate 2). These access towers were erected in plots adjacent to the main plots discussed previously. Each of the towers in the second replicate allow access to portions of the south side of two trees and the north side of two additional trees.

Table 4-1. Biometrical characteristics of 12-year-old loblolly pine plantation study plots in June 1993. (Data from U.S.D.A. Forest Service, Southern Forest Experiment Station, Pineville, LA, used with permission by James D. Haywood)

Treatment	Mean dbh (cm)	Mean Height (m)	Density (#/ha)	BA (m^2/ha)
Unfertilized, Unthinned	13.2	13.05	2786	39.0
Fertilized, Unthinned	13.8	13.96	2682	41.3
Unfertilized, Thinned	16.4	12.31	731	15.8
Fertilized, Thinned	18.3	13.17	711	19.3

Sampling Scheme

For each of the four treatment combinations (thinned/unfertilized, thinned/fertilized, un-thinned/unfertilized and unthinned/fertilized) a standard sampling scheme was established. The trees in the treatment plots showed no obvious dominate and codominate individuals, owing to the even-age planting and relatively young stand age. The lateral branches of the upper and lower one-third of the canopy were delineated into two canopy levels for sampling. For the purpose of comparison between canopy levels and treatments, only the most recent, fully expanded, terminal shoots or adjacent lateral shoots foliage were sampled from the south side of the tree. Mid-way through the growing season, July 1993, sampling was stopped on the last flush of the 1992 growing season (92-Last-Flush foliage) and in August 1993 sampling on the first flush of the 1993 growing season (93-First-Flush foliage) was begun. The change in foliage sampling was necessary to compliment the physiological study performed on the most recent, fully expanded, flush of needles. The results from this study will later be used to correlate nutrient content with measured physiological parameters.

Foliar Nutrient Analyses

Needles were sampled from trees in the stands monthly, April through October 1993, as previously described. The sampling months of April through July 1993 were represented by the last flush of the 1992 growing season (92-Last-Flush foliage). Themonths of August through October represent the foliage of the first flush of the

1993 growing season (93-First-Flush foliage). Only mature (fully elongated) fascicles were used in the samples. Two needle samples were collected from each of the canopy levels and treatment combinations then pooled, from each of the two replicates, seven times during the growing season. This sampling scheme was initiated so that the findings could be later correlated with physiological measurements taken on the same flush of needles. The needle samples were dried (65°C), then ground to a fine powder (20 to 40 mesh) in a Wiley mill. Foliar nutrient analyses for nitrogen, phosphorus, potassium, calcium, magnesium, manganese copper, iron, boron, and zinc were performed. Total nitrogen was analyzed using a LECO (Model FP-428) Nitrogen Analyzer. The other elements were analyzed using an argon plasma spectrometer (ICP) after following proper protocol for sample preparation by the L.S.U. Agricultural Center Plant Analysis Lab, in the laboratory of Dr. Paul F. Bell and analyses were run in the laboratory of Sam E. Feagley by Mrs. Joanie Haigler. Nitrogen concentrations are expressed on a percent of dry weight and the other nutrients on an element weight per tissue dry weight basis.

Needle Chlorophyll

Loblolly pine needles were sampled monthly, May through September 1993, and analyzed for chlorophyll *a* and *b* by methods described by Moran (1982) and Inskeep and Bloom (1985). Four samples, two from each of two different branches, were taken from each of the canopy levels (lower and upper) and treatment combinations from each of the two replicates. Samples that could not be immediately

analyzed were stored at -70° C until processing. A sample of the extract was placed in a spectrophotometric tube, and the absorbance at 647 nm and 665 nm was recorded. The concentrations of chlorophyll *a* and *b* were calculated as

chlorophyll *a* = 12.70 A_{665} - 2.79 A_{647}

chlorophyll *b* = 20.70 A_{647} - 4.62 A_{665}.

The concentration in μg ml^{-1} was multiplied by the total amount of chlorophyll solution to determine the total amount of chlorophyll in the sample.

RESULTS

Nitrogen

The seasonal patterns of foliar nitrogen concentration were similar among the thinning treatments and canopy positions but differed between fertilizer treatments (Figures 4-1A & 4-2A). The 92-Last-Flush foliage in the lower canopy level, of all treatment combinations generally decreased in nitrogen content. The 93-First-Flush foliage in the upper canopy level generally increased from the August to the October sampling months. Foliar nitrogen concentration was significantly higher ($p= 0.02$; Table 4-2) in the unfertilized plots. Averaged across the sampling months, and canopy positions, mean nitrogen concentrations by weight were 1.11% and 1.26% for fertilized and unfertilized plots, respectively ($p \leq 0.05$). Nitrogen concentration was not significantly different for the main effect of thinning treatment in this study. Generally, foliage from the unthinned (1.22 %) plots had a higher mean foliar nitrogen content than the thinned (1.16 %) plots. A general decline, over time, in nitrogen

concentration was found in the 92-Last-Flush foliage from the upper and lower canopy foliage, fertilized treatment and the upper canopy foliage, unfertilized treatment. However, the nitrogen concentration of the lower canopy foliage, unfertilized treatment did not differ across the sampling months. For the 93-First-Flush, upper canopy foliage, in the unfertilized treatment, nitrogen concentration increased over time in the thinned and unthinned treatments. In contrast, the 93-First-Flush foliage of the upper and lower canopy, fertilized treatment did not significantly differ across the same period.

Canopy position had a significant impact on foliar nitrogen concentrations ($p= 0.026$; Table 4-2). Foliar nitrogen concentration was significantly less in the lower canopy than in the upper canopy of the thinned (1.21 % and 1.36 %, respectively) and unthinned (1.18 % and 1.29 %, respectively), unfertilized plots for 92-Last-Flush foliage. In contrast, no significant difference was found between the lower and upper canopy positions in the fertilized plots, in either the thinned (1.01 % and 1.04 %, respectively) or unthinned (1.15 % and 1.17 %, respectively) treatments for the 92-Last-Flush foliage. However, the foliar nitrogen concentrations were lower in the fertilized than that of the fertilized treatments. The 93-First-Flush foliage showed a similar pattern except that the upper canopy foliage, thinned and fertilized treatment had higher nitrogen concentration than that of the lower canopy foliage.

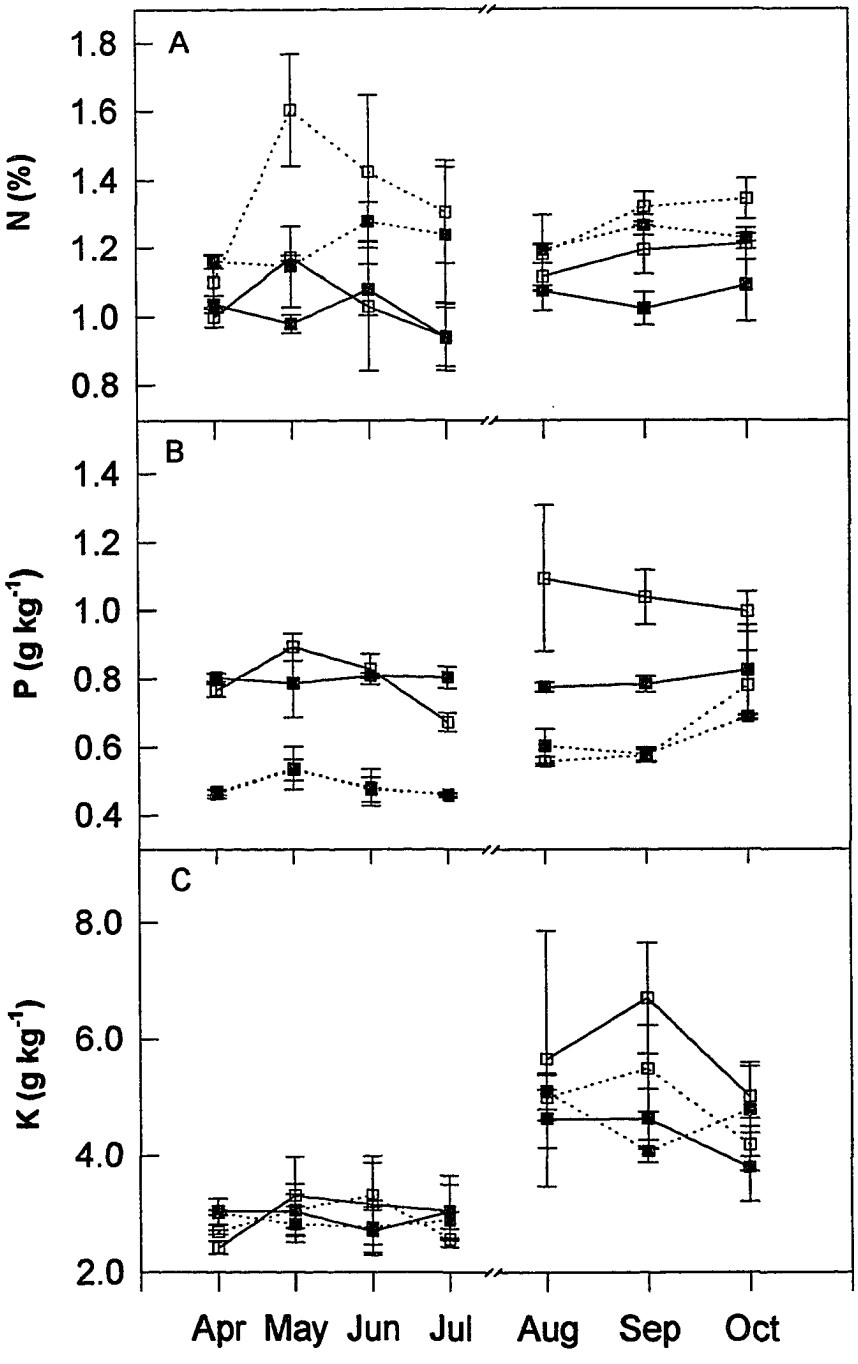


Figure 4-1. Foliar (A) nitrogen, (B) phosphorous and (C) potassium concentrations of 92-Last Flush foliage (April-July) and 93-First Flush foliage (August-October) of loblolly pine in the thinned plots. (n=2; ■ = lower, □ = upper, ····· = unfertilized, —— = fertilized).

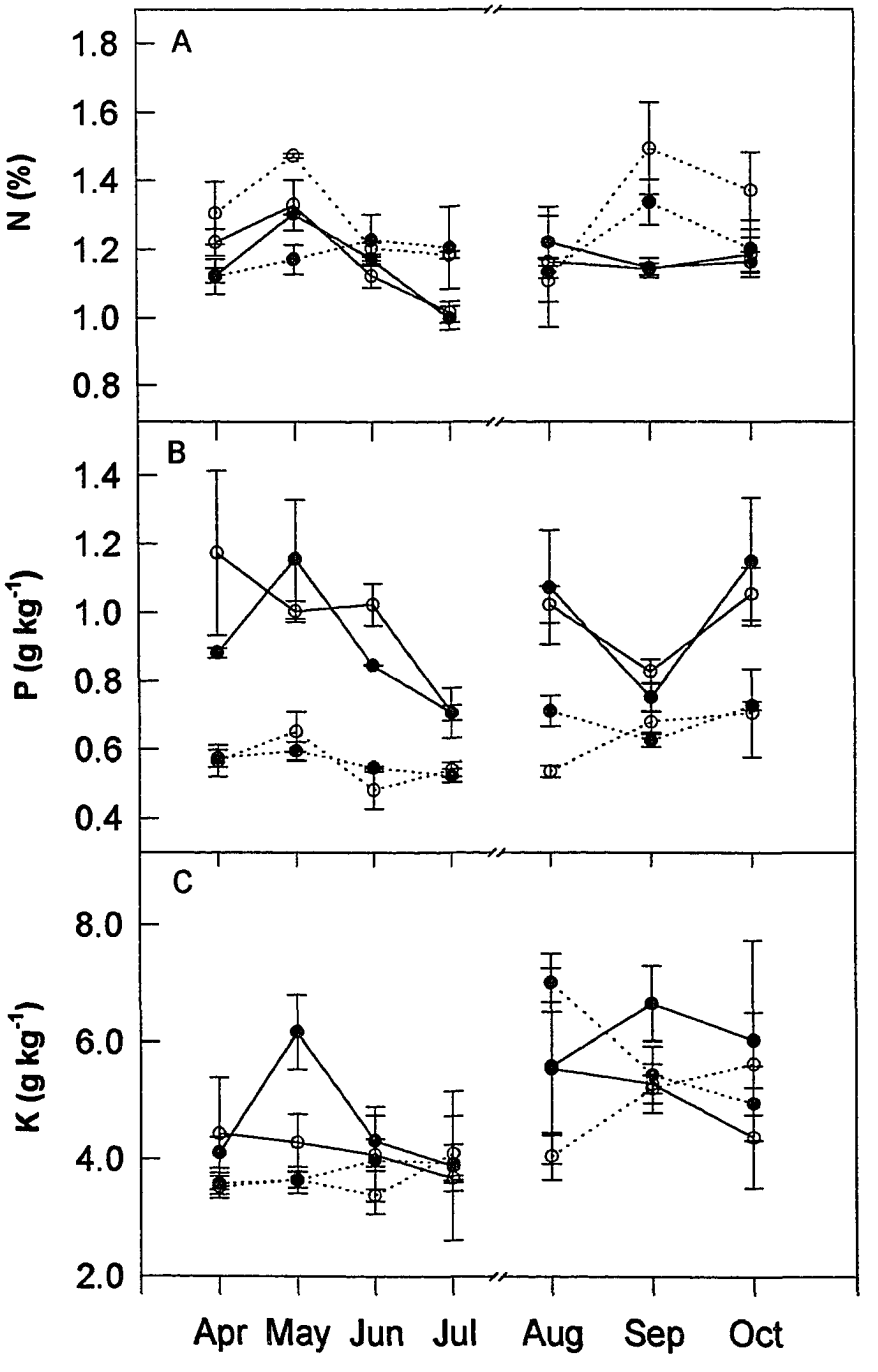


Figure 4-2. Foliar (A) nitrogen, (B) phosphorous and (C) potassium concentrations of 92-Last Flush foliage (April-July) and 93-First Flush foliage (August-October) of loblolly pine in the unthinned plots. (n=2; ● = lower, ○ = upper, ····· = unfertilized, —— = fertilized).

Table 4-2. ANOVA (Pr > F) table for needle nutrient analyses of loblolly pine from the 1993 growing season.

Source	df	N	P	K	Ca	Mg	B
Fertilization (F)	1	0.0217	0.0001	0.3151	0.0302	0.0426	0.1842
Thinning (T)	1	0.3776	0.0075	0.0317	0.8944	0.4244	0.0233
F x T	1	0.2064	0.2051	0.6370	0.9457	0.4695	0.4111
Error A	4						
Month (M)	6	0.0102	0.0001	0.0001	0.0001	0.2092	0.0014
M x F	6	0.1473	0.0423	0.7119	0.2874	0.1310	0.8112
M x T	6	0.6488	0.0330	0.7329	0.2869	0.7025	0.9399
M x F x T	6	0.3713	0.0331	0.8099	0.9056	0.7112	0.4842
Error B	24						
Canopy Level (Lev)	1	0.0260	0.1925	0.5033	0.1365	0.0122	0.0020
Lev x F	1	0.1476	0.1305	0.7190	0.9860	0.5694	0.0735
Lev x T	1	0.4842	0.3047	0.0252	0.3772	0.5646	0.4040
Lev x F x T	1	0.6148	0.6515	0.2115	0.8545	0.9058	0.9799
Error C	4						
M x Lev	6	0.0121	0.6527	0.7227	0.0283	0.1321	0.1522
M x Lev x F	6	0.4105	0.3764	0.5255	0.1623	0.0796	0.3017
M x Lev x T	6	0.1531	0.0883	0.0925	0.0741	0.0398	0.3497
M x Lev x F x T	6	0.5377	0.1329	0.3669	0.0531	0.0930	0.0960
Error D	24						
Total	111						

Phosphorous

Foliar phosphorus concentrations differed significantly throughout the sample months. The seasonal pattern was dependent on the fertilizer and thinning treatment, as is evident by the significant interaction of sampling month, fertilizer and thinning treatments ($p= 0.03$, Table 4-2). Foliar phosphorus concentration of fertilized, unthinned treatment foliage tended to decrease with time for the 92-Last-Flush needles, however, the 93-First-Flush needles showed an erratic pattern (Figures 4-1B & 4-2B). The 92-Last-Flush foliage phosphorous content appeared stable during the sampling months. The 93-First-Flush, unfertilized treatment foliage tended to increase from August to October for both thinning treatments and canopy positions.

Main treatment effects of fertilization and thinning were significant for foliar phosphorous concentration. Fertilization increased phosphorus concentration while thinning resulted in a decrease in foliar phosphorus concentration. A significant sampling month, by fertilizer, by thinning treatment interaction effect, influenced when the fertilizer and thinning treatments had an impact. The 92-Last-Flush foliage from the unthinned, fertilized treatment had significantly higher phosphorous concentration than did the thinned, fertilized foliage. However, for the thinned, unfertilized versus unthinned, unfertilized 92-Last-Flush foliage no significant difference was found. For 93-First-Flush foliage, phosphorous concentration was generally higher in the unthinned, fertilized treatment foliage than in the thinned, fertilized treatment foliage. A slight tendency for phosphorous accumulation was found for the 93-First-Flush, unfertilized treatment foliage from August to October.

Mean phosphorous concentrations, averaged across sampling month and foliage age class, were 0.85, 0.95, 0.54 and 0.61 g kg^{-1} for fertilized/thinned, fertilized/unthinned, unfertilized/thinned and unfertilized/unthinned treatments, respectively.

Potassium

Potassium concentration between the sampling months was significantly different and tended to separate based on the age class of the foliage (Figures 4-1C & 4-2C). The 92-Last-Flush foliage had a lower potassium concentration than that of the 93-First-Flush foliage. Fertilization did not have an impact on the foliar potassium concentration. However, the main effect of thinning was significant, and the foliage from branches in the thinned treatment generally had a lower foliar potassium concentration than that of the unthinned treatment.

A significant interaction of thinning and canopy position was found in both the 92-Last-Flush and 93-First-Flush foliage. The general pattern was for the upper canopy foliage to have a higher potassium concentration than that of the lower canopy foliage, within the thinned treatment. In contrast, within the unthinned treatment, the opposite effect was found; lower canopy foliage was generally higher in potassium than that of the upper canopy foliage.

Calcium

Foliar calcium concentration increased from the first sampling month to the last sampling month within each flush for all canopy positions and treatments (Figures 4-

3A & 4-4A). Fertilization significantly increased calcium concentration in 92-Last-Flush foliage and had a lesser impact on 93-First-Flush foliage. Thinning did not have a significant impact on foliar calcium concentration. While not statistically significant, the lower canopy position foliage tended to have a higher calcium concentration than that of the upper canopy.

Magnesium

Seasonal patterns of foliar magnesium concentrations were highly variable compared to the other mineral nutrients examined, and the patterns were significantly different among sampling months (Figures 4-3B & 4-4B). Fertilization significantly increased the foliar magnesium content in 92-Last-Flush needles. The 93-First-Flush foliage was similar with the exception of the depressed magnesium concentration of the fertilized treatment foliage in the September sampling month. Canopy position also had a significant impact on magnesium content. Magnesium concentration of 92-Last-Flush foliage was higher in the lower canopy than in the upper canopy position. For the 93-First-Flush foliage, the same was true for the unfertilized treatment but not for the fertilized during the September period. There also was an interaction of sampling month, canopy position and thinning for magnesium concentration.

Boron

Foliar boron concentration decreased in 92-Last-Flush foliage with age but no such seasonal pattern was found in the 93-First-Flush foliage (Figures 4-3C & 4-4C).

Thinning significantly decreased the foliar boron concentration of foliage in the upper and lower canopy positions. No pattern of fertilization impact was found in this study. Foliar boron content of lower canopy foliage was generally higher than that of the upper canopy.

Sulphur

Seasonal patterns of foliar sulphur concentrations were highly variable compared to the other mineral nutrients examined, and the patterns were not significantly different among sampling months or treatments. Mean foliar sulphur concentration was 757mg kg^{-1} (s.e.= 56.8, n=144) averaged across all periods and treatments.

Nutrient Ratios

Phosphorous to nitrogen ratios varied with sampling month without any clear pattern developing over the time sampled. The relative values for P:N did not significantly change, with respect to thinning, fertilization and canopy position, over time or age class of foliage. Therefore, the ratios presented are those averaged across sampling months (Figure 4-5). Fertilization significantly increased the P:N ratio in the thinned and unthinned treatments. However, P:N ratios were still below the optimum levels suggested by Adams and Allen (1984). No significant effect of canopy level

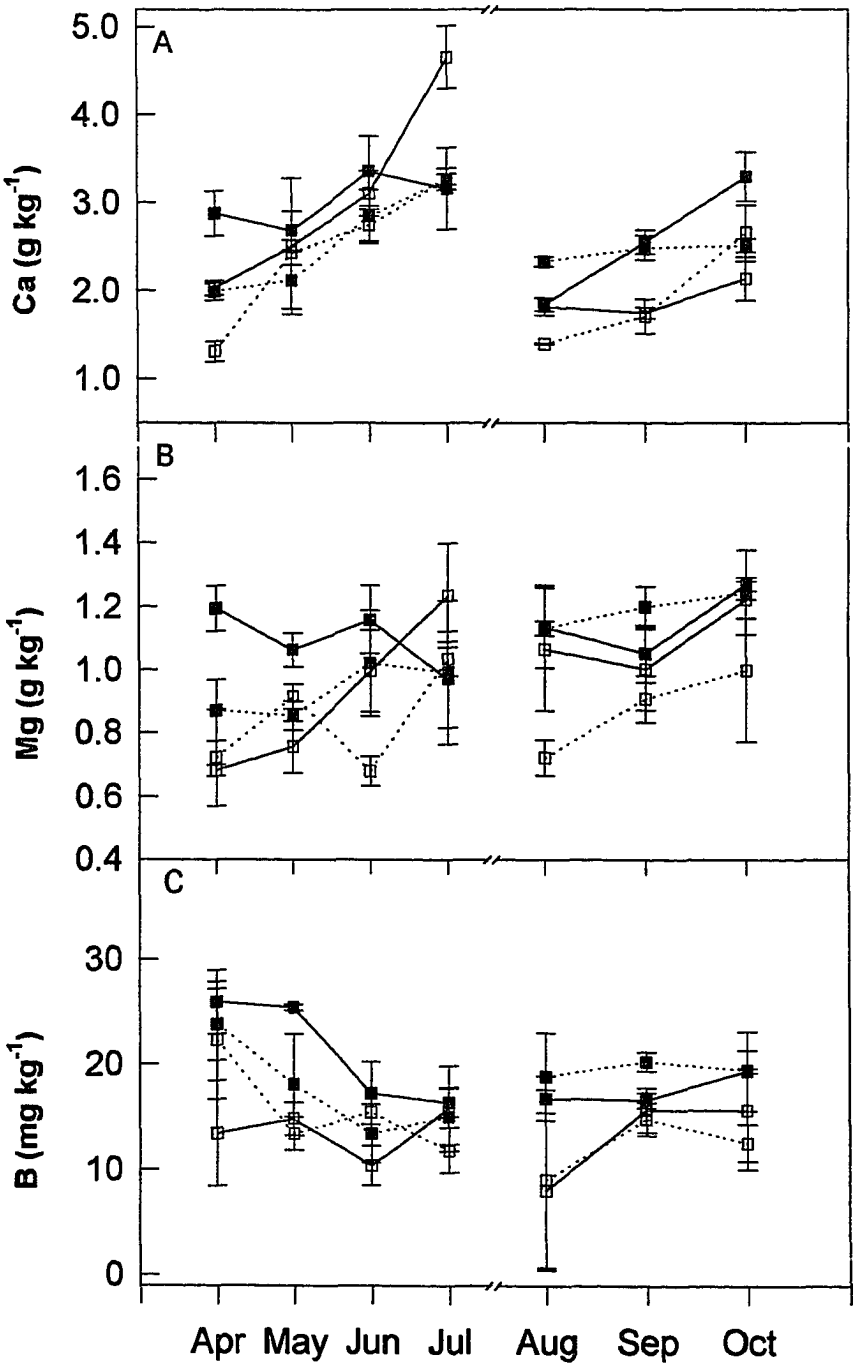


Figure 4-3. Foliar (A) calcium, (B) magnesium and (C) boron concentrations of 92-Last Flush foliage (April-July) and 93-First Flush foliage (August-October) of loblolly pine in the thinned plots. (n=2; ■ = lower, □ = upper, ····· = unfertilized, —— = fertilized).

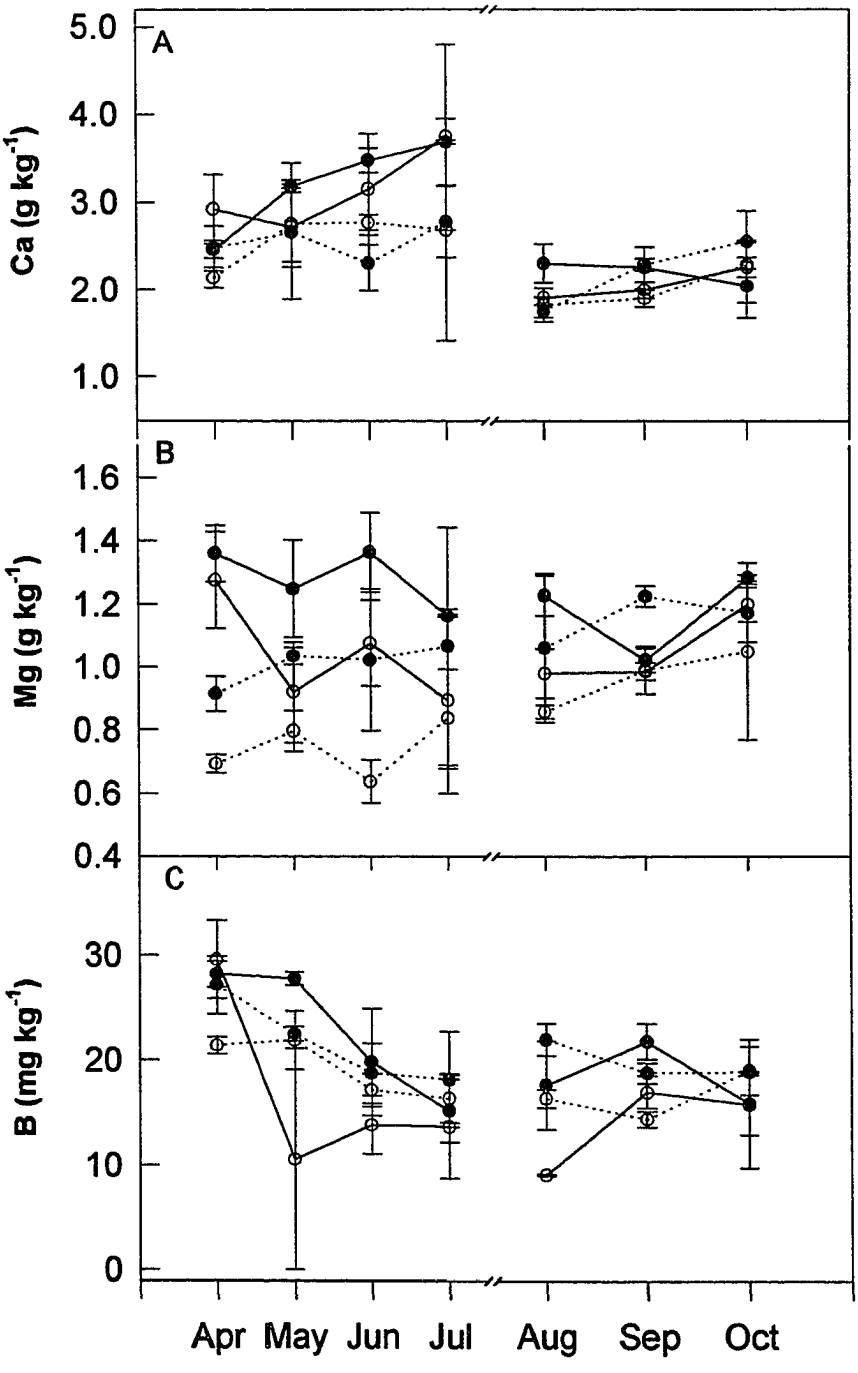


Figure 4-4. Foliar (A) calcium, (B) magnesium and (C) boron concentrations of 92-Last Flush foliage (April-July) and 93-First Flush foliage (August-October) of loblolly pine in the unthinned plots. (n=2; ● = lower, ○ = upper, ····· = unfertilized, —— = fertilized).

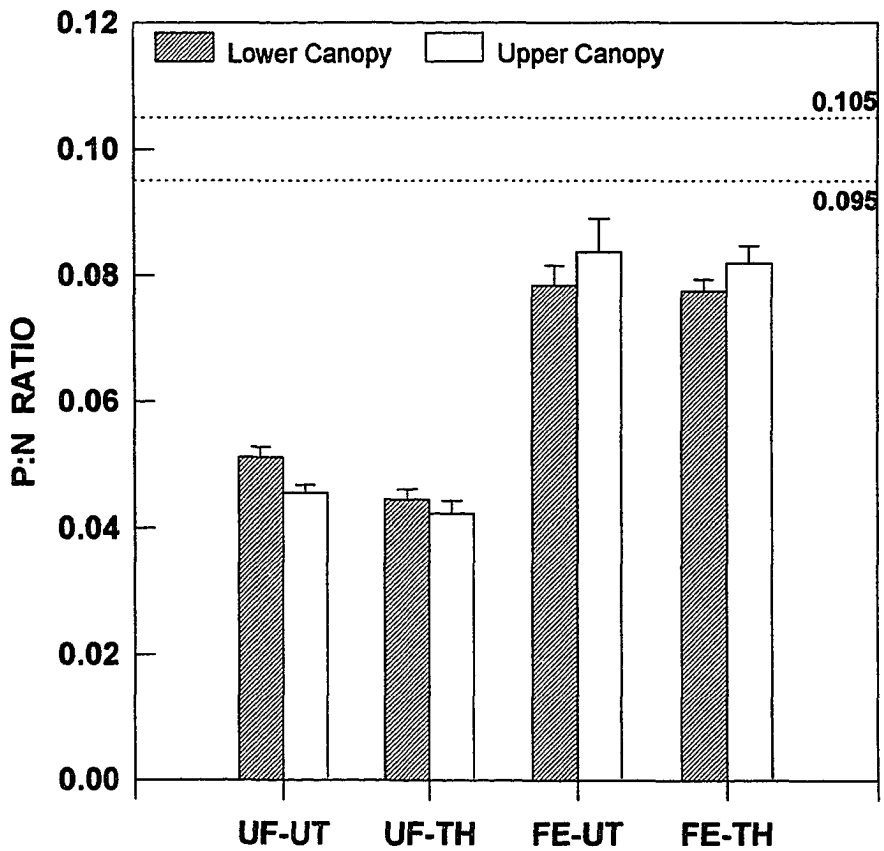


Figure 4-5. Mean foliar P:N ratios averaged across the 1993 growing season. Dotted lines indicate optimum P:N ratios, upper and lower bounds (Adams & Allen 1985). (n=14; UF = unfertilized, FE = fertilized, UT = unthinned, TH = thinned).

was evident, nor was thinning effective in changing the proportion of phosphorous and nitrogen five years after the treatments were applied.

Nutrient ratios for the upper canopy position did not differ significantly for fertilized/thinned (100N:8P:38K:23Ca:9Mg) versus fertilized/unthinned treatments (100N:8P:40K:23Ca:9Mg), and the unfertilized/thinned (100N:4P:30K:16Ca:7Mg) did not differ significantly from the unfertilized/unthinned treatments (100N:5P:32K:17Ca:6Mg). Fertilization led to increased K:N, Ca:N and Mg:N ratios, whereas thinning reduced the K:N ratio. Thinning did not have an impact on any of the other nutrient proportions. No interaction of fertilization with thinning was found in any of the nutrient ratios, nor with the concentration of mineral nutrients.

Needle Chlorophyll Content

Fertilization and thinning main effect treatments did not have an impact on chlorophyll concentrations (Table 4-3, Figure 4-6). Chlorophyll concentrations (Chl *a*, total Chl and Chl *a/b* ratio) did vary significantly ($p \leq 0.01$) over the growing season. Chlorophyll concentrations declined in 92-Last-Flush foliage (May-July) but did not differ significantly between sampling months in the 93-First-Flush foliage (Figure 4-6). Chlorophyll *a/b* ratios for the 93-First-Flush foliage were greater than the last sampling month for the 92-Last-Flush foliage.

Significant effects of canopy level on all needle chlorophyll concentrations (Chl *a*, Chl *b*, total Chl, and Chl *a/b* ratio) were found (Table 4-3). Generally, chlorophyll concentrations in the lower canopy foliage were greater than that of the upper canopy

Table 4-3. ANOVA (Pr > F) table for needle chlorophyll content of loblolly pine from the 1993 growing season. (Chl *a* = chlorophyll *a*, Chl *b* = chlorophyll *b*, Total = total chlorophyll (a + b), *a/b*ratio = chlorophyll *a/b* ratio).

Source	df	Chl *a*	Chl *b*	Total	*a/b* ratio
Fertilization (F)	1	0.5410	0.5119	0.5320	0.3935
Thinning (T)	1	0.1469	0.1320	0.1421	0.1456
F x T	1	0.8543	0.8628	0.8567	0.7506
Error A	4				
Measurement Period (MP)	4	0.0005	0.1799	0.0041	0.0001
MP x F	4	0.8040	0.9184	0.8484	0.4134
MP x T	4	0.8101	0.9101	0.8508	0.8909
MP x F x T	4	0.7155	0.9094	0.7803	0.9105
Error B	16				
Canopy Level (Lev)	1	0.0417	0.0207	0.0338	0.0051
Lev x F	1	0.6747	0.7004	0.6812	0.5974
Lev x T	1	0.8635	0.7515	0.9711	0.1571
Lev x F x T	1	0.9629	0.8197	0.9226	0.5486
Error C	4				
MP x Lev	4	0.8769	0.7093	0.8360	0.5969
MP x Lev x F	4	0.3827	0.1082	0.2817	0.6125
MP x Lev x T	4	0.1420	0.2080	0.1488	0.7392
MP x Lev x F x T	4	0.2993	0.1988	0.2692	0.4410
Error D	16				
Total	79				

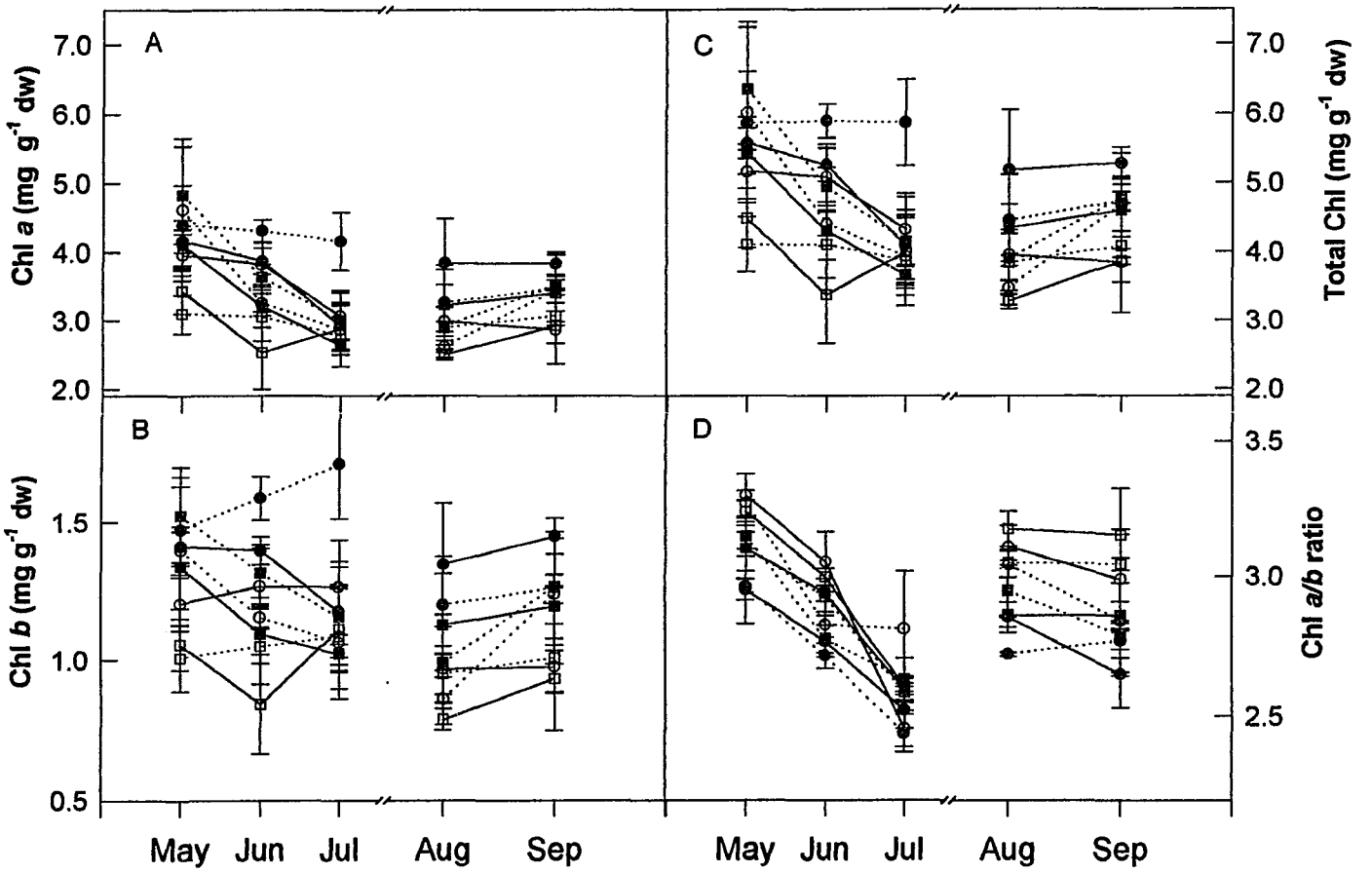


Figure 4-6. Foliar (A) chlorophyll *a*, (B) chlorophyll *b*, (C) total chlorophyll and (D) chlorophyll *a/b* ratio for 92-Last Flush (May-July) and 93-First Flush (August-September) foliage. Bars indicate plus or minus one standard error of the mean. (● = unthinned-lower, ○ = unthinned-upper, ■ = thinned-lower, □ = thinned-upper, ····· = unfertilized, —— = fertilized).

foliage. The chlorophyll *a/b* ratio of upper canopy foliage was significantly greater than that of the lower canopy foliage. No significant interactions of main effects were found.

Correlation analyses were conducted to investigate possible relationships between nutrient concentrations and chlorophyll content using the mean concentrations for each sampling month. Correlations between nitrogen concentrations and total chlorophyll content were not significant for any of the treatment combinations (Figures 4-7 & 4-8). However, when the lower canopy foliage of the thinned and unthinned, fertilized plots were combined, a positive correlation was found between nitrogen and total chlorophyll content (R^2= 0.49; p = 0.025; Y = 0.524 + 0.121X). Lower, thinned fertilized treatment foliage tended to separate out from the lower, unthinned fertilized foliage. The lower unthinned, fertilized plots had a greater nitrogen and total chlorophyll content.

In contrast to total chlorophyll concentration, a significant correlation ($p \leq 0.01$) of nitrogen content versus chlorophyll *a/b* ratio was found for foliage from thinned and unthinned, fertilized treatments (Figure 4-9). However, no significant correlation was found for foliage in the thinned or unthinned, unfertilized treatment (Figure 4-10).

DISCUSSION

The concentration of mobile nutrients, nitrogen, phosphorous and potassium are usually highest in younger foliage because of the stronger sink created by their high

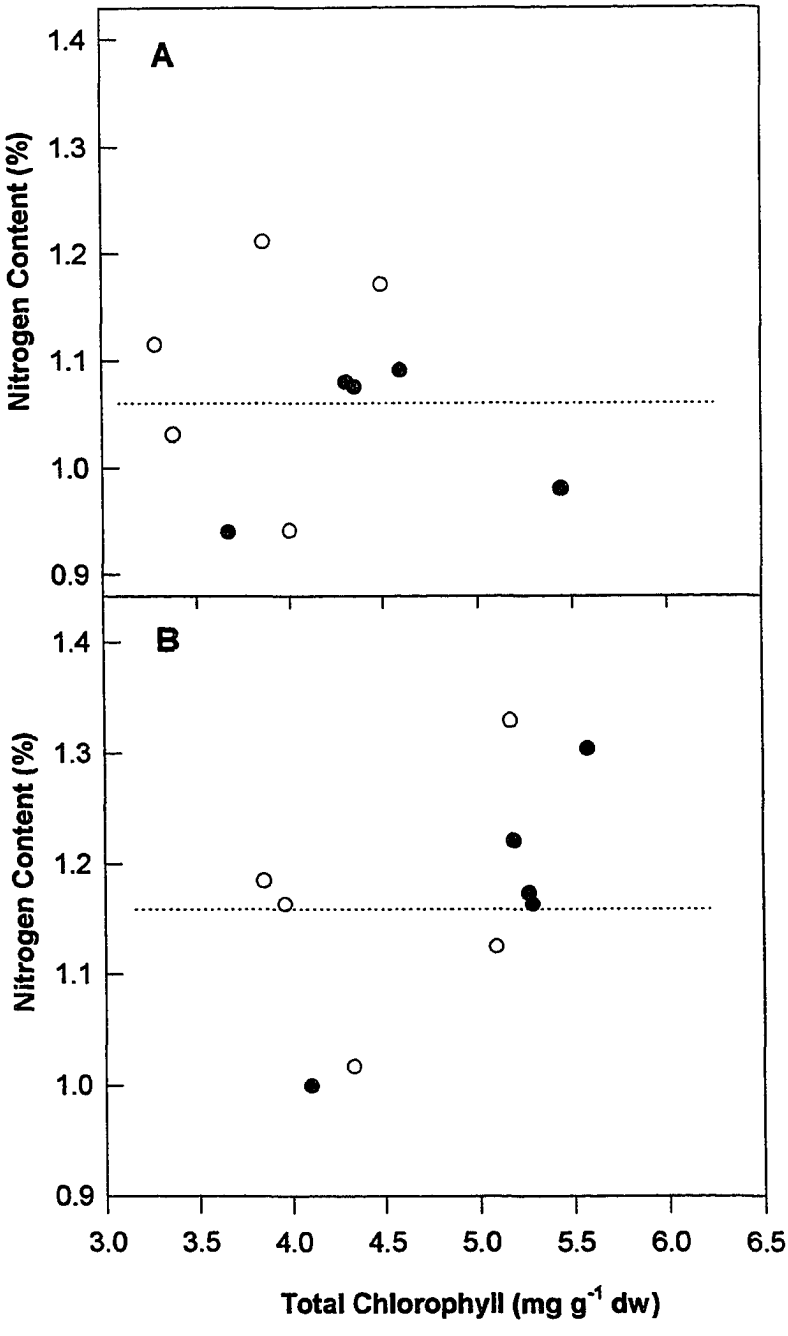


Figure 4-7. Fertilized treatment mean foliar nitrogen concentration vs. mean total foliar chlorophyll concentration for (A) thinned and (B) unthinned loblolly pine stands. (● = lower canopy, ○ = upper canopy).

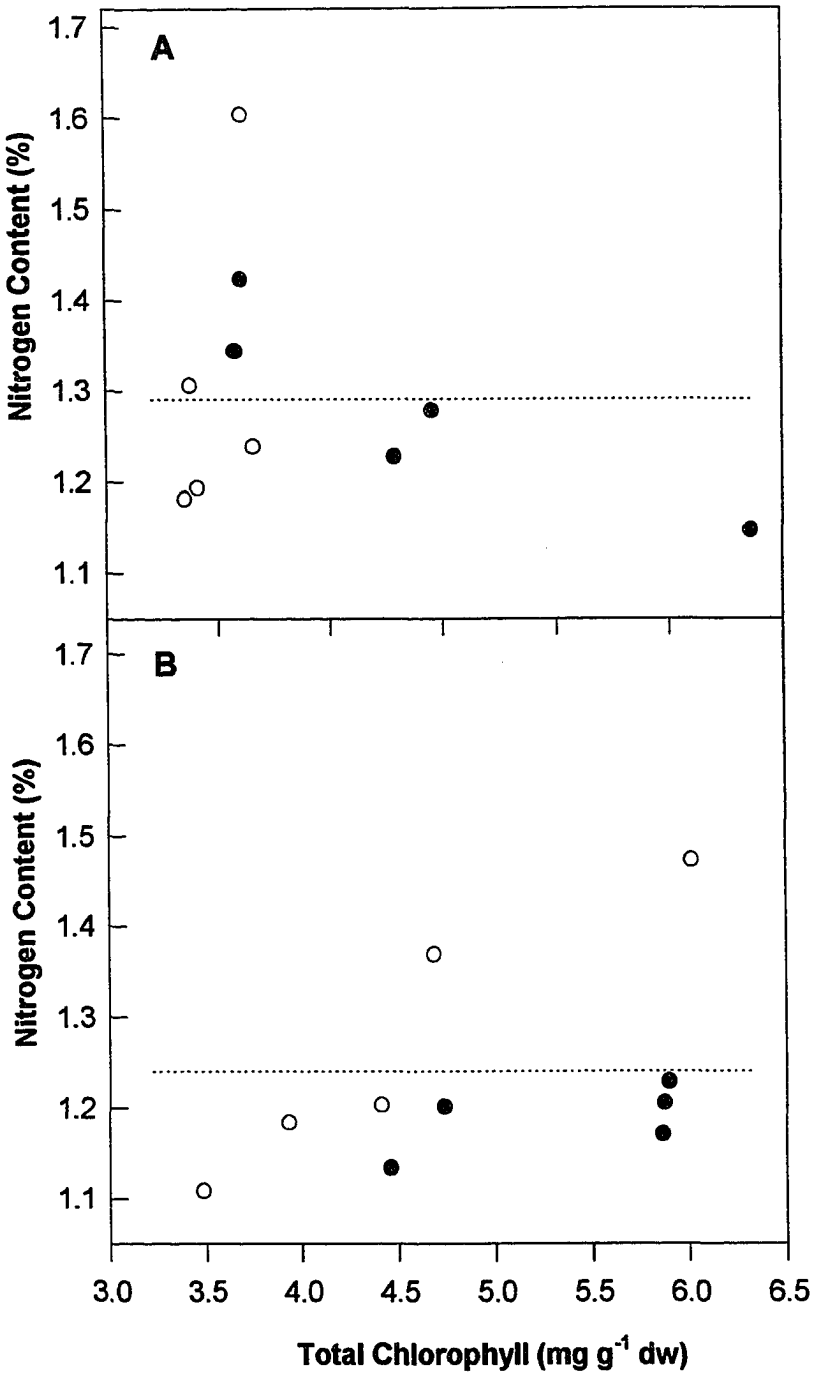


Figure 4-8. Unfertilized treatment mean foliar nitrogen concentration vs. mean total foliar chlorophyll concentration for (A) thinned and (B) unthinned loblolly pine stands. (● = lower canopy, ○ = upper canopy).

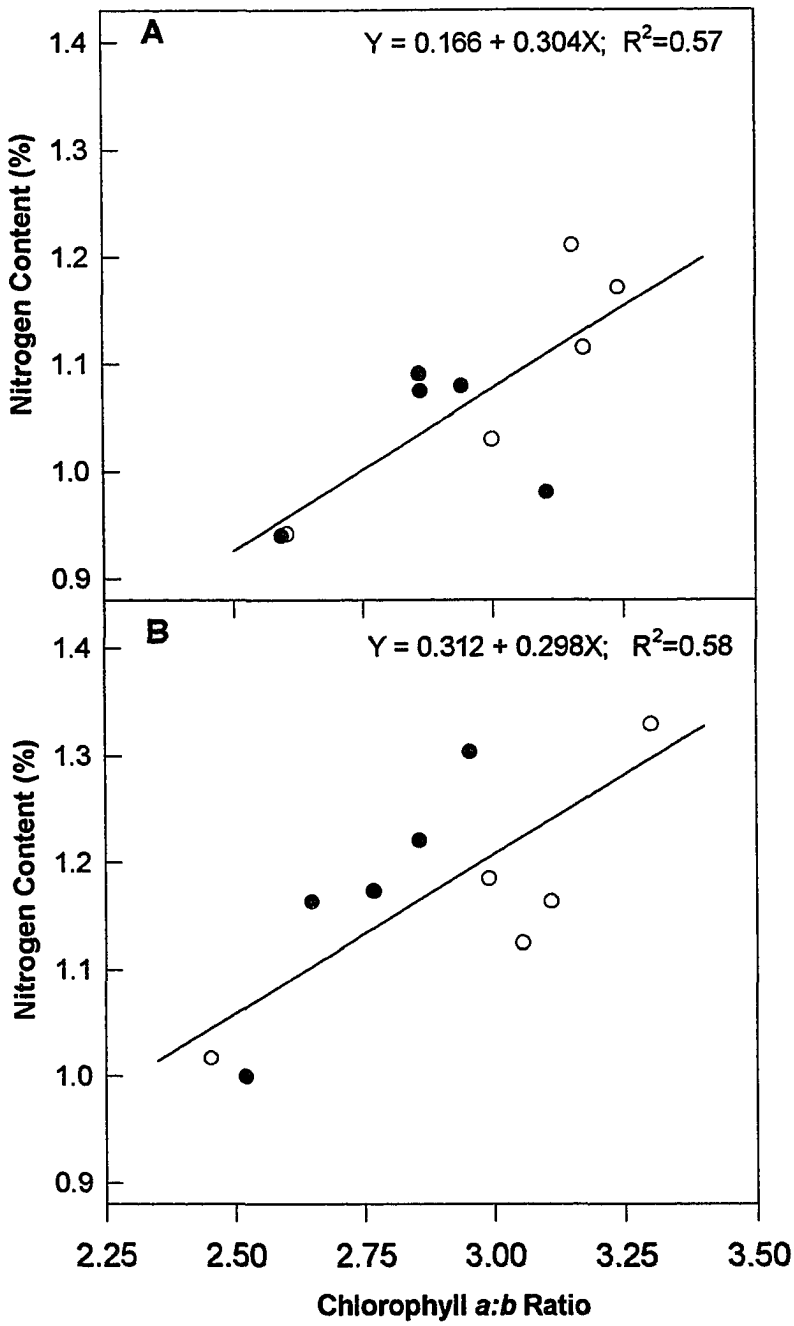


Figure 4-9. Fertilized treatment mean foliar nitrogen concentration vs. mean foliar chlorophyll *a:b* ratio for (A) thinned and (B) unthinned loblolly pine stands. (● = lower canopy, ○ = upper canopy).

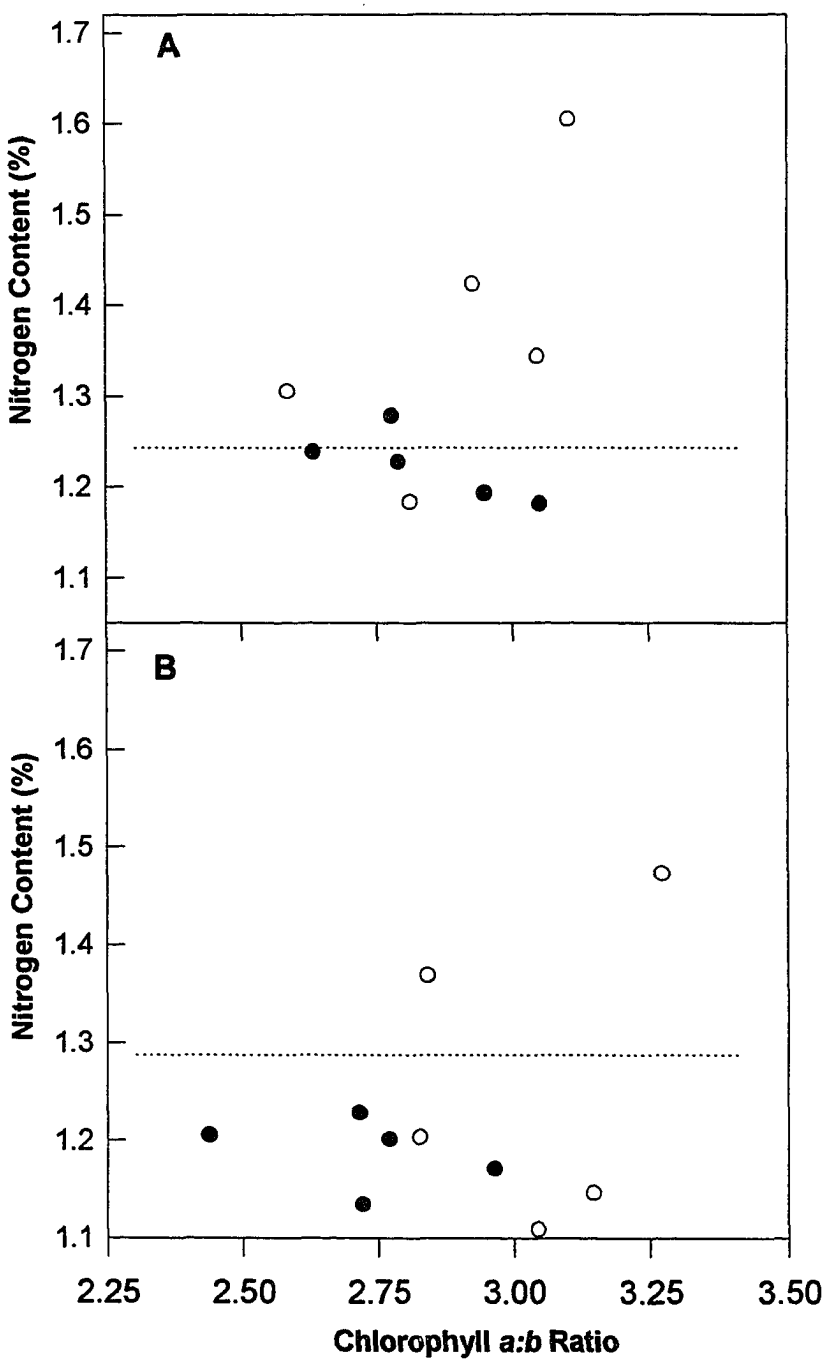


Figure 4-10. Unfertilized treatment mean foliar nitrogen concentration vs. mean foliar chlorophyll *a:b* ratio for (A) thinned and (B) unthinned loblolly pine stands. (● = lower canopy, ○ = upper canopy).

metabolic activity (Mengel and Kirkby 1982, Smith et al. 1971). Presumably nitrogen and phosphorous are translocated from the older to the younger foliage (Smith et al. 1971, Vitousek 1982). In this study, this appeared to be true for phosphorous and potassium, but not for nitrogen. Nitrogen concentration of 92-Last-Flush foliage showed only a slight decline and no accumulation in the 93-First-Flush foliage. This may be, due in part, to the sampling scheme used in this study. Sampling of 93-First-Flush foliage was postponed until the needles had reached their maximum length. Thus, the sampling scheme may have missed the lower nitrogen concentration in the younger, 93-First-Flush foliage at the beginning of their activity and the decline in 92-Last-Flush foliage after their last sampling month. On the other hand, Smith et al. (1971) did not find a depletion in nitrogen content of previous flushes during the development of subsequent flushes. They suggested that nitrogen is probably being received from other sources: older foliage prior to senescence, stems, branches and roots. This study tends to support their findings.

Phosphorous and potassium levels of 92-Last-Flush foliage generally did not decline with time, except for phosphorous in the unthinned, fertilized treatment foliage. Phosphorous and potassium foliar nutrient concentration for the 92-Last-Flush foliage, at the last sampling month (July), were generally lower than that of the 93-First-Flush foliage. This stable pattern of nutrient concentration would indicate that the development of the 93-First-Flush foliage has drawn nutrients from other sources other than the previous years foliage. Based upon physiological work with this species, this was expected. Foliage from a previous growing season continues to function

photosynthetically, at rates usually exceeding those of the 93-First-Flush growth, well into the current growing season. This would explain the maintenance of foliar nutrient concentrations (N, P, & K) found in the 92-Last-Flush foliage during the last month of sampling. Keep in mind that the 92-Last-Flush foliage was the second flush from the 1992 growing season. That is, this flush may have itself been nutrient limited and thus, the foliage had a lowered nutrient concentration, developing after a previous flush had drained winter-stored nutrient reserves. An alternative explanation could be that the nitrogen is translocated from the older, 92-First-Flush foliage (Wells and Metz 1963, Rathfon et al. 1993) which was not measured in this study.

Fertilization with nitrogen and phosphorous are generally believed to increase the foliar concentration of each of these minerals (Allen 1987, Switzer and Nelson 1963). However, some studies have found a reduction in foliar phosphorous and potassium levels in Douglas-fir after fertilization (Heilman and Gessel 1963). In the current study, phosphorous levels were increased significantly by fertilization in both the thinned and unthinned treatment foliage. Lower canopy foliage was significantly higher in phosphorous content than upper canopy foliage. In stark contrast, foliar nitrogen concentration was reduced by fertilization. It is hypothesized that the relatively lower nitrogen concentration found in the fertilized plots is caused by a dilution phenomenon. The application of the fertilizer was five years prior to this study and increased mean tree size (see Table 4-1), as well as foliage biomass (personal observation), thus diluting foliar nitrogen content per unit dry weight. Switzer et al. (1966) demonstrated loblolly pine stands continue to accumulate nitrogen

at the mean rates of 6.3 and 5.0 kg ha^{-1} $year^{-1}$, for good and poor sites, respectively, during the initial thirty years of stand establishment. Since nitrogen levels are currently lower in the fertilized stands, re-fertilization of these stands, with nitrogen and/or phosphorous, may again produce a measurable response in tree growth and nutrient concentration.

Thinning significantly reduced foliar phosphorous and potassium concentrations in fertilized treatments. Averaged across sampling months and canopy positions, phosphorous levels were 0.85 and 0.95 g kg^{-1}, and potassium levels were 3.9 and 4.9 g kg^{-1} for thinned and unthinned stands, respectively. The reason for the lower concentration of each of these nutrients may be related to the release of growing space by thinning. The creation of growing space may have led to a reduction in nutrient concentration, again through dilution because of increased foliage biomass. The positive correlation of nitrogen and total chlorophyll in the lower canopy foliage, from thinned and unthinned, fertilized treatments, further reflected the impact that thinning has on nutrient content. No such relationship between nitrogen and chlorophyll was found in the upper canopy levels. Thus, stand density is a strong modifying factor, particularly on lower canopy branches, and should be looked at closely when determining a stands potential response to fertilization.

The more immobile nutrients, such as calcium, tended to increase with age for all foliage studied as found in other studies (Wells and Metz 1963, Rathfon et al. 1993). The accumulation of immobile nutrients is the result of calcium being taken up with soil water and transported to the needles, through transpiration, and deposited in

the needles where it accumulates predominately in the cell walls. Once the foliage is mature, most of the calcium required should have already been acquired by the needles. Furthermore, the continued accumulation of calcium may indicate adequate amounts of calcium continue to be found in the soil solution.

Canopy position had a significant impact on nitrogen, magnesium and boron concentrations. Nitrogen concentration was generally less, and magnesium concentration greater, in lower canopy foliage. The lower foliar nitrogen concentration found in the lower canopy is probably related to the lower light penetrating to the lower crown (Evans 1989). Canopy position, however, did not have a significant impact on the phosphorous level in the foliage. Higher foliar magnesium concentration coincided with higher concentration of chlorophyll in the lower canopy. The significantly greater concentration of chlorophyll and lower chlorophyll *a/b* ratio confirm the shaded conditions of the lower canopy foliage of the thinned and unthinned stands and fall within the range reported for other species with C_3 photosynthesis (Larcher 1983).

The presence of a significant positive correlation of nitrogen content versus chlorophyll *a/b* ratio in some of the stands was expected. The unthinned, fertilized stand had a closed canopy and the thinned, fertilized stand had begun to approach canopy closure once again. Therefore, both of these stands had distinguishable sun- and shade-exposed foliage within their crowns. Thus, increases in the needle chlorophyll *a/b* ratio with concomitant increases in nitrogen content, of upper canopy foliage can be explained in terms of light availability. Chlorophyll *a/b* ratios have

been suggested as a good predictor of light availability (Dale and Causton 1992), and *a/b* ratios are known to decrease in shade-grown foliage in Sitka spruce (Lewandowska et al. 1976). Sun-adapted foliage have greater concentrations of ribulose bisphosphate carboxylase (Rubisco), primarily because CO_2 availability and its incorporation by Rubisco, are the limiting step in carbon fixation (Black 1973, Evans 1989, Osmond 1978). The low availability of light in the lower canopy affects the physiology of the needles causing them to invest more energy into the light capturing pigments, including chlorophyll (Evans 1989) and less in the relatively nitrogen expensive enzyme Rubisco. Rubisco is a nitrogen costly enzyme to construct so the higher nitrogen concentration in the upper canopy foliage also was expected.

Boron is an essential element reported to be important in shoot expansion and has been suggested to be important in plant growth and development (Dugger 1983). Foliage in the thinned stands and in the upper canopy position were significantly lower in boron content. However, the levels of boron found in this study never fell below that of those reported to be a critical level (Dugger 1983).

No one nutrient can act alone in producing physiological changes leading to growth responses. Studies on fertilization in loblolly pine have shown that nitrogen and phosphorous produce a greater response when used together than when applied alone (Allen 1987, Vose and Allen 1988, Wells and Allen 1987). The balance between foliar nutrients is an important diagnostic tool in determining tree growth response to fertilization. Comerford and Fisher (1984) reported that the proportion of nitrogen to phosphorous was a more reliable and accurate method for determining

nitrogen deficient soils. Adams and Allen (1985) later reported critical phosphorous to nitrogen proportions that were convenient for determining which foliar nutrient is most limiting, and can be extremely useful in determining which mineral nutrient, nitrogen or phosphorous, to apply to produce a growth response.

Relative nutrient proportions for the upper canopy foliage were 100N:8P:38K:23Ca:9Mg (fertilized/thinned), 100N:8P:40K:23Ca:9Mg (fertilized/ unthinned) , (100N:4P:30K:16Ca:7Mg (unfertilized/thinned) 100N:5P:32K:17Ca:6Mg (unfertilized/unthinned). Fertilization increased P:N, K:N, Ca:N and Mg:N proportions. The proposed optimum ratio of P:N is 0.095 to 0.105 (Adams and Allen 1985) for loblolly pine. Above this range, nitrogen is most limiting to growth and a strong nitrogen response would be expected. Below this range a strong phosphorous response is expected. Results from this study suggest that while the P:N ratio was increased by fertilization these stands still fall below the optimum level suggested in the literature. In addition, foliar nutrient proportions were not affected by the thinning treatment, but an increase in calcium and magnesium in the lower canopy position was found.

CONCLUSIONS

Foliar concentrations of nitrogen and phosphorous tended to decrease with age in 92-Last-Flush needles. The nutrient content of 93-First-Flush foliage did not display a discernable pattern of nutrient accumulation over the sampling times or may have already increased to maximum levels by the time they were measured. Some

evidence of nutrient retranslocation from 92-Last-Flush needle to new growth was found for nitrogen under both fertilized and unfertilized treatments but most of the nitrogen probably came from other sources, perhaps the 92-First-Flush needles.

Fertilization significantly decreased foliar nitrogen concentrations but increased foliar phosphorous, calcium and magnesium levels. Thinning significantly reduced foliar phosphorous and potassium concentrations in fertilized stands. The decrease in nitrogen, in response to fertilization, and the decrease in phosphorous and potassium, in response to thinning, may have been the result of a dilution effect caused by the increased biomass of these trees (Haywood 1993). Fertilization and thinning did not have an impact on foliar chlorophyll content, but canopy level played a significant role in determining foliar chlorophyll content and followed the classic sun-shade physiological patterns.

Fertilization had a significant impact on foliar nutrient content. Fertilization in this study led to a decrease in nitrogen content, primarily through the process of dilution as a result of increases in biomass, and an increase in phosphorous content. In contrast, thinning primarily impacted only the lower canopy foliage by affecting the light availability to the lower canopy branches. Therefore, greater variability in physiology and concomitant nutrient content between upper and lower canopy foliage is expected in dense canopies. Thus, to adequately sample foliage in dense stands, it would be necessary to sample upper and lower canopy branches. If, however, crown closure has not occurred, or trees are open-grown, it may be acceptable to sample from almost any standardized position within the crown.

Atmospheric CO_2 concentrations have already risen from a preindustrial level of 280 ppm to approximately 360 ppm. If the climatic changes become more favorable, e.g., increased CO_2 levels, nutrient supply may impose a serious limit to growth. Tissue et al. (1993) observed the photosynthetic response of loblolly pine seedlings to elevated CO_2 was correlated with nitrogen availability. The photosynthetic rates were higher only for those seedlings grown at elevated CO_2 with supplemental nitrogen. This study suggests that increases in foliar biomass will lead to a reduction in foliar nutrient contents. The current literature suggests that loblolly pine grown in native soils (low nitrogen) will acclimate to long-term CO_2 enrichment and maintain current photosynthetic rates unless nitrogen is added to the stands (Tissue et al. 1993, Tschaplinski et al. 1993).

This study points out the need to consider the sampling date, the silivicultural practices of thinning and fertilization, the age class of the foliage, and foliage canopy position when diagnosing nutrient concentrations and proportions. Furthermore, it is important to consider sampling during peak periods of growth as suggested by Wells and Metz (1963) and Rathfon et al. (1993).

CHAPTER 5

FOLIAR LIGHT RESPONSE CURVE ANALYSIS

INTRODUCTION

It has been known for a long time that plants from sun and shade environments have different photosynthetic characteristics (Böhning and Burnside 1956, Björkman 1968, Lewandowska et al. 1976, Boardman 1977). Additionally, many species adapt to the light environment during growth. Thus, in many species the photosynthetic characteristics are modified in an individual plant during development. Physiological and morphological modifications are important in the success of many plant species (Larcher 1983). Morphological adaptations involve increased leaf thickness and mesophyll cell volume with increasing levels of light (Nobel 1977 & 1991, Patterson 1980). The adaptability of the photosynthetic system to changes in the light environment during growth is of interest when considering predictive models of tree canopy foliage photosynthesis.

In general, increased illumination of foliage leads to a concurrent increase in photosynthetic carbon uptake per unit of leaf tissue. There are several key characteristics found in such a light response curve. The initial part of the light-dependent curve in dim light reflects the net loss of CO_2 since more CO_2 is lost through respiration than is fixed by photosynthesis. At the light compensation point (I_C), photosynthesis exactly fixes as much CO_2 as is released by respiration. The initial

linear phase of the light response curve indicates the apparent quantum efficiency (ϕ) of the photosynthetic system. In this region of the light response curve, the speed of the light reactions is the limiting factor for the overall process. The greater the slope, the higher the quantum efficiency (δ CO_2 uptake/ δ quanta absorbed), until at very high light intensity, the yield of photosynthesis continues to increase only slightly or not all. At the point of little or no increase in photosynthesis, with increasing light, the reaction is light-saturated (I_S), and the rate of CO_2 uptake is now limited by enzymatic or by the supply of CO_2 , rather than by the photochemical processes.

A comparison of light response curves and the cardinal points, I_C and I_S ,reflect the light environment under which the foliage developed. Foliage adapted to shade respire less than high-light adapted foliage, and therefore, it has a lower light compensation point (I_C) (Marshal and Biscoe 1980, Björkman 1981). Carbon dioxide uptake by sun foliage is saturated at a higher light intensity than that of shade foliage (Boardman 1977). In a study of *Pinus taeda* L., Cregg (1990) investigated photosynthetic light response of foliage under three levels of shade treatment. He found that I_S and maximum photosynthesis decreased with increasing levels of shade. Similar results have been found in *Pinus radiata* D. (Warrington et al. 1988) where low light reduced maximum photosynthesis from 2.0 to 1.3 μmol m^{-2} s^{-1}. Ginn et al. (1991) found a reduction is light saturated photosynthesis, from 4.17 μmol m^{-2} s^{-1} for upper canopy (sun adapted) to 3.22 μmol m^{-2} s^{-1}, of lower canopy foliage (shade adapted) in an unthinned stand of nine-year-old *P. taeda*.

Theoretically, the quantum efficiency (ϕ) of foliage from different species should not vary using identical mechanisms of energy conversion, e.g., C_3 photosynthesis (Ehleringer and Pearcy 1983). Björkman's (1981) review of the literature found that ϕ did not differ between species with the same photosynthetic pathway. Furthermore, based on his earlier studies (Björkman et al. 1972a, Björkman et al. 1972b, Ehleringer and Björkman 1977) as well as work by Ludlow and Wilson (1971), he concluded that no differences exist between sun and shade leaves of the same species.

In loblolly pine, Cregg (1990) found widely varying values, though none were significantly different, for ϕ in response to shade treatments in July, 1989. In October, 1989 the values observed were identical across all shade treatments. For *Pinus sylvestris*, Leverenz and Öquist (1987) found little variation in ϕ of seedlings during the course of a year. Similarly, no difference was found in values of ϕ for detached shoots of open- and forest-grown *Picea abies* L. shoots (Kull and Koppel 1987).

The convexity term (θ, a measure of the degree of bending) in the light response curve equation has been suggested to be an indicator of chlorophyll content (Leverenz 1987). The convexity term determines the transition from one major limitation of photosynthesis to another as light increases (Ögren 1993). This assumption is based on the gas exchange model of Farquhar et al. (1980) which predicts the rate limitation at high light is exerted by ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco). Some investigators have tried to assign a physical

interpretation to θ , with a decease in θ attributable to an increased capacity of Rubisco relative to electron transport (Ögren 1993).

Previous studies involving the impact of silvicultural treatments of loblolly pine foliage have concentrated on the effect of the light environment on the maximum rate of photosynthesis as measured under light saturated conditions (Ginn et al. 1989, Ginn et al. 1991), or ambient light conditions (Nowak et al. 1990). However, because the light environment changes within the crown and light levels are most often below light saturation (Sinclair and Knoerr 1982, Smolander 1984), the photosynthetic response to light is important. The objective of this study was to investigate the variation in light response curves of upper and lower canopy loblolly pine foliage in response to fertilization and thinning. Information from this study could be useful in modeling photosynthetic carbon gain in similar stands of trees given the ambient light conditions within the crowns.

MATERIALS AND METHODS

Site and Stands

Loblolly pine (*Pinus taeda* L.) used for this study were planted in 1981 and are located in the West Pasture, Johnson Tract, Palustris Experimental Forest, Rapides Parish, Louisiana as described in previous chapters. In the fall of 1988, two cultural treatments were randomly assigned to the twelve plots in a two-by-two factorial design with three replicates. Silvicultural treatments included thinning and fertilization with plots measuring 23.7 by 23.7 meters. Thinning consisted of either leaving the trees at

their original planting density of 2990 trees per hectare, or plots were thinned to 731 trees per hectare. The fertilizer treatment was either no fertilizer applied or diammonium triple superphosphate was applied at 744 kg per hectare (150 kg phosphorous and 134 kg nitrogen per hectare).

Photosynthesis Measurements

Photosynthetic light response curves were developed with the LI-COR LI6200 Portable Photosynthesis System. Light response curves, along with dark respiration rates, allowed the calculation of light compensation points (Givnish 1988). Using shade cloths and accessory lighting (Sylvania 400 watt metal halide lamp), photosynthesis measurements were taken at different PPFD levels for fascicles on detached branches. Ten to twelve light levels, ranging from zero to two thousand μmol $m^{-2}s^{-1}$ PPFD, were used to produce each light curve. The PPFD levels were such that the light was increased from darkness to 2000 μmol $m^{-2}s^{-1}$ PPFD. The PPFD levels were obtained by changing the height of the lamp from the cuvette containing the two fascicles. Foliar dark respiration rates were measured after the fascicles had equilibrated to the dark condition created by covering the gas-exchange cuvette with black cloth. Fascicle within the cuvette were maintained in a horizontal position so that the needles did not shade each other. Sampling times were restricted to the period from 10:00 to 14:00 hours in order to minimize potential diurnal effects. Apparent quantum efficiency (ϕ) and convexity (θ) were determined from the curves.

All measurements were taken under ambient conditions on detached branches using the most recent fully expanded fascicles (current-year needles). The sample branches were recut under water and a 30 cm section of vinyl tubing, filled with water, was attached to the cut end of the stem to provide an adequate supply of water to the foliage. Special precautions to minimize exposure chamber heating under artificial lighting were taken. A hot mirror, which reflects ~85% of the infrared radiation, and a small external fan were used to help dissipate the heat. A single light response curve, generally was completed within thirty minutes. Ginn et al. (1991) showed that photosynthetic rates of individual needles were not effected by removal from the tree for up to thirty minutes. A series of sample runs were performed on these trees and confirmed their findings. The projected surface area of the needles was determined by a LI-COR LI3000 Leaf Area Meter on the portion of the needles enclosed in the gas exchange chamber. Light levels, as photosynthetically active radiation (PPFD), were measured using the quantum sensor incorporated on the LI6200 Photosynthesis System (LI-COR Inc., Lincoln, NB).

Modelling Light Response Curves

Light response curves for photosynthesis were fit to the data by a two parameter nonlinear regression analysis using the PROC NLIN (SAS Institute, Inc., Cary, NC) procedure in SAS. The regression model was based on the non-rectangular hyperbola equation presented by Marshall and Biscoe (1980). The equation for this model is presented in equation 1.

Equation 1: $$P_{net}=\frac{(\phi Q+P_{max}+R_d) - \sqrt{(\phi Q+P_{max}+R_d)^2-4\phi Q\theta(P_{max}+R_d)}}{2\theta} - R_d$$

P_{net} = net photosynthetic rate in μmol m^{-2} s^{-1}
Q = photosynthetic photon flux density in μmol m^{-2} s^{-1}
ϕ = apparent quantum efficiency
θ = convexity term
P_{max} = net photosynthetic rate at asymptote
R_d = dark respiration rate

Experimental Design

The experimental design was that of a split-plot. The main plot treatments were thinning and fertilization, with a sub-plot treatment of canopy position. All treatment combinations of one replicate were sampled on a given day. The remaining replicates were sampled on the following days. In 1992, three replicates were sampled during July 21- 23, 1992 and September 11-13, 1992. In 1993, two replicates were sampled during each of the sampling periods, June 5-6, 1993 and October 1-7. The procedure was done on fully mature foliage in the early and late summer.

Needle Properties

After returning to the laboratory, needle material used in the light response experiment was measured for projected leaf area using a leaf area meter (LI-COR model LI-3000, Lincoln, NE) and the tissue oven-dried at 65° C for 48 hours. Projected leaf area and dry weights were recorded and specific leaf areas (SLA) calculated on the portion of each fascicle enclosed in the LI-COR cuvette.

Specific leaf area (cm^{-2} g^{-1} dw) of needles from each branch were determined on the sample of needles used in the light response, chlorophyll and nitrogen content

experiments. Total needle surface area was determined in a separate experiment from this study using the method described by Johnson (1984). An approximate conversion from projected leaf area to total surface area can be obtained by dividing by 2.68 (Vose and Allen 1988).

Needle Chlorophyll Content

Loblolly pine needles sampled from the branches used for gas exchange work, were analyzed for chlorophyll *a* and *b* by methods described by Moran (1982) and Inskeep and Bloom (1985) using dimethylformamide. Samples that could not be immediately analyzed were stored at -70° C. A sample of each extract was placed in a spectrophotometric tube and the absorbance at 647 and 665 nm was recorded. The concentrations of chlorophyll *a* and *b* were calculated as:

$$\text{chlorophyll } a = 12.70\ A_{665} - 2.79\ A_{647}$$

$$\text{chlorophyll } b = 20.70\ A_{647} - 4.62\ A_{665}$$

The concentration in $\mu g\ ml^{-1}$ was multiplied by the total amount of chlorophyll solution to determine the total amount of chlorophyll in the sample. Chlorophyll *a:b* ratios were used to detect sun-shade differences in the forest canopy foliage (Dale and Causton 1992).

Nitrogen-Use Efficiency

Needles were subsampled from branches used in the light response experiment in 1993. Only mature, fully elongated fascicles were used in the samples. Forty to

fifty fascicles were taken from each sample branch and pooled. The needle samples were oven dried (65°C), then ground to a fine powder (20 to 40 mesh) in a Wiley mill. Total nitrogen was analyzed using a LECO (Model FP-428) Nitrogen Analyzer by the L.S.U. Agricultural Center Plant Analysis Lab, in the laboratory of Dr. Paul F. Bell. Nitrogen concentrations are expressed on a percent of dry weight. Nitrogen-use efficiency was calculated on a mg CO_2 per mg^{-1} N basis.

Statistical Analyses

Analysis of variance was used to test for significance of differences among treatments for maximum photosynthesis, apparent quantum efficiency, the convexity term and other physiological parameters using PC-SAS statistical software (PROC GLM, SAS Institute, Inc.). Plots of ground were the experimental unit for physiological and environmental comparisons. The experimental design is a repeated measure with a split plot. The main plot consists of the thinning and fertilization treatments, and subplot treatment of canopy level (upper and lower), with the measurement periods (early and late summer) as the repeated measure. A separate analysis using this model was done for each of the two growing seasons (1992 and 1993).

RESULTS

Environmental conditions during measurements were not dramatically different between the two sampling periods during the 1992 or 1993 growing seasons (Table

5-1). Predawn fascicle xylem water potential did differ between the months sampled, but did not differ significantly among the silvicultural treatments within the sample periods (Table 5-2). The thinned plots generally had higher mean predawn water potentials than that of the unthinned plots. Overall, mid-day xylem water potential did not differ significantly between the sampling months or silvicultural treatments and never fell below -1.5 MPa. However, needle fascicle mid-day xylem water potential from the upper canopy level (-1.5 MPa) were significantly lower than mid-day xylem water potentials of the lower canopy (-1.2 MPa).

Light Response Curves

Light response curves of photosynthesis were fitted, using a non-rectangular hyperbola (equation 1), to the data collected during the 1992 and 1993 periods. The estimates of the mean light response curve parameters for 1992 and 1993 sampling years are presented in Table 5-3. Since the pattern among treatment was similar for both years, the mean values for the two sample years were used to graphically present the mean response of photosynthesis to light for the lower canopy (Figure 5-1) and upper canopy foliage (Figure 5-2). Potential maximum rates of photosynthesis (P_{max}) on a projected leaf area basis were significantly different between months in 1992 ($p= 0.0001$) and had a significant ($p= 0.0498$) fertilizer by canopy level interaction (Table 5-3). However, neither thinning, fertilizer, month, canopy level, nor their interactions had a significant impact on mean P_{max} during the 1993 sampling year.

Table 5-1. Mean (s.e.) air temperature, relative humidity and carbon dioxide concentration in the LI-COR cuvette during the 1992 and 1993 light response measurements.

Date	Air Temperature	Relative Humidity	CO_2 Concention
m-yr	°C	%	$\mu l\ l^{-1}$
7-92	32.2 (0.34)	65 (1.7)	365 (2.6)
9-92	30.1 (0.51)	62 (1.5)	368 (2.5)
6-93	30.8 (0.49)	64 (1.5)	372 (2.7)
10-93	28.3 (0.72)	61 (3.4)	378 (2.5)

Table 5-2. Mean (s.e.) predawn fascicle xylem water potentials for plots sampled during the 1992 and 1993 light response measurements (FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned; n=6).

Treatment	Date 7-92	9-92	6-93	10-93
	MPa			
FE - TH	-0.46 (0.036)	-0.64 (0.029)	-0.50 (0.015)	-0.42 (0.018)
FE - UT	-0.56 (0.023)	-0.66 (0.035)	-0.58 (0.009)	-0.46 (0.024)
UF - TH	-0.56 (0.036)	-0.65 (0.044)	-0.54 (0.013)	-0.48 (0.028)
UF -UT	-0.58 (0.058)	-0.66 (0.020)	-0.59 (0.018)	-0.54 (0.016)

Table 5-3. Mean parameters of light response curves for the 1992 and 1993 sampling periods (FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned, L=Lower, U=Upper).

1992 (n=6)					
Treatment	Canopy Level	ϕ	θ	P_{max}	Rd
FE-TH	L	0.055	0.500	10.639	2.644
FE-UT	L	0.062	0.499	10.691	1.773
UF-TH	L	0.065	0.504	15.091	2.428
UF-UT	L	0.063	0.501	12.660	1.769
FE-TH	U	0.051	0.505	14.537	2.562
FE-UT	U	0.056	0.504	13.549	2.548
UF-TH	U	0.054	0.506	13.854	2.760
UF-UT	U	0.050	0.506	13.974	2.443
1993 (n=4)					
Treatment	Canopy Level	ϕ	θ	P_{max}	Rd
FE-TH	L	0.047	0.506	12.508	1.232
FE-UT	L	0.049	0.504	10.637	1.396
UF-TH	L	0.053	0.508	12.819	1.194
UF-UT	L	0.052	0.505	11.864	1.582
FE-TH	U	0.051	0.506	11.737	1.387
FE-UT	U	0.049	0.509	13.640	1.609
UF-TH	U	0.036	0.505	11.461	2.186
UF-UT	U	0.044	0.509	14.423	1.696

ϕ = apparent quantum efficiency
θ = convexity term
P_{max}= net photosynthetic rate (μmol m^{-2} s^{-1}) at 2000 μmol m^{-2} s^{-1} PPFD
R_d = dark respiration rate (μmol m^{-2} s^{-1})

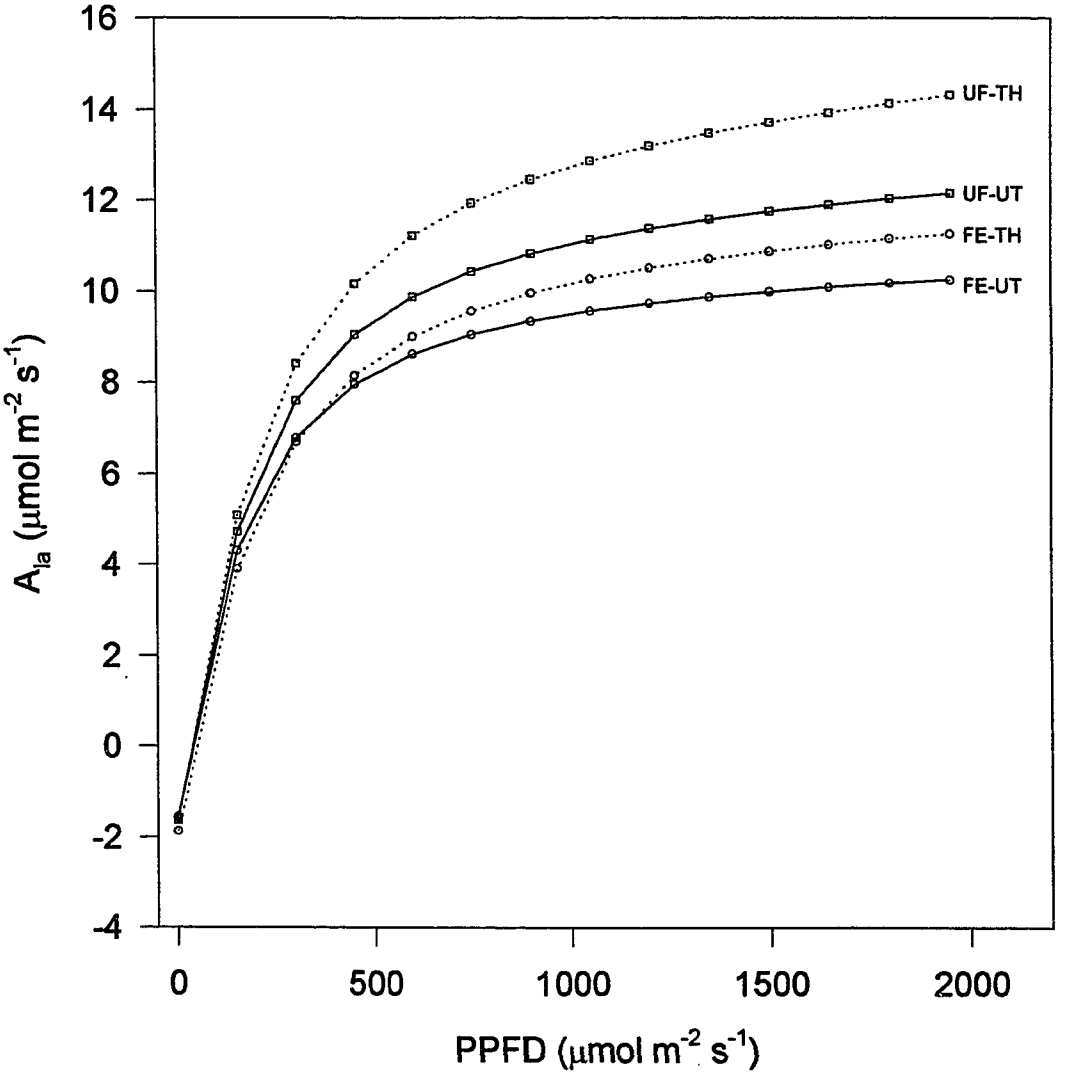


Figure 5-1. Lower canopy level mean photosynthetic light response curves on a projected leaf area basis. Data for 1992 and 1993 combined for these curves. (FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned).

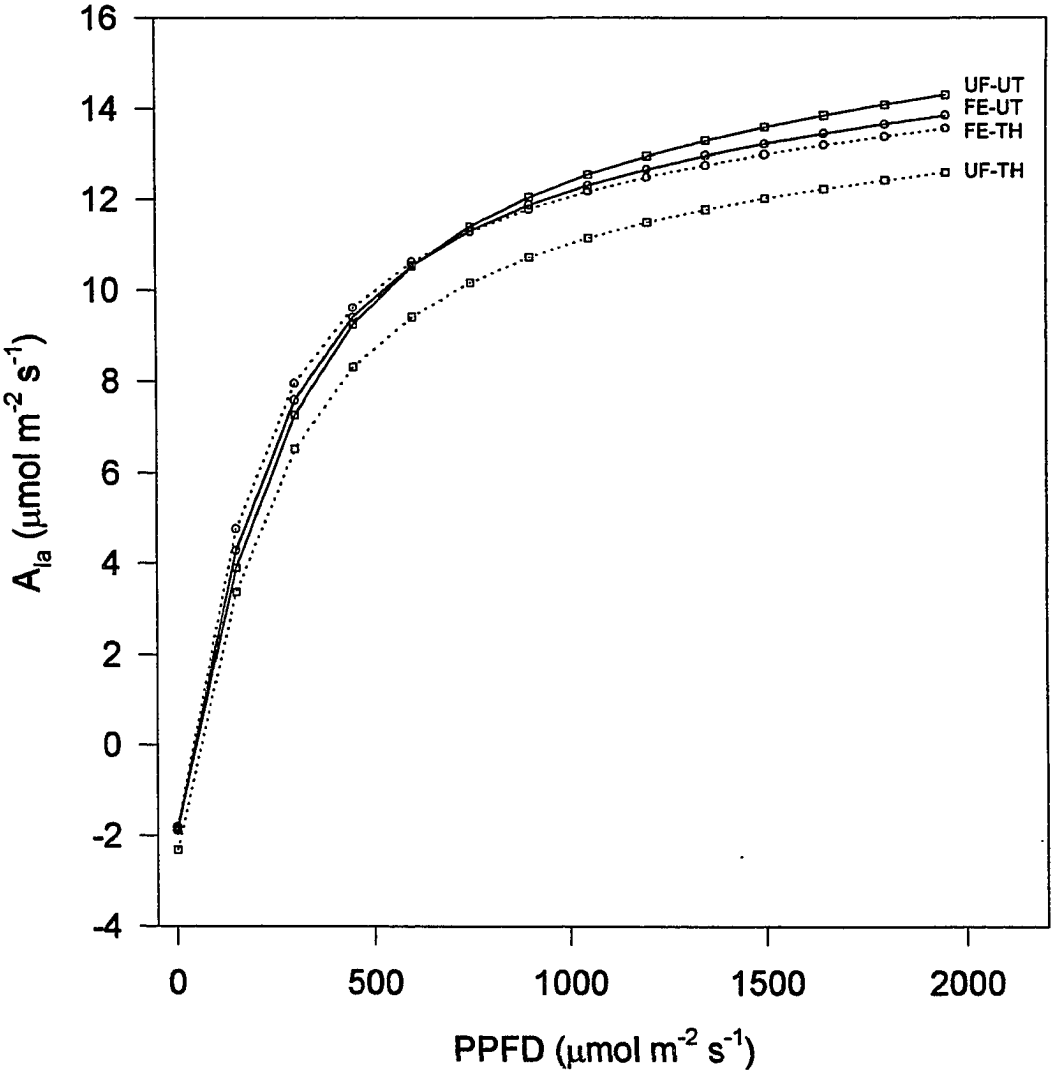


Figure 5-2. Upper canopy level mean photosynthetic light response curves on a projected leaf area basis. Data for 1992 and 1993 combined for these curves. (FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned).

Generally, upper canopy foliage had a higher P_{max} than lower canopy foliage within both fertilizer treatments. An exception is that within the unfertilized plots the thinned, lower canopy foliage tended to have a greater P_{max} than did the thinned, upper canopy foliage (Table 5-3).

The initial slopes of photosynthesis response to light, a measure of apparent quantum efficiency (ϕ), when calculated on a leaf area basis, were nearly significant ($p= 0.06$) for a fertilizer by canopy level interaction in the 1993 sampling year. The interaction is evident by the lower ϕ value of upper, unfertilized canopy foliage in the 1993 sampling year (Table 5-3). The 1992 sampling year showed a similar pattern but also was not significant. The general pattern was for the lower canopy foliage to have a greater ϕ than that of the upper canopy foliage.

A significant overall effect of canopy level ($p= 0.0328$) was found for the convexity term (θ) of the light response curves estimated from the 1992 data and generally showed the same pattern in 1993 (Table 5-3).

Dark respiration rates, on a leaf area basis, measured on needles sampled during the light response curve varied widely between the two sampling years (Table 5-3). In 1992, a nearly significant ($p= 0.056$) overall effect of level was found. In general, upper canopy foliage had a greater rate of respiration than that of the lower canopy foliage. In both sampling years, the foliage from the unfertilized, unthinned upper canopy had the highest rate of dark respiration.

In order to further assess the degree of shade acclimation, linear regression of data from the lower part of the light response curve (0-300 $\mu mol\ m^{-2}\ s^{-1}$ PPFD) was

used to calculate the predicted light compensation points. No significant differences were found with respect to canopy level by treatment combinations for any of the light compensation points.

Foliage Characteristics

Overall, specific leaf area (SLA) was significantly greater for needles from the lower canopy than from the upper canopy level during the 1992 ($p= 0.0001$) and 1993 ($p= 0.0272$) sampling years (Table 5-4). During the 1992 sampling year, there was a significant thinning by canopy level interaction ($p=0.005$). The unthinned, lower canopy foliage had a greater mean SLA than that of the thinned, lower canopy foliage. In contrast, during the 1993 sampling year, there was a significant overall effect of fertilization ($p= 0.0406$) and month ($p= 0.0021$) on SLA. Mean specific leaf area was greater for the June 1993 sampling period than on the October, 1993 period. Needles from the fertilized plots also had greater SLA's than that of the unfertilized plot needles.

Chlorophyll Content

Total chlorophyll content and chlorophyll *b*, on a leaf area basis, were significantly impacted by the overall effects of fertilization ($p= 0.001$), month ($p= 0.05$) and canopy level ($p= 0.01$, Tables 5-5 and 5-6). Foliage from unfertilized, plots had higher total chlorophyll and chlorophyll *b* concentrations than that of the fertilized plots. Likewise, lower canopy foliage generally had higher concentrations of

Table 5-4. Mean specific leaf area of needles sampled for light response curves during the 1992 (n=6) and 1993 sampling dates (n=4; FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned, L=Lower, U=Upper).

Treatment	Canopy Level	7-92	9-92	1992 Mean	6-93	10-93	1993 Mean
		---------- $cm^2\ g^{-1}$ dw ----------					
FE-TH	L	46.8	53.1	**50.0**	105.7	51.4	**78.5**
FE-UT	L	55.6	30.6	**58.9**	115.0	61.0	**88.0**
UF-TH	L	45.8	51.9	**48.9**	68.5	50.4	**59.5**
UF-UT	L	32.4	53.4	**52.6**	95.4	49.8	**72.6**
FE-TH	U	46.4	47.1	**46.7**	80.4	38.6	**59.5**
FE-UT	U	51.4	30.6	**50.3**	107.0	46.0	**76.5**
UF-TH	U	43.9	46.1	**45.0**	71.7	32.2	**51.9**
UF-UT	U	42.8	44.4	**43.6**	68.9	41.9	**55.4**

Table 5-5. Mean chlorophyll content on a projected needle area basis from the 1992 sampling dates. (FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned, L=Lower, U=Upper).

July 1992 (n=6)					
Treatment	Canopy Level	Chl *a*	Chl *b*	Total	*a*:*b* Ratio
		---------- mg cm^{-2} ----------			
FE-TH	L	59.6	28.3	87.9	2.10
FE-UT	L	55.8	24.6	80.4	2.27
UF-TH	L	68.8	30.5	99.2	2.26
UF-UT	L	67.4	32.0	99.4	2.13
FE-TH	U	54.8	23.4	78.2	2.34
FE-UT	U	57.9	24.4	83.3	2.29
UF-TH	U	70.4	29.9	100.3	2.37
UF-UT	U	70.2	29.2	99.4	2.43

September 1992 (n=6)					
Treatment	Canopy Level	Chl *a*	Chl *b*	Total	*a*:*b* Ratio
		---------- mg cm^{-2} ----------			
FE-TH	L	40.7	20.2	60.9	2.01
FE-UT	L	46.3	21.4	67.6	2.16
UF-TH	L	56.3	24.5	80.8	2.29
UF-UT	L	46.8	20.6	67.4	2.28
FE-TH	U	41.4	16.9	58.4	2.48
FE-UT	U	39.0	16.8	55.7	2.33
UF-TH	U	50.1	20.3	70.4	2.47
UF-UT	U	45.7	18.1	63.7	2.55

Table 5-6. Mean chlorophyll content on a projected needle area basis from the 1993 sampling dates. (FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned, L=Lower, U=Upper).

June 1993 (n=4)					
Treatment	Canopy Level	Chl *a*	Chl *b*	Total	*a:b* Ratio
		---------- mg cm^{-2} ----------			
FE-TH	L	55.6	18.9	74.5	2.94
FE-UT	L	52.9	19.0	71.9	2.77
UF-TH	L	62.6	22.6	85.2	2.78
UF-UT	L	71.6	26.5	98.1	2.72
FE-TH	U	47.6	15.9	63.5	3.00
FE-UT	U	56.8	18.9	75.7	3.05
UF-TH	U	56.2	19.2	74.5	2.93
UF-UT	U	55.0	19.5	74.5	2.83
October 1993 (n=4)					
Treatment	Canopy Level	Chl *a*	Chl *b*	Total	*a:b* Ratio
		---------- mg cm^{-2} ----------			
FE-TH	L	56.4	19.7	76.1	2.86
FE-UT	L	55.4	20.9	76.2	2.65
UF-TH	L	55.1	19.7	74.8	2.79
UF-UT	L	57.6	20.8	78.4	2.77
FE-TH	U	58.0	18.4	76.3	3.15
FE-UT	U	52.7	17.9	70.6	2.99
UF-TH	U	57.4	18.8	76.2	3.05
UF-UT	U	68.3	24.8	93.2	2.84

total chlorophyll and chlorophyll *b* than that of the upper canopy foliage. As a measure of light availability (Dale and Causton 1992), chlorophyll *a:b* ratios were calculated. The chlorophyll *a:b* ratios were significantly less for the lower canopy foliage in 1992 (p= 0.004) and nearly significant in 1993 (p= 0.06).

Nitrogen-Use Efficiency

Nutrient content data was measured on foliage from the same branch and flush as that used in the determination of the 1993 foliage light response curves. Foliage from unfertilized plots had significantly higher nitrogen concentrations than the fertilized plots in June and October 1993 (Table 5-7). Nitrogen-use efficiency (NUE) of photosynthesis was calculated at 1500 μmol m^{-2} s^{-1} for the 1993 data (Table 5-8). A significant impact of sampling month was found (p= 0.048) with a greater NUE in the June 1993 sampling period than in the October, 1993 sampling period. A significantly higher NUE was also found for the fertilized plots, relative to the unfertilized plots (p= 0.01).

DISCUSSION

Examination of the mean light response curves for the upper canopy foliage of the unfertilized, thinned plots revealed this foliage tended to have the lowest maximum rate of photosynthetic carbon gain per unit leaf area over the range of light levels provided (Figure 5-2). In contrast, lower canopy foliage of the unfertilized, thinned treatment plots tended to have the highest rate of photosynthesis, per unit leaf area,

Table 5-7. Mean (s.e.) nitrogen content for lower and upper canopy foliage sampled during the 1993 light response sampling dates (FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned; n=2).

Treatment	June 1993		October 1993	
	Lower	Upper	Lower	Upper
	% N			
FE - TH	1.08 (0.07)	1.03 (0.19)	1.09 (0.11)	1.21 (0.05)
FE - UT	1.17 (0.01)	1.12 (0.03)	1.16 (0.03)	1.18 (0.05)
UF - TH	1.28 (0.06)	1.24 (0.04)	1.23 (0.01)	1.34 (0.06)
UF -UT	1.23 (0.07)	1.20 (0.02)	1.20 (0.08)	1.37 (0.11)

Table 5-8. Mean (s.e.) nitrogen-use efficiency (NUE) at saturated light conditions (1500 μmol m^{-2} s^{-1} PPFD) for lower and upper canopy foliage sampled during the 1993 light response sampling periods (FE=Fertilized, UF=Unfertilized, TH=Thinned, UT=Unthinned; n=2).

Treatment	June 1993		October 1993	
	Lower	Upper	Lower	Upper
	mg CO_2 mg^{-1} N			
FE - TH	11.44 (0.92)	6.88 (1.71)	6.27 (0.06)	4.60 (0.77)
FE - UT	11.64 (1.99)	12.57 (2.66)	4.95 (1.53)	5.91 (0.50)
UF - TH	7.14 (0.74)	6.14 (2.98)	5.46 (2.06)	3.03 (0.26)
UF -UT	9.88 (2.26)	9.19 (1.24)	4.31 (0.39)	4.20 (0.45)

across the range of light levels (Figure 5-1). Lower canopy foliage light response curves tended to segregate out more and were associated with the observed lower *in situ* light levels previously reported (Chapter 2). Thinning increased overall foliar biomass per tree (personal observation) which resulted in a decrease in the available light, and thus, photosynthetic capacity of the lower canopy foliage. In addition, fertilizer further modified the response of lower canopy foliage also by increasing foliage within the crown and decreasing the amount of light reaching the lower canopy level. Shade adapted foliage generally tends to have a lower light saturation point as shown by three of the four lower crown curves. The one exception is the lower canopy foliage of the unfertilized, thinned plot. The unfertilized, thinned stand remained open, and thus, the light saturation response was not lowered.

The apparent quantum efficiency (ϕ), measured as the slope of the linear portion of the light response curve, is an indication of the efficiency of radiation use. As was found in other studies, ϕ did not vary with respect to natural light conditions within the same species (Wilson 1971, Björkman et al. 1972a, Björkman et al. 1972b, Ehleringer and Björkman 1977, Cregg 1990).

Overall, fertilization significantly decreased chlorophyll *a*, *b* and total chlorophyll concentrations in the 1992 sampling periods. The reduction in chlorophyll is primarily a result of the dilution effect associated with increased needle thickness as seen in the specific leaf area data (Table 5-3) and is discussed below. The 1993 sampling periods, although not significant, showed the same pattern. Lower and upper canopy level foliage chlorophyll *a*:*b* ratios differed significantly during both the 1992

and 1993 sampling periods. Chlorophyll *b* was significantly greater in the lower canopy and accounted for the shift in *a:b* ratio between lower and upper canopy foliage. Upper canopy foliage had greater *a:b* ratios and was indicative of the previously measured higher light levels measured on *in situ* branches (Chapter 2), and may represent a convenient bioassay in predicting the light environment as suggested by Dale and Causton (1992).

Specific leaf area (SLA) was significantly different for sampling dates and canopy level in both years. Additionally, in 1993, fertilization had a significant impact on SLA. Specific leaf area did not display a discernable pattern between sampling dates with respect to treatments. This variability may be more a measure of the high natural variation in needle morphology caused by treatments. However, fertilization increased SLA while thinning led to a decrease in SLA. Plots which were fertilized but unthinned had increased SLA, relative to the other combination of treatments. In contrast, when unfertilized but thinned the SLA decreased. Furthermore, the two treatments in combination, fertilized thinned and unfertilized unthinned, counteracted each other producing intermediate SLA levels. Canopy level influenced the SLA over all silvicultural treatments by increasing the SLA of lower canopy foliage relative to that of the upper canopy foliage. Thus, specific leaf area followed similar patterns to the light response curves, with foliage responding morphologically to the available light (i.e., increasing SLA with decreasing natural light conditions).

Nitrogen content in 1993 was significantly lower in those plots which received the fertilization treatment. This reduction in nitrogen probably was the result of a

dilution effect with the increased needle size. However, nitrogen-use efficiency (NUE) at saturated light levels was significantly greater in the plots which received the fertilizer treatment on the June 1993 sampling date. The NUE declined dramatically across all treatments and canopy levels during the October 1993 sampling date. This reduction in NUE was not the result of a decrease in nitrogen content (Table 5-7) but rather a reduction in photosynthetic rate. Photosynthesis has previously been demonstrated to decline with leaf age for loblolly pine (McGregor and Kramer 1963, Higginbotham 1974, Drew and Ledig 1981).

Physiological light response curve data for loblolly pine obtained here indicates that fertilization increased the quantity and efficiency (relative to the nitrogen content) at which carbon is sequestered by the foliage in the upper canopy. Lower canopy foliage showed a tendency for a fertilizer by thinning interaction. Lower canopy foliage of the unfertilized, thinned plots had a greater photosynthetic rate over the range of light levels measured than the other lower canopy by treatment combinations. This is the result of the more open canopy architecture allowing greater radiation on the foliage of the unfertilized, thinned stand. Fertilized, thinned and fertilized, unthinned foliage light response curves were similar, and both were lower than that of the unfertilized because of the increased foliar biomass and resultant decreased canopy light levels. The increased foliar biomass is the result of nearly five years of growth since the thinning treatments were applied. Growing space for new foliage had been reoccupied by the more rapidly expanding crowns of the fertilized plots. Thus, lower maximum photosynthesis and overall response curves of foliage in the lower crowns of

fertilized plots is probably the result of decreased light availability and nitrogen content. In contrast, despite the higher nitrogen and chlorophyll content of unfertilized, thinned upper canopy foliage, a reduction in photosynthetic potential (i.e., light response curve) was found. Differences in SLA does not explain the reduced photosynthetic rate on a per unit leaf area. On the contrary, the lower SLA would tend to accentuate the difference even more if photosynthesis was expressed on a per unit dry weight basis.

Data from this study indicates that if foliage in the lower crown of unthinned plots is exposed to increased light levels by a thinning treatment they will not show an immediate increase in photosynthetic rates. A period of acclimation may be necessary for the older needles to adapt or new foliage produced to make use of the increase in light availability in the lower canopy of recently thinned plots.

Photosynthetic rates of loblolly pine seedlings in response to increased CO_2 have been positively correlated with nitrogen concentration (Tissue et al. 1993). In their study, seedlings grown under low nitrogen availability had decreased rubisco content under elevated CO_2, with the nitrogen being reallocated to the light reaction components without a concomitant increase in photosynthesis. Limited water supply also has been reported to limit any potential increase in photosynthesis due to elevated CO_2 levels (Tschaplinski et al. 1993). This suggests that the photosynthetic response to higher CO_2 will depend on soil fertility (nitrogen availability), water availability and other limiting factors in the stand.

Tissue et al. (1993) suggested that plants occurring in low light environments proportionally shift more nitrogen to light capturing processes and take advantage of sunflecks or other transitory high light. This may be the case for foliage in the lower canopy of the fertilized plots where additional nutrients may enable them to more efficiently capture light and take advantage of sun flecks.

Canopy level was most important in determining the morphology and physiological attributes of individual fascicles in this study. Silvicultural treatments such as fertilization and thinning acted as further modifiers. The key factor in all these instances is the quantity of incident radiation reaching the foliage. Therefore it appears that fertilization has accelerated canopy closure with respect to the physiology of individual fascicles.

Upper canopy foliage, regardless of fertilizer or thinning treatment, as light saturated at approximately 900 μmol m^{-2} s^{-1} PPFD, with no differences in the photosynthetic rate among three of the treatments. The one exception was the lower photosynthetic rate at light saturation and photosynthetic response curve of the foliage from the unfertilized, unthinned plot. Based on the previous *in situ* study, natural light levels in the upper canopy are well above this level. The natural variation in PPFD found in the upper canopy is not likely to dramatically change *in situ* photosynthetic rates for a particular fertilizer and thinning treatment. In contrast, light saturation points of lower canopy foliage were dependent on the treatment. Photosynthesis for foliage from the lower canopy of the unthinned plots saturated at a significantly lower level (400 μmol m^{-2} s^{-1}) than that of the lower, thinned (650 μmol m^{-2} s^{-1}) plots.

Significant differences in the light saturation points among the lower canopy level foliage indicates the need to use separate light response functions when modeling tree canopies based on thinning treatments.

CHAPTER 6

OVERALL CONCLUSIONS

Early attempts to scale carbon and water vapor exchange from the individual leaf level to the whole canopy level have focused on horizontally homogeneous crop canopies (de Wit 1965, Monteith 1965). However, microenvironmental conditions are rarely uniform in the canopy because the natural environment fluctuates and plant canopies are rarely horizontally uniform. Recent physiological evidence suggests that the forest canopy is a heterogeneous structure (Sinclair and Knoerr 1982, Smolander 1984, Leverenz and Hinckley 1990, Nowak et al. 1990, Ford 1992).

Scaling up from the individual fascicle level to the forest canopy will require modeling of various physiological parameters in response to microenvironmental changes. This dissertation examined selected physiological processes which occurred in a loblolly pine stand in response to *in situ* and *in vitro* microenvironmental conditions in order to understand the variability of foliage physiology within the canopy. A study was designed that allowed for the comparison of selected physiological responses between upper and lower crown positions for loblolly pine in thinned and unthinned plots. An *in situ* study was conducted in order to characterize diurnal and temporal physiological response patterns of upper and lower canopy foliage in response to thinning (Chapter 2). The results indicate that five years after thinning small microenvironmental differences still existed between the lower canopies of the thinned

and unthinned plots. Physiological metabolism of the lower canopy, in thinned plots tended to be greater than that of the lower canopy, of unthinned plots. However, physiological variability was high and made it difficult to statistically detect silivicultural treatment differences. Fertilization treatments were not directly compared in this study due to constraints on sampling time during a measurement day, but it appears that the application of fertilizer accelerated canopy closure in the thinned stands. Important temporal differences were detected within the lower crowns of the unthinned stands. Lower crown foliage had greater physiological activity in the afternoon than in the morning sampling period.

Physiological differences between foliage from northern and southern exposed branches were found to be greater in thinned than in the unthinned plots (Chapter 3). Thinning, increased light on the south exposed branches more than north exposed branches. East-west physiological parameter responses are not likely to be mirror images, and although not studied these differences have the potential to exceed those of the north-south variation. Thus, it is important in the development of physiological models, particularly in thinned stands, to keep in mind the potential differences caused by differences in branch exposure within each canopy level.

Edaphic factors, such as soil moisture and nutrient availability, are likely to influence stand productivity. Generally, this study found that the predawn water status of the thinned stands was only marginally better than that of the unthinned stands, and neither treatment fell below -0.8 MPa. Substantial drought effects may be found on more droughty sites or with climate shifts. Based on stomatal conductance and

predawn water potential data from Cregg et al. (1990), it is unlikely that the small differences (Δ = 0.06 MPa) between the thinned and unthinned plots in the current study contributed to any physiological differences related to water stress.

Overall, fertilization significantly increased foliar phosphorous, calcium and magnesium levels (Chapter 4). However, thinning significantly decreased foliar phosphorous and potassium levels within the fertilized plots. Foliar nitrogen content was significantly decreased five years after thinning. This is probably primarily an effect caused by the increase in needle biomass of trees in the fertilized plots. The relatively poor performance of the unfertilized, thinned plots was due to an increase in growing space with thinning but without a concomitant increase in nutrients. Because of the long-lived nature of loblolly pine needles (usually two growing seasons) no depletion of foliar nutrient concentration was detected during the development of a subsequent flush of needles. This finding may support results by Smith et al. (1971). Chlorophyll concentrations were not affected by fertilization or thinning treatments but were significantly greater in the lower canopy level.

Light-response curve analysis revealed that foliage from the upper and lower crown positions, especially within the unthinned plots, appeared to show the classic sun-shade physiological patterns (Chapter 5). Potential rates of maximum photosynthesis (P_{max}) were greater in the foliage from the upper canopy level than the foliage from the lower canopy levels. Likewise, the foliage from the lower, thinned canopy had a greater P_{max} than that of the lower, unthinned canopy, following a positive relationship with growth light availability. Quantum efficiency did not differ

for any of the foliage studied suggesting that the basic unit of the photosynthetic apparatus is the similar. However, the quantity of light and resources invested in capturing that light is likely to be different for foliage from the different canopy positions and treatments.

Previous studies have shown a positive relationship between canopy light interception and stand productivity (Waring 1983, Leverenz and Hinckley 1990, Dalla-Tea and Jokela 1991). The within crown variation in photosynthesis was strongly dependent on canopy PPFD levels and supported previous findings (Dalla-Tea and Jokela 1991). Net CO_2 uptake (NCE) on a per unit leaf area basis was similar for upper canopy foliage for both the fertilizer and thinning treatments. Within the upper canopy, net CO_2 uptake was generally higher for foliage with a southern exposure, in both the *in situ* and detached branch, light saturated studies. A strong interaction of canopy level with thinning was apparent for net CO_2 uptake. Thinned, lower canopy level foliage had higher NCE than that of the unthinned, lower canopy. Light response curve analysis confirmed the higher potential carbon uptake of the lower canopy level, thinned treatment foliage as compared to lower canopy, unthinned treatment foliage. Furthermore, southern exposed foliage, within the thinned treatment had higher NCE than that of the northern exposed foliage in the *in situ* and light saturated studies.

Potential carbon uptake for foliage from different treatments and portions of the canopy can thus be ranked from highest to the lowest rate. These ranking for *in situ*

net CO_2 uptake are summarized below where UF=unfertilized, FE=fertilized, UT=unthinned, TH=thinned:

1) Foliage net carbon exchange per unit leaf area from upper canopy level was greater than foliage net carbon exchange from lower canopy level.

2) Within the upper canopy level of the fertilized plots: FE UT = FE TH.

3) Within the lower canopy level of the fertilized plots: FE TH = FE UT.

4) Within the upper canopy level of the unfertilized plots: UF UT > UF TH.

5) Within the lower canopy of the unfertilized plots: UF TH > UF UT

6) Within the thinned, lower canopy level: south exposed > north exposed.

The pattern of CO_2 uptake parallelled canopy PPFD patterns at the needle level.

Climatic changes, of the order of magnitude, predicted by climate change models for a doubling of CO_2 are potentially enough to produce significant physiological response changes in forest trees. Increases in atmospheric CO_2 concentrations should lead to an increase in photosynthetic rates within forests. A resulting increase in biomass might be expected assuming that maintenance respiration, decomposition and tissue longevity remain unaffected. Studies on loblolly pine have shown carbon assimilation can be increased, over the short-term, with increases in CO_2 (Tissue et al. 1993, Tscheplinski et al. 1993). However, Tissue et al. (1993) observed that the photosynthetic response of loblolly pine seedlings to elevated CO_2 was correlated with nitrogen availability. The photosynthetic rates were higher only for those seedlings grown at elevated CO_2 with supplemental nitrogen. The current study suggests that increases in foliar biomass caused by thinning and fertilization will lead

to a reduction in foliar nutrient contents. The current literature suggests that loblolly pine grown in native soils (low nitrogen) will acclimate to long-term CO_2 enrichment and maintain current photosynthetic rates unless nitrogen is added to the stands (Tissue et al. 1993, Tschaplinski et al. 1993).

Data from this dissertation indicates that increases in needle photosynthetic rates caused by the increased light levels resulting from thinning led to increases in individual tree biomass and accelerated canopy closure. Concomitant with the increase in tree biomass a decrease in foliar nutrient content was observed. Consequently, the growth rate of individual trees over the long-term is in question should nutrients become limiting. If elevated CO_2 concentrations do result in long-term growth enhancement, increases in productivity would be more likely to occur in commercial forests where input of additional nutrients may be practical. If stands of loblolly pine remain unthinned or are not thinned with more frequency, lowered light levels within forest canopies may reduce the expected increases in photosynthesis and thus, productivity.

Most process models partition the crown by horizontal layers (e.g., lower-, mid- and upper canopy). This study indicates that potential differences exist between north versus south exposed foliage. In addition, east-west differences could potentially be equally important. In the north-south study light, and not the inherent physiology of the foliage, was the primary factor determining photosynthesis rates. Thus, the forest canopy may need to be separated based on the vertical distribution of foliage and hence light capture.

Differences in the branch carbon exchange index (BCEI) indicate that branch leaf area is important in determining whole branch carbon uptake. Fertilization and thinning treatments, modified by light levels, led to an increase in the amount of foliage biomass per branch. Thus, foliar biomass is not distributed uniformly throughout horizontal positions in the canopy in these stands. Thus, when scaling up from the individual needle level to the whole stand, the vertical distribution of foliage will need to be used as the basic unit determining forest productivity

In summary, the increase in nutrient availability from fertilization accelerated leaf area development in the previously thinned plots. Accelerated development of the canopy during stand growth, caused by silvicultural treatments (fertilizer and thinning) enhanced light interception through an increase in leaf area. Studies investigating the timing for response and recovery from thinning and fertilization are needed to determine if current practices optimize stand productivity. Based on the physiological differences found in this study, information on within crown variation in PPFD, vapor pressure gradient, CO_2 concentration and air temperature need to be described and quantified for modeling. Physiological responses related to thinning and fertilization can be attributed primarily to changes in foliage distribution and amounts. These changes in foliar biomass modify light interception and produce changes in physiological responses such as photosynthesis and stomatal conductance.

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APPENDIXES

APPENDIX A: STUDY SITE.

● Weather Station

Treatment 4 Rep 1 Plot 1	Treatment 1 Rep 1 Plot 2	Treatment 2 Rep 3 Plot 3	Treatment 3 Rep 3 Plot 4		
Treatment 3 Rep 1 Plot 7	Treatment 2 Rep 1 Plot 8		Treatment 4 Rep 2 Plot 10		
	Treatment 2 Rep 2 Plot 14	Treatment 3 Rep 2 Plot 15	Treatment 1 Rep 2 Plot 16	Treatment 1 Rep 3 Plot 17	Treatment 4 Rep 3 Plot 18

Treatment codes are:

1. No fertilizer and unthinned
2. Fertlizer and unthinned
3. No fertilizer and thinned
4. Fertilizer and thinned

APPENDIX B: NORTH - SOUTH PARAMETER MEANS.

Appendix B-1. Means (s.e.) for physiological parameters measured during the *in situ* experiment for northern and southern aspect foliage within the thinned and unthinned treatments for the combined measurement periods of April and September 1993. (n=4).

PPFD ($\mu mol\ m^{-2}\ s^{-1}$)

	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	370 (97)	508 (141)	1470 (133)	1607 (99)
Thinned	804 (164)	1132 (172)	1435 (121)	1536 (182)

A_{la} ($\mu mol\ m^{-2}\ s^{-1}$)

	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	5.99 (1.63)	6.19 (1.76)	17.77 (2.08)	19.09 (1.30)
Thinned	10.10 (1.29)	12.43 (1.44)	15.76 (1.66)	18.71 (1.49)

A_{dw} ($nmol\ g^{-1}$ dw)

	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	31.69 (6.76)	32.64 (7.80)	64.59 (6.45)	70.10 (4.03)
Thinned	44.46 (5.66)	55.09 (4.16)	48.38 (3.16)	67.48 (6.91)

g_C ($mol\ m^{-2}\ s^{-1}$)

	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	0.1005 (0.0209)	0.1135 (0.0246)	0.2737 (0.0477)	0.2616 (0.0256)
Thinned	0.1607 (0.0291)	0.1628 (0.0259)	0.2454 (0.0513)	0.2284 (0.0252)

(table con'd.)

E(mmol m^{-2} s^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	1.561 (0.198)	1.773 (0.328)	3.889 (0.196)	5.146 (0.616)
Thinned	2.914 (0.574)	3.594 (0.555)	4.144 (0.723)	4.177 (0.496)

VPG (mbars)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	18.48 (2.88)	17.85 (3.10)	15.99 (3.15)	17.97 (3.29)
Thinned	18.96 (3.62)	21.61 (3.59)	17.22 (3.73)	18.77 (3.59)

WUE (mg CO_2 gH_2O^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	8.67 (1.24)	7.98 (1.14)	11.65 (1.80)	10.24 (1.43)
Thinned	11.52 (2.54)	10.59 (2.79)	11.56 (2.23)	12.53 (2.04)

Ψ (bars)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	-12.80 (0.93)	-13.30 (0.58)	-14.13 (0.84)	-14.39 (1.05)
Thinned	-14.09 (0.55)	-14.34 (0.43)	-15.58 (0.45)	-15.49 (0.62)

Appendix B-2. Means (s.e.) for physiological parameters measured during the detached shoot experiment for northern and southern aspect foliage within the thinned and unthinned treatments for the April 1993 measurement period. (n=2)

A_{la} (μmol m^{-2} s^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	9.05 (1.86)	7.26 (2.13)	11.45 (1.17)	13.79 (0.96)
Thinned	8.37 (0.79)	8.45 (0.39)	10.00 (2.27)	10.25 (1.50)

A_{dw} (nmol g^{-1} dw)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	51.60 (7.58)	54.99 (7.51)	49.45 (4.65)	57.27 (8.12)
Thinned	37.22 (2.29)	38.25 (7.32)	44.82 (11.59)	37.71 (5.92)

g_C (mol m^{-2} s^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	0.1636 (0.0242)	0.1589 (0.0234)	0.2447 (0.0235)	0.2576 (0.0161)
Thinned	0.1602 (0.0091)	0.1769 (0.0150)	0.1793 (0.0365)	0.2385 (0.0181)

(table con'd.)

E(mmol m^{-2} s^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	3.028 (0.731)	3.007 (0.746)	3.579 (0.343)	3.831 (0.597)
Thinned	2.889 (0.532)	3.097 (0.483)	2.783 (0.169)	3.698 (0.499)

VPG (mbars)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	17.38 (2.97)	18.01 (2.89)	15.53 (2.66)	15.67 (2.89)
Thinned	17.44 (2.48)	17.57 (2.60)	16.88 (2.65)	16.10 (2.38)

WUE (mg CO_2 gH_2O^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	8.87 (2.76)	9.86 (2.90)	8.08 (1.30)	9.47 (1.54)
Thinned	7.75 (1.35)	7.67 (2.18)	8.81 (2.07)	7.12 (1.30)

Ψ (bars)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	-11.28 (1.83)	-11.95 (1.69)	-13.05 (1.47)	-12.75 (1.73)
Thinned	-12.03 (1.42)	-12.58 (1.34)	-12.25 (1.42)	-13.13 (1.46)

Appendix B-3. Means (s.e.) for physiological parameters measured during the detached shoot physiology experiment for northern and southern aspect foliage within the thinned and unthinned treatments for the September 1993 measurement period. (n=2).

A_{la} (μmol m^{-2} s^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	8.03 (0.41)	9.67 (1.09)	12.71 (0.83)	10.56 (0.57)
Thinned	10.63 (1.29)	9.17 (1.95)	10.44 (1.04)	11.52 (0.83)

A_{dw} (nmol g^{-1} dw)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	46.28 (5.99)	46.55 (5.41)	43.98 (1.78)	42.86 (0.65)
Thinned	47.21 (1.23)	48.13 (5.68)	35.87 (4.33)	40.15 (5.13)

g_c (mol m^{-2} s^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	0.1692 (0.0168)	0.1969 (0.0306)	0.3463 (0.0156)	0.3024 (0.0468)
Thinned	0.2540 (0.0373)	0.2153 (0.0493)	0.2902 (0.0065)	0.3021 (0.0213)

(table con'd.)

E(mmol m^{-2} s^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	3.320 (0.182)	4.173 (0.643)	5.988 (0.189)	6.241 (0.241)
Thinned	4.947 (1.814)	4.999 (1.957)	5.749 (1.173)	6.953 (1.700)

VPG (mbars)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	16.75 (1.97)	18.74 (1.68)	15.84 (0.73)	19.90 (1.62)
Thinned	14.49 (5.47)	17.10 (5.14)	17.05 (4.68)	19.28 (4.66)

WUE (mg CO_2 gH_2O^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	5.98 (0.48)	6.05 (1.17)	5.21 (0.34)	4.16 (0.27)
Thinned	7.71 (2.58)	6.48 (1.75)	5.33 (1.47)	4.92 (1.27)

Ψ (bars)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	-11.03 (0.76)	-10.18 (0.41)	-14.03 (0.29)	-14.15 (0.48)
Thinned	-10.75 (2.17)	-10.80 (2.54)	-12.10 (1.92)	-13.15 (2.20)

Appendix B-4. Mean (s.e.) dark respiration rates (Rd), measured on a per unit needle area and per unit dry weight basis, during the detached branch physiology experiment for northern and southern aspect foliage within the thinned and unthinned treatments for the September 1993 measurement period. (n=2).

Rd (μmol m^{-2} s^{-1})				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	2.14 (0.26)	2.11 (0.13)	2.90 (0.27)	2.51 (0.30)
Thinned	1.81 (0.48)	1.86 (0.32)	2.85 (0.11)	2.56 (0.15)

Rd (nmol g^{-1} dw)				
	Lower-North	Lower-South	Upper-North	Upper-South
Unthinned	11.79 (0.29)	10.16 (0.70)	10.04 (0.75)	10.25 (1.30)
Thinned	7.83 (1.34)	10.00 (1.39)	9.85 (0.97)	8.82 (0.59)

Appendix B-5. Mean (s.e.) chlorophyll content of needles for foliage sampled during the April 1993 measurement period for northern and southern aspect foliage. Means followed by a different small letter are significantly different ($P \leq 0.05$) for upper versus lower canopy level within thinning, and those followed by a different capital letter are significantly different for northern versus southern aspect within a canopy level and thinning treatment. (n=4).

Chlorophyll *a* (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	2.33 (0.16) aA	2.32 (0.16) aA
Unthinned	Upper	1.93 (0.20) bA	1.77 (0.20) bB
Thinned	Lower	1.79 (0.08) aA	1.69 (0.14) aB
Thinned	Upper	1.36 (0.08) bA	1.20 (0.06) bB
Chlorophyll *b* (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	0.84 (0.06) aA	0.85 (0.04) aA
Unthinned	Upper	0.65 (0.06) bA	0.67 (0.17) bA
Thinned	Lower	0.61 (0.03) aA	0.58 (0.04) aA
Thinned	Upper	0.43 (0.02) bA	0.36 (0.02) bA
Total Chlorophyll (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	3.18 (0.23) aA	3.17 (0.20) aA
Unthinned	Upper	2.58 (0.26) bA	2.44 (0.37) bB
Thinned	Lower	2.41 (0.12) aA	2.26 (0.17) aA
Thinned	Upper	1.79 (0.10) bA	1.56 (0.08) bB
Chlorophyll *a*:*b* Ratio			
Thinning	Level	North	South
Unthinned	Lower	2.77 (0.02) aA	2.72 (0.07) aA
Unthinned	Upper	2.98 (0.05) aA	2.85 (0.32) aA
Thinned	Lower	2.93 (0.03) aA	2.91 (0.07) aA
Thinned	Upper	3.21 (0.09) aA	3.36 (0.05) bA

Appendix B-6. Mean (s.e.) chlorophyll content of needles for foliage sampled during the September 1993 measurement period for northern and southern aspect foliage. Letters following means represent statistical significance as described in appendix 2-5. (n=4).

Chlorophyll *a* (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	2.99 (0.18) aA	2.93 (0.34) aA
Unthinned	Upper	2.56 (0.19) bA	2.35 (0.40) bA
Thinned	Lower	2.22 (0.16) aA	2.43 (0.28) aA
Thinned	Upper	2.28 (0.25) aA	2.37 (0.25) aA

Chlorophyll *b* (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	1.10 (0.08) aA	1.11 (0.14) aA
Unthinned	Upper	0.92 (0.08) bA	0.87 (0.17) bA
Thinned	Lower	0.79 (0.06) aA	0.88 (0.12) aA
Thinned	Upper	0.79 (0.10) aA	0.85 (0.10) aA

Total Chlorophyll (mg g^{-1} dw)			
Thinning	Level	North	South
Unthinned	Lower	4.10 (0.26) aA	4.04 (0.48) aA
Unthinned	Upper	3.49 (0.27) bA	3.23 (0.56) bA
Thinned	Lower	3.01 (0.22) aA	3.31 (0.40) aA
Thinned	Upper	3.07 (0.34) aA	3.22 (0.34) aA

Chlorophyll *a*:*b* Ratio			
Thinning	Level	North	South
Unthinned	Lower	2.73 (0.04) aA	2.67 (0.03) aA
Unthinned	Upper	2.82 (0.05) bA	2.84 (0.12) bA
Thinned	Lower	2.84 (0.06) aA	2.82 (0.10) aA
Thinned	Upper	2.91 (0.07) aA	2.84 (0.05) aA

APPENDIX C: CHLOROPHYLL EXTRACTION (DMF) PROCEDURE

note: procedure following that of W.P. Inskeep & P.R. Bloom (1985) and R. Moran (1982) with modifications to deal with phenolic compounds in plant extract.

Prepare DMF Extraction Buffer:

-- add 2.5 mg/ml PVP_i (plolyvinylpolypyrrolidone) to 100% DMF (N,N-Dimethylformamide).

Tissue Preparation:

1) Cut needles in small pieces (approx. 2 mm long.
2) Weigh plant material to be assayed (100-200 mg fw.
3) Place needles in a small vial and add 4 ml of the DMF extraction buffer.
4) Incubate, in the dark, at 4°C until all of the chlorophyll is visibly removed. This will be species specific.
5) Dilute 1:1 with fresh DMF buffer.
6) If not immediately assayed with spectrophotometer place in the dark at 4°C. DO NOT leave in "cold storage" for more than 5 days.

Spectrophotometer Procedure:

7) Allow samples to reach room temperature in the dark.

8) Using a blank, zero the spectrophotometer at 647, 665 & 750nm.

9) Place 3 ml of the plant extract into a cuvette.

10) Take a reading at 750 nm, if the Absorbance is > 0.01 then dilute the solution 1:1 with fresh DMF buffer, remeasure at 750 nm.

11) After checking Abs @ 750 then;

Read Abs at 665 nm and record the reading

Read Abs at 647 nm and record the reading

11) Calculate chlorophyll using the following equations:

$Chl_a = 12.70\ A^{665} - 2.79\ A^{647}$

$Chl_b = 20.70\ A^{647} - 4.62\ A^{665}$

$Chl_{a+b} = 17.90\ A^{647} + 8.08\ A^{665}$

note: chlorophyll concentrations are in μg/ml plant extract.

Dry weight:

1) Carefully drain off DMF buffer into approved container.

2) Dry tissue, in vials, at 65°C. Reweigh.

VITA

Dennis Albert Gravatt was born April 29, 1957, in Kansas City, Kansas. He was the first of three children born to Albert F. and Helen J. Gravatt.

Dennis graduated from Washington High School, Kansas City, Kansas in 1975. Dennis married Patricia Ann Eggleston on June 18, 1977. With the support of his wife, Dennis began to attend college evening classes.

Dennis attended Kansas City Kansas Community College where he received a Associate of Arts degree in 1981. He continued to work as a Production Supervisor for Farmland Industries, Inc., Kansas City, MO until he became a full-time student at the University of Kansas in 1986. While attending the University of Kansas he earned a Bachelor of Science degree in Systematics and Ecology in 1988.

Dennis entered graduate school at the University of Kansas where he earned a Masters of Art degree, in Botany, in 1991.

Dennis was offered a Graduate Fellowship to study in the field of Forest Biology at Louisiana State University in 1991. In December of 1991 he and his wife moved to Baton Rouge, LA to pursue his doctoral degree in Forest Biology.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Dennis A. Gravatt

Major Field: Forestry

Title of Dissertation: Physiological Variation in Loblolly Pine (Pinus taeda L.) as Related to Crown Position and Stand Density

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

October 28, 1994