

**Influences of Elevated Atmospheric CO₂ and Water Stress on
Photosynthesis and Fluorescence of Loblolly Pine,
Red Maple, and Sweetgum.**

by

Philip J. Lenham

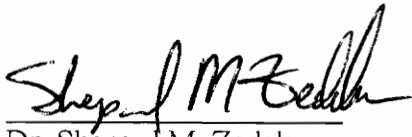
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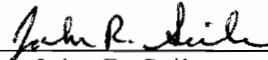
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(Abstract)

Changes in light harvesting ability and other physiological responses could influence the competitive outcomes of tree species in a future elevated CO₂ atmosphere. Photosynthetic light response curves were constructed with a closed photosynthesis system (LI-COR, Inc. Lincoln, NB) in order to investigate the effect of growth in elevated CO₂ (746 $\mu\text{l l}^{-1}$) and ambient CO₂ (379 $\mu\text{l l}^{-1}$) on light responses, and seedlings were allowed to dry out to examine physiological changes to water stress. While drying out, photosynthesis was measured with a closed system (LI-COR, Inc. Lincoln, NB) and fluorescence was measured with a portable fluorescence measurement system (P.K. Morgan Instruments, Inc. Andover, MA). No species showed significant increases in quantum yields or decreases in light-compensation points as a result of elevated CO₂. Photosynthesis declined in all species due to water stress, but seedlings grown in elevated CO₂ maintained photosynthesis longer. Loblolly pine and red maple grown in elevated CO₂ showed signs of photosynthetic acclimation. Photochemical efficiency of PSII declined with water stress in loblolly pine. Red maple and sweetgum showed no relationship between photochemical efficiency of PSII and simulated drought. Growth in elevated CO₂ did not influence this response in loblolly pine, but sweetgum started with a lower photochemical efficiency of PSII which increased significantly. Photosynthesis and

photochemical efficiency of PSII were weakly but positively correlated in loblolly pine and red maple. Exposure to elevated CO₂ increased this correlation for red maple. Sweetgum showed a correlation only for those seedlings grown in elevated CO₂. This research suggests that, under moderately increased CO₂ levels, the three species will not change their photosynthetic responses to light and will tolerate dryer conditions longer. For loblolly pine, photochemical efficiency of PSII declines under water stress which may partially explain the decline in photosynthesis during water stress.

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Table of Contents

Abstract	ii
Acknowledgements	iv
List of Tables	vi
List of Figures	vii
CHAPTER 1. Introduction and Justification	1
CHAPTER 2. Literature Review	4
Photosynthetic and Chlorophyll Fluorescence Responses to Elevated CO ₂	5
Water Stress and Elevated CO ₂ Effects on Photosynthesis Characteristics and Chlorophyll Fluorescence.....	9
CHAPTER 3. The Influence of Elevated CO₂ Concentration on the Light Compensation Points, Light-saturated Net Photosynthetic Rates, and Quantum Yields of Loblolly Pine, Sweetgum, and Red Maple	14
Introduction.....	14
Materials and Methods.....	16
Results and Discussion.....	19
Conclusion.....	27
CHAPTER 4. The Influence of Water Stress and Elevated CO₂ Concentration on Gas Exchange and Photochemical Efficiency of PSII of Loblolly Pine, Sweetgum, and Red Maple	30
Introduction.....	30
Materials and Methods.....	34
Results and Discussion.....	37
Conclusion.....	53
Summary and Conclusions	58
Literature Cited	60
Vita	65

List of Tables

CHAPTER 3.

Table 3.1: Quantum yield, light-compensation point, and light-saturated photosynthetic rate obtained from light curves, constructed under ambient CO₂ levels on 16 September 1993 (loblolly pine) 20 December 1993 (sweetgum) and 4 January 1993 (red maple) following 12 (loblolly pine), 5 (sweetgum), or 4 (red maple) months at a CO₂ level..... 21

CHAPTER 4.

Table 4.1: Coefficient of determination values (r^2), slopes, intercepts, and p-values describing the relationship between water potential (Independent Variable) and photosynthesis (Dependent Variable) for loblolly pine and day (Independent Variable) and Ps for sweetgum and red maple..... 40

Table 4.2: Coefficient of determination values (r^2), slopes, intercepts, and p-values describing the relationship between water potential (Independent Variable) and photochemical efficiency of PSII (Dependent Variable) for loblolly pine and time (Independent Variable) and Fv/Fm for sweetgum and red maple.....45

Table 4.3: Coefficient of determination values (r^2), slopes, intercepts, and p-values describing the relationship between photosynthesis (Independent Variable) and photochemical efficiency of PSII (Dependent Variable) for loblolly pine, sweetgum, and red maple.....49

List of Figures

CHAPTER 2.

Figure 2.1: Characteristic chlorophyll fluorescence induction kinetics of a dark adapted maize leaf. A, fast kinetics; B, slow kinetics. The characteristic fluorescence parameters are denoted OI, DP, SMT. The digitally displayed fluorescence values are inserted: F_0 - minimal fluorescence level; F_m - maximal fluorescence level; F_v ($F_m - F_0$) - variable fluorescence; F_t - terminal fluorescence level; F_q ($F_m - F_t$) - quenching capacity (From Greaves et al. 1991)..... 6

CHAPTER 3.

Figure 3.1: A representative light curve, constructed at ambient CO_2 , using a 12-month-old loblolly pine grown in $379 \mu l l^{-1} CO_2$ and the predicted fit from the nonlinear regression equation..... 20

Figure 3.2: Mean photosynthetic rates at different light levels, constructed at ambient CO_2 , for 12-month-old loblolly pine seedlings grown at $379 \mu l l^{-1} CO_2$ or $746 \mu l l^{-1} CO_2$. Means at the different light levels do not differ significantly..... 24

Figure 3.3: Mean photosynthetic rates at different light levels, constructed at ambient CO_2 , for 5-month-old sweetgum seedlings grown at $379 \mu l l^{-1} CO_2$ or $746 \mu l l^{-1} CO_2$. Means at the different light levels do not differ significantly..... 25

Figure 3.4: Mean photosynthetic rates at different light levels, constructed at ambient CO_2 , for 4-month-old red maple seedlings grown at $379 \mu l l^{-1} CO_2$ or $746 \mu l l^{-1} CO_2$ 26

CHAPTER 4.

Figure 4.1: Photosynthesis ($\mu mol CO_2 m^{-2} s^{-1}$), measured at ambient CO_2 , for 5-month-old sweetgum seedlings grown in 379 or $746 \mu l l^{-1} CO_2$ as influenced by increasing water content (%)..... 38

Figure 4.2: Photosynthesis ($\mu mol CO_2 m^{-2} s^{-1}$), measured at ambient CO_2 , for 12-month-old loblolly pine seedlings grown in 379 or $746 \mu l l^{-1} CO_2$ as influenced by decreasing water potential (MPa)..... 41

Figure 4.3: Photosynthesis ($\mu mol CO_2 m^{-2} s^{-1}$), measured at ambient CO_2 , for 5-month-old sweetgum seedlings grown in 379 or $746 \mu l l^{-1} CO_2$ as influenced by increasing time (Day) with water withheld..... 42

Figure 4.4: Photosynthesis ($\mu mol CO_2 m^{-2} s^{-1}$), measured at ambient CO_2 , for 4-month-old red maple seedlings grown in 379 or $746 \mu l l^{-1} CO_2$ as influenced by increasing time (Day) with water withheld..... 43

Figure 4.5: Photochemical efficiency of PSII (unitless) for 12-month-old loblolly pine seedlings grown in 379 or 746 $\mu\text{l l}^{-1}$ CO_2 as influenced by decreasing water potential (MPa).....	47
Figure 4.6: Photochemical efficiency of PSII (unitless) for 5-month-old sweetgum seedlings grown in 379 or 746 $\mu\text{l l}^{-1}$ CO_2 as influenced by increasing time (Day).....	48
Figure 4.7: Photochemical efficiency of PSII (unitless) for 12-month-old loblolly pine seedlings grown in 379 or 746 $\mu\text{l l}^{-1}$ CO_2 correlated with increasing photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).....	50
Figure 4.8: Photochemical efficiency of PSII (unitless) for 4-month-old red maple seedlings grown in 379 or 746 $\mu\text{l l}^{-1}$ CO_2 correlated with increasing photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).....	51
Figure 4.9: Photochemical efficiency of PSII (unitless) for 5-month-old sweetgum seedlings grown in 379 or 746 $\mu\text{l l}^{-1}$ CO_2 correlated with increasing photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).....	52

CHAPTER 1

Introduction and Justification

Changes in photosynthetic responses to light in elevated CO₂ could greatly influence the competitive outcomes of tree species. Due to increases in atmospheric CO₂ concentrations, the influence of elevated CO₂ concentrations on plants has become an important question. Present and past research has shown both short-term and long-term effects on photosynthesis of plants grown in elevated atmospheric CO₂. However, little research has investigated specifically how the photosynthetic characteristics of C₃ plants change in response to elevated CO₂. Specifically, does elevated CO₂ influence quantum yields, light-saturated net photosynthetic rates, or light-compensation points of loblolly pine (*Pinus taeda* L.), sweetgum (*Liquidambar styraciflua* L.), or red maple (*Acer rubrum* L.)?

Loblolly pine, sweetgum, and red maple were chosen for this research because of their importance in the southern forest ecosystem as crop trees and competitors. Loblolly pine is the largest timber producing tree in the southeast and sweetgum and red maple are major competitors to loblolly pine, in the southern and more northern portion of loblolly pines range, respectively (Baker and Langdon 1990, Kormanik 1990, Walters and Yawney 1990). An increase or decrease in the light-compensation point or the quantum yield of a species could change the growth responses of the species under low light conditions. These increases or decreases could alter seedling survival for loblolly pine or understory species interference by red maple or sweetgum. Similarly, an increase or decrease in the light-saturated photosynthetic rate could change the competitive outcomes of the species. An increase in the light-saturated photosynthetic rate might allow for greater growth and biomass accumulation while a decrease, termed acclimation by researchers, would lower

growth and biomass accumulation under light-saturating conditions. An increased light-saturated net photosynthetic rate would be beneficial to loblolly pine because it could grow up and away from competition faster while a decreased light-saturated photosynthetic rate could be harmful since red maple and sweetgum could more easily overtop it. Overtopping by red maple and sweetgum would be more of a concern if they showed an increase in their light-saturated net photosynthetic rate.

Accompanying the increase in atmospheric CO₂ concentrations may be a decrease in precipitation. It is well documented that water deficits have a negative effect on the photosynthetic process. Two explanations for this impact on photosynthesis are stomatal and non-stomatal inhibition. Regardless of whether the decrease in photosynthesis is due to stomatal or non-stomatal limitations, photosynthesis declines with declining water availability. The direct impact of water stress on chlorophyll fluorescence in loblolly pine, sweetgum, and red maple is not known. An increase in chlorophyll fluorescence during water stress would suggest a direct impact on the photosynthetic apparatus. Alternatively, no change would leave open the hypothesis that water stress impacts on photosynthesis are largely stomatal in nature. In addition, how does growth in elevated CO₂ interact with these responses?

Maintaining photosynthesis and the functioning of photosystems under water stress, resulting from growth in elevated CO₂, would be advantageous and could also change competitive relationships between the three species. An increase in drought tolerance could enhance seedling survival and may extend a seedlings range into drier sites. A change in the photosystems could alter light-harvesting abilities of the three species and could alter their photochemical efficiency.

With these possible competitive changes in mind, the general hypotheses tested by this research were:

- Ho: Growth in elevated CO₂ concentrations will not change the light compensation points of loblolly pine, red maple, or sweetgum.
- Ho: Growth in elevated CO₂ concentrations will not change the light-saturated net photosynthetic rates of loblolly pine, red maple, or sweetgum.
- Ho. Growth in elevated CO₂ concentrations will not change the quantum yields of loblolly pine, red maple, or sweetgum.
- Ho: Chlorophyll fluorescence will not correlate with gas exchange during water stress in loblolly pine, red maple, and sweetgum.
- Ho: Growth in elevated CO₂ concentrations will not change the relationship between chlorophyll fluorescence and gas exchange during water stress.

Two experiments were performed to test these hypotheses. The first experiment examined the photosynthetic response of loblolly pine, sweetgum, and red maple to increasing irradiance. The second experiment involved analyzing photosynthesis and chlorophyll fluorescence characteristics of loblolly pine, sweetgum, and red maple during water stress.

CHAPTER 2

Literature Review

The rising levels of atmospheric CO₂ has become an important issue in scientific research. Increases in atmospheric CO₂ may influence light responses and fluorescence responses of plants which could influence competitive relationships between species.

One useful way to provide information concerning photosynthetic responses to environmental conditions is to construct photosynthetic light response curves. Each light curve depicts the light-compensation point, the light-saturation point, and the quantum yield of the plant. The light-compensation point is the irradiance at which net photosynthesis equals zero, offsetting dark respiration in the plant. The light-saturation point is the irradiance at which light is no longer the limiting factor but rather the ability to fix carbon is. The quantum yield, which is the initial slope of the regression between irradiance and net photosynthesis, signifies the efficiency of the photosynthetic apparatus under light-limiting conditions (Kozlowski et al. 1991).

Chlorophyll fluorescence, which is primarily associated with Photosystem II (PSII), is an efficient way to determine if environmental factors modify photosynthetic capacity (Bolhàr-Nordenkampf et al. 1989). With the recent development of chlorophyll fluorescence measuring instruments, it can now be measured rapidly and non-destructively. There are many different abbreviations used for chlorophyll fluorescence parameters, but most are similar. F_v/F_m is the ratio of variable fluorescence (F_v) to maximal fluorescence (F_m) and estimates the efficiency of electron transfer from the light harvesting pigments, where F_v originates, to the electron acceptors of PSII. Maximal fluorescence is the point at which F_v is at a maximum, when all electron acceptors are fully reduced. Alternatively, F_v can be determined by subtracting the initial rise in chlorophyll fluorescence (F_o) from F_m .

F_0 is the initial fluorescence of the light harvesting pigments while the electron acceptors are in an oxidized state (Greaves et al. 1991; Figure 2.1). The photochemical efficiency of PSII (estimated by F_v/F_m) is an ideal measurement for determining the influence of environmental conditions, such as water stress and elevated CO_2 , on fluorescence. An F_v/F_m ratio of 1.0 (unitless) indicates that all electrons gathered by the light harvesting pigments are being passed to the electron acceptors of PSII. A reduction in the F_v/F_m ratio to 0.50 (unitless) signifies that more fluorescence is originating from the light harvesting pigments and, therefore, only half of the electrons are reaching the electron acceptors of PSII. Therefore, F_v/F_m measurements provide information on inhibition or damage to transfer of light energy to electrons in PSII, and are a sensitive indicator of photoinhibition (Bolh ar-Nordenkampf et al. 1989).

This review will examine information on how photosynthetic and chlorophyll fluorescence responses have changed due to an increase in atmospheric CO_2 and water stress.

PHOTOSYNTHETIC AND CHLOROPHYLL FLUORESCENCE RESPONSES TO ELEVATED CO_2

Ehleringer and Bj orkman (1977) found that the quantum yield of various C_3 plants is dependent on temperature, CO_2 , and O_2 concentrations. They found that, at 21% O_2 , the quantum yield of a C_3 plant is strongly dependent on both the intercellular CO_2 concentration and leaf temperature. As intercellular CO_2 increases the quantum yield of the C_3 plants increases. At low intercellular CO_2 , the quantum yield extrapolates to zero at the CO_2 compensation point (Ehleringer and Bj orkman 1977).

Bazzaz et al. (1990) found that shade-tolerant tree species showed a greater stimulation to elevated CO_2 than shade-intolerant species under the light and nutrient conditions used in their study. They hypothesized that, given their peak light levels of 520

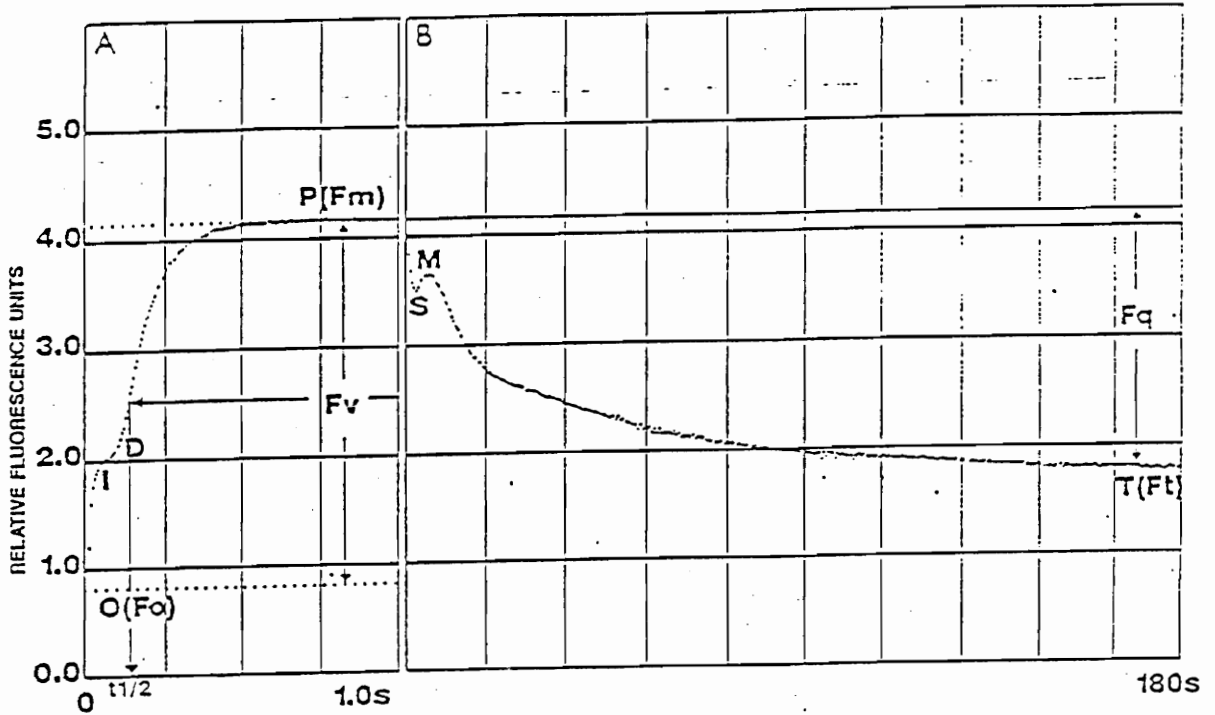


Figure 2.1: Characteristic chlorophyll fluorescence induction kinetics of a dark adapted maize leaf. A, fast kinetics; B, slow kinetics. The characteristic fluorescence parameters are denoted OI D P S M T. The digitally displayed fluorescence values are inserted: F_o - minimal fluorescence level; F_m - maximal fluorescence level; F_v ($F_m - F_o$) - variable fluorescence; F_t - terminal fluorescence level; F_q ($F_m - F_t$) - quenching capacity (From Greaves et al. 1991).

$\mu\text{mol m}^{-2} \text{s}^{-1}$ were only equivalent to understory conditions, the shade-intolerant species acclimated to the lower light and increased in quantum yield but decreased in maximum photosynthetic rates (Bazzaz et al. 1990). The shade-tolerant species, since they presumably were not limited by light but rather by CO_2 , were able to take advantage of the increase in CO_2 and showed a higher photosynthetic rate. Therefore, shade-tolerant and moderately shade-tolerant species such as red maple and sweetgum may show a greater photosynthetic increase to elevated CO_2 under low light and may be more competitive in the understory of forests composed of shade-intolerant species such as loblolly pine.

Loblolly pine, sweetgum, and red maple all contain the C_3 pathway for photosynthesis. A study by Long and Drake (1991) examined the effect of long-term CO_2 concentrations on quantum yield of photosynthesis and PSII of the C_3 sedge *Scirpus olneyi* Gray. They found that shoots grown and measured in elevated CO_2 ($680 \mu\text{l l}^{-1}$) showed a significant increase in quantum yield from $0.065 (\mu\text{mol photons } \mu\text{mol CO}_2^{-1})$, for shoots grown at ambient CO_2 ($351 \mu\text{l l}^{-1}$), to $0.078 (\mu\text{mol photons } \mu\text{mol CO}_2^{-1})$. They also found that the light-compensation point of photosynthesis decreased from 51 to $31 \mu\text{mol m}^{-2} \text{sec}^{-1}$ for stems grown in ambient and elevated CO_2 , respectively. The photochemical efficiency of PSII, which they inferred from chlorophyll fluorescence, was unchanged by growth in elevated CO_2 (Long and Drake 1991). These findings suggest C_3 species, such as loblolly pine, sweetgum, and red maple, will become more photosynthetically efficient under light-limiting conditions in a future elevated CO_2 environment without altering the photochemical efficiency of PSII. In a simulation, Long (1991) found both quantum yield and light-saturated rates of CO_2 uptake increased with an increase in CO_2 concentration. The increase was greater as temperature rose.

Curtis and Teeri (1992) studied the seasonal response of leaf gas exchange to elevated CO₂ in bigtooth aspen (*Populus grandidentata* Michx.). Seedlings were exposed to ambient and elevated CO₂ concentrations for 70 days. At 28, 45, and 68 days after exposure to elevated CO₂, they measured photosynthetic light and CO₂ response characteristics. In the ambient-grown seedlings, light-saturated photosynthetic rates increased from day 28 to day 45 but decreased at day 68. The seedlings grown in elevated CO₂, however, showed no decline in photosynthesis, which suggested to them that growth under elevated CO₂ caused a delay in the onset of late-season declines in photosynthetic capacity (Curtis and Teeri 1992). They concluded that the forest may respond positively to an increase in atmospheric CO₂.

Other studies, reviewed by Farrar and Williams (1991), support the initial increase in photosynthesis under increased CO₂ conditions. However, many reports have shown that the initial increase in rates of carbon fixation of C₃ species may not be maintained in the long term (Farrar and Williams, 1991). This decrease in the initial increase in rates of carbon fixation has been termed acclimation. The presence of acclimation in one species, but not in another, would also likely contribute to changes in species composition in a future CO₂ environment.

Wullschleger et al. (1992) found that leaves of seedlings grown in elevated CO₂ had reduced chlorophyll concentrations. A reduction in the amount of chlorophyll would suggest that the rate of photosynthesis might decline. However, they found that this was not the case. Total chlorophyll content of yellow-poplar (*Liriodendron tulipifera* L.) and white oak (*Quercus alba* L.) grown at elevated CO₂ was reduced by 27 and 55% respectively when compared with ambient-grown seedlings (Wullschleger et al. 1992). Wullschleger et al.'s results are in agreement with Houpiis et al. (1988), who examined chlorophyll and carotenoid concentrations in two varieties of ponderosa pine (*Pinus*

ponderosa Dougl. ex Laws.) seedlings subjected to long-term elevated CO₂. Houpis et al. found that growth in elevated CO₂ concentrations (greater than 425 µl l⁻¹) decreased chlorophyll *a* and chlorophyll *b* concentrations (Houpis et al. 1988). In the experiment by Wullschleger et al., carbon exchange rates increased by 60% for yellow poplar and 35% for white oak with respect to ambient-grown seedlings when measured in elevated CO₂. This implies that leaves of plants grown in CO₂-enriched air are more efficient in the capture or use of light for photosynthesis than ambient-grown plants (Wullschleger et al. 1992). Wullschleger et al. also found that the quantum yield increased for both species by over 35% when grown in elevated CO₂, which is in agreement with the research done by Long and Drake (1991). Wullschleger et al. also completed some preliminary experiments on photochemical quenching. They used pulse-modulated fluorescence techniques and found that leaves of both yellow poplar and white oak grown in elevated CO₂ were able to take in more light-energy through photochemical quenching. These results suggest an increase in PSII involvement in light-dependent processes, and that, in turn, suggests there is an increase in light-use efficiency of plants grown in elevated CO₂ (Wullschleger et al. 1992).

WATER STRESS AND ELEVATED CO₂ EFFECTS ON PHOTOSYNTHESIS CHARACTERISTICS AND CHLOROPHYLL FLUORESCENCE

Many studies have focused on the influence of drought stress on the photosynthetic processes of plants. Jones and Fanjul (1983), in their work with apple (*Malus spp.* Mill.), found that all components of the photosynthetic system were affected under mild or moderate water stress. Ögren and Öquist (1985) performed an experiment on the effects of drought on photosynthesis and chlorophyll fluorescence in intact willow leaves under ambient CO₂ concentrations. They found that the maximum photosynthetic rate in willow leaves became less as water stress increased at high and low intercellular CO₂ levels. They

found that the decrease in photosynthesis at leaf water potentials of -1 MPa was equally attributable to stomatal or non-stomatal factors but decreases in photosynthesis below -1 MPa was only attributable to non-stomatal factors (Ögren and Öquist 1985).

Tolley and Strain (1985) studied the effects of CO₂ enrichment and water stress on gas exchange of sweetgum and loblolly pine. General findings were that the water-stressed plants had lower photosynthetic rates than the well-watered plants. With loblolly pine, CO₂ enrichment had little effect on the photosynthetic rates of either the well-watered or the water-stressed plants. However, the effects of water stress were delayed for sweetgum when grown at elevated CO₂. Sweetgum seedlings grown in elevated CO₂ maintained higher photosynthetic rates throughout the water-stress cycle than sweetgum seedlings grown in ambient CO₂ and the loblolly pine grown in either elevated or ambient CO₂. The sweetgum also showed an increase in water use efficiency. Tolley and Strain concluded that sweetgum should tolerate longer exposure to water stress, and therefore have greater survival on drier sites.

In 1984, Björkman and Bowles used chlorophyll fluorescence to analyze the influence of water stress on photosynthetic reactions of the desert shrub (*Nerium oleander* L.). They found both stomatal and non-stomatal limitations to photosynthesis and inactivation of light reactions associated with PSII under water stress (Björkman and Bowles 1984).

Ögren and Öquist (1985), found that at severe water stress, chlorophyll fluorescence oscillations are changed. Before fluorescence reaches a steady state level, a fluorescence oscillation occurs with a peak, minimum, and maximum level. According to Walker (1981) the minimum, maximum, and steady state level oscillation marks signify the beginning of carbon assimilation. In Ögren and Öquist's experiment, drought stress caused the half-rise time from the minimum level to the maximum level to increase, which

they interpreted to mean that water stress affected carbon metabolism and possibly photophosphorylation.

A study by Conroy et al. (1986) on chlorophyll *a* fluorescence and photosynthetic and growth responses of radiata pine (*Pinus radiata* D. Don) to P deficiency, drought stress, and high CO₂ showed that the light-saturated rate of photosynthesis in needles with adequate P was higher at elevated CO₂ than at ambient CO₂. They found that the maximal rates of quenching of fluorescence and induced fluorescence were reduced in droughted plants grown at ambient CO₂. Constant yield fluorescence was not changed. From their results they concluded that PSII was affected by drought stress. When plants grown at elevated CO₂ were studied, the maximal rate of quenching of fluorescence did not decrease. This was taken to mean that plants grown in elevated CO₂ may have an improved ability to tolerate drought.

Not all researchers agree that the functioning of PSII is influenced by water stress. Downton et al. (1988) used chlorophyll fluorescence to determine if water stress caused photosynthesis to decline because of stomatal or non-stomatal limitations. They concluded that the decline in photosynthesis was due to stomatal closure and not non-stomatal limitations. They further suggested that much of the apparent non-stomatal water stress effects reported in plants could be due to non-uniform stomatal closure during water stress.

Sharkey and Badger (1982) examined the effects of water stress on electron transport, photophosphorylation, and metabolite levels of *Xanthium strumarium* L. mesophyll cells. They found that photophosphorylation was sensitive to water stress but that electron transport was unaffected by water stress even at very low water potentials. This is in agreement with Genty et al. (1987), Epron and Dreyer (1990), Jefferies (1992), and Havaux (1992).

Genty et al. (1987) studied the effects of drought on primary photosynthetic processes of cotton leaves (*Gossypium hirsutum* L., cv Reba). They found that PSII photochemistry is unaffected by drought stress. Epron and Dreyer (1990) studied stomatal and non-stomatal limitations by leaf water deficits in three oak species; they also compared gas exchange and chlorophyll fluorescence data. They found, that in all three species, the chlorophyll fluorescence kinetics displayed similar shapes above -4.0 MPa and concluded that the functioning of PSII was not influenced by drought stress.

Jefferies, in 1992, studied the effects of drought on chlorophyll fluorescence in potato (*Solanum tuberosum* L.). He found that constant and variable fluorescence declined with time in both the irrigated and non-irrigated treatments but declined more in the droughted plants. But, the yield of variable fluorescence, or the efficiency of PSII, was unaffected by the drought treatment. More importantly, he found that the changes in chlorophyll fluorescence were not closely related to changes in leaf water potential.

Havaux (1992) examined the stress tolerance of PSII and the antagonistic effects of water, heat, and photoinhibition stresses on several plant species. He found that PSII was highly drought resistant. Even a highly desiccated leaf showed only minimal effects on the photochemical efficiency of PSII. Havaux found that, when well-watered leaves were exposed to high temperature, PSII was irreversibly damaged; But, when droughted leaves were exposed to high temperature, PSII was not irreversibly damaged. He also found that photoinhibition damage of PSII was greater in water-stressed leaves than well-watered leaves.

Cornic and Briantais (1991) partitioned the photosynthetic electron flow between CO₂ and O₂ reduction in a C₃ leaf (*Phaseolus vulgaris* L.) at different CO₂ concentrations and during water stress. They used many fluorescence parameters to analyze how the light energy was being used and to determine if a decline in photosynthesis was due to stomatal

or non-stomatal effects. They reasoned that, under a mild stress, the decline in photosynthesis is due to closing stomata. They concluded that, although photosynthesis declined and the internal CO₂ concentration declined, the allocation of electrons to O₂ increased during the desiccation of the leaf. They used the fluorescence parameter $\Delta F/F_m$ (where ΔF is the difference between the maximum fluorescence, F_m , and the steady-state fluorescence emissions) to determine that the allocation of electrons to O₂ increased during the desiccation of the leaf. They found these rates to be in agreement with measurements of leaf O₂ absorption using ¹⁸O₂ and the kinetic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase (Cornic and Briantais 1991). Cornic et al. (1989) also found that the PSII reaction center was hardly modified by water stress.

Past research has investigated the influence of changing environmental conditions, such as increased atmospheric CO₂ and water stress, on light and fluorescence responses of plant and tree species. Results from these experiments, investigated in the literature review, indicate that the potential is there for loblolly pine, sweetgum, and red maple to have their light and fluorescence responses changed in response to elevated CO₂ and water stress. These possible changes could influence the competitive interactions of these species leading to changes in their relative dominance in the southern forest ecosystem.

CHAPTER 3

The Influence of Elevated CO₂ Concentration on the Light-Compensation Point, Light-Saturated Net Photosynthetic Rates, and Quantum Yield of Loblolly Pine, Sweetgum, and Red Maple.

INTRODUCTION

Changes in photosynthetic responses to light in elevated CO₂ could influence the competitive outcomes of tree species. The chosen species for this experiment were loblolly pine (*Pinus taeda* L.), sweetgum (*Liquidambar styraciflua* L.), and red maple (*Acer rubrum* L.) because of their importance in the southern forest ecosystem as crop trees and competitors. Loblolly pine is the most important timber-producing tree in the southeast (Baker and Langdon 1990). Sweetgum and red maple are major competitors to loblolly pine, in the southern and more northern portion of loblolly pines range, respectively (Kormanik 1990 and Walters and Yawney 1990).

One useful way to provide information concerning photosynthetic responses to environmental conditions is to construct light-response curves. From a properly constructed light curve the light-compensation point, the light-saturation point, and the quantum yield of the plant can be obtained. The light-compensation point is the irradiance at which net photosynthesis equals zero, offsetting dark respiration in the plant. The light-saturation point, or light-saturated net photosynthetic rate, is the irradiance at which light is no longer the limiting factor but rather the ability to fix carbon is. The quantum yield is calculated from the initial slope of the relationship between light and net photosynthesis. It reveals the efficiency of the photosynthetic apparatus under light-limiting conditions (Kozłowski et al. 1991).

An increase or decrease in the light-compensation point or the quantum yield of a species could change the growth responses of the species under low light conditions. These increases or decreases could alter seedling survival for loblolly pine or understory

species interference by red maple or sweetgum. Similarly, an increase or decrease in the light-saturated photosynthetic rate could change the competitive outcomes of the species. An increase in the light-saturated photosynthetic rate would potentially allow for greater growth and biomass accumulation, while a decrease would lower growth and biomass accumulation under light-saturating conditions. An increased-light saturated photosynthetic rate would be beneficial to loblolly pine, which could grow up and away from competition faster, while a decreased light-saturated photosynthetic rate could be harmful, since red maple and sweetgum could more easily overtop it.

Due to increases in atmospheric CO₂ concentrations, the influence of elevated CO₂ concentrations on plants has become an important question. Present and past research has shown both short-term and long-term effects on photosynthesis of plants grown in elevated atmospheric CO₂. Little research has investigated specifically how the photosynthetic characteristics of co-occurring tree species change in response to elevated CO₂. However, there have been several related studies which have found growth in elevated CO₂ to change characteristics of the photosynthetic response to light. A study by Bazzaz et al. (1990) found that shade-tolerant tree species showed a greater response to elevated CO₂ than shade-intolerant species. Ehleringer and Björkman (1977), Long (1991), Long and Drake (1991), and Wullschleger et al. (1992) have found that the quantum yield of various plants increases under growth in elevated CO₂. Long and Drake (1991) and Valle et al. (1985) found that the light-compensation point of photosynthesis decreased for plants grown in elevated CO₂. The affect of elevated CO₂ on light-saturated photosynthetic rates is less clear. It is well known that short-term and long-term exposure to elevated CO₂ increases photosynthetic rates, but long-term exposure to elevated CO₂ has other influences. The initial rise in light-saturated photosynthetic rates of plants grown in elevated CO₂ is maintained in some species, (Gunderson et al. 1993, Wullschleger et al. 1992), but the

majority of the studies have found the initial high rates to decrease after prolonged exposure to elevated CO₂ (Farrar and Williams 1991, Tissue et al. 1993). This photosynthetic decrease has been termed acclimation.

With these past studies and possible competitive alterations in mind, the influence of elevated CO₂ on the photosynthetic characteristics of loblolly pine, sweetgum, and red maple was analyzed to determine if growth in elevated CO₂ concentrations changes the light compensation point, light-saturated net photosynthetic rates, or the quantum yield of loblolly pine, red maple, or sweetgum.

MATERIALS AND METHODS

Seedling Culture

Seed from loblolly pine (Virginia Department of Forestry, mixed stock), sweetgum (Georgia, mixed stock), and red maple (Erie County, PA, mixed stock) were germinated in a greenhouse under natural light using covered flats which contained Pro-Mix®. Prior to germination, the seeds were first stratified by soaking overnight in water, draining, and chilling (2°C) for 4 weeks (loblolly pine) and 8 weeks (red maple). The sweetgum seed was not stratified. After the seeds germinated and seedlings reached a height of approximately 5 cm, twenty seedlings of each species were transplanted into 1-L pots containing a 2:1:1 mixture (V/V) of peat moss (Fisons Horticulture Inc., Bellevue, WA), perlite (Silbrico Corporation, Silbrico, IL), and vermiculite (Palmetto Co. Inc.). The loblolly pine were transplanted into the 1-L pots on 1 September 1992, the sweetgum on 21 July 1993, and the red maple on 27 August 1993. The loblolly pine were placed into the chambers on 1 October 1992, the sweetgum on 9 August 1993, and the red maple on 10 September 1993. CO₂ chambers located at Virginia Tech were used for this study as described by Samuelson (1992). The two chambers measured 1.5m x 0.91m x 0.91 m and

were covered with teflon film plastic. Light transmittance for each chamber was over 90% of greenhouse light and air-conditioners in each chamber controlled temperature and facilitated air mixing (Samuelson 1992). The ambient CO₂ chamber averaged $360 \pm 41 \mu\text{l l}^{-1}$ CO₂ during the day, $395 \pm 44 \mu\text{l l}^{-1}$ CO₂ during the night, and $379 \pm 47 \mu\text{l l}^{-1}$ CO₂ overall. The elevated CO₂ chamber averaged $725 \pm 74 \mu\text{l l}^{-1}$ CO₂ during the day, $774 \pm 80 \mu\text{l l}^{-1}$ CO₂ during the night, and $746 \pm 87 \mu\text{l l}^{-1}$ CO₂ overall. Temperature in the ambient CO₂ chamber averaged $25 \pm 2^\circ\text{C}$ during the day and $23 \pm 3^\circ\text{C}$ during the night and relative humidity averaged $67 \pm 12\%$ during the day and $76 \pm 13\%$ during the night. Temperature in the elevated CO₂ chamber averaged $25 \pm 2^\circ\text{C}$ during the day and $23 \pm 1^\circ\text{C}$ during the night and relative humidity averaged $68 \pm 9\%$ during the day and $80 \pm 17\%$ during the night. Once in the chambers, the seedlings were rotated one position clockwise every other day, and the CO₂ treatment and the seedlings were moved between chambers once a week. This was compensate for possible variations within and between the chambers. The seedlings were kept well-watered and, every week, received 20 ml of Peters general purpose 20-20-20 fertilizer at a concentration of 200 ppm N, 87 ppm P, and 166 ppm K. (Peters, Fogelsville, PA).

Measurements

Light curves were constructed on six seedlings per CO₂ treatment for each species at the end of 1 year (loblolly pine), 5 months (sweetgum), and 4 months (red maple) of growth in the CO₂ treatments. Seedlings within a CO₂ treatment were selected for uniformity of leaf area and general size.

As in Nowak (1991), light curves for each species were constructed using a 400 watt sodium vapor lamp and various amounts of shade cloth and aluminum foil to achieve five different light levels plus complete darkness. The five light levels averaged $1189 \pm$

93, 483 ± 61 , 149 ± 11 , 44 ± 6 , and $14 \pm 3 \mu\text{mol m}^{-2} \text{s}^{-1}$. A water bath located above the measurement area was used to prevent the cuvette from overheating. Gas exchange was measured using one fully expanded fascicle or leaf with the LI-6200 Portable Photosynthesis System (LI-COR, Inc. Lincoln, NB) under ambient CO_2 concentrations. The temperature and relative humidity inside the cuvette during the experiments averaged $25 \pm 2 \text{ }^\circ\text{C}$, and $42 \pm 18\%$ relative humidity. Relative humidity was similar for sweetgum and red maple but relative humidity was higher for loblolly pine because the loblolly pine were measured in the late summer. All plants were measured at ambient CO_2 ($361 \pm 6 \mu\text{l l}^{-1} \text{CO}_2$) since any differences between treatments would be the same or larger at elevated CO_2 , thus making differences between treatments conservative.

For final analysis, photosynthesis was determined on an area basis for all species. Leaf area for loblolly pine was determined using the formula

$$\text{LA} = d l (3.142 + n)$$

where d equals the fascicle diameter, l equals the needle length, and n equals the number of needles in a fascicle (Nowak 1991). Leaf area for red maple and sweetgum was determined using the LI-3000 Portable Area Meter (LI-COR, Inc. Lincoln, NB).

Statistical Design and Analysis

The experiments were arranged in a completely randomized design with six replicates of each species and two CO_2 treatments. Estimates of light-saturated net photosynthetic rates (P_{max}) and light compensation point (LCP) were calculated as in Hanson et al. (1988) using the following equation and nonlinear regression

$$P_s = P_{\text{max}} \{ 1 - (1 - R_d/P_{\text{max}}) [1 - (\text{PPFD}/\text{LCP})]^z \}$$

where P_s equals photosynthesis, PPFD equals the photosynthetic photon flux density, R_d equals dark respiration, and z is an exponential multiplier to allow for a better curved fit.

The apparent quantum yield was obtained from the initial slope of each curve using the first derivative of the above function with respect to PPFD = LCP (Hanson et al. 1988). After determining estimates, individually for each seedling, the influence of CO₂ treatment on estimated dark respiration, light compensation point, light-saturated net photosynthetic rates, and quantum yield, was analyzed using analysis of variance. Each species was analyzed separately and not compared statistically.

RESULTS AND DISCUSSION

A typical light response curve is shown in Figure 3.1. The nonlinear regression predictions of fit performed quite well. Values obtained from the analysis of the light response curves, constructed at ambient CO₂, showed that no significant differences in the photosynthetic light responses were evident for either loblolly pine or sweetgum in response to elevated CO₂ (Table 3.1). Similarly, no change in quantum yield or light compensation point was seen for red maple in response to elevated CO₂. However, a significant decrease in light saturated photosynthetic rates did occur for red maple in response to elevated CO₂ (Table 3.1).

Long and Drake (1991) found that, in shoots grown and measured in elevated CO₂, quantum yield increased from 0.065 ($\mu\text{mol photons } \mu\text{mol CO}_2^{-1}$) to 0.078 ($\mu\text{mol photons } \mu\text{mol CO}_2^{-1}$) and light compensation point decreased from 51 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to 31 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in stems grown and measured in 351 $\mu\text{l l}^{-1}$ and 680 $\mu\text{l l}^{-1}$ CO₂ respectively. Valle et al. (1985) found that, for soybean, quantum yield increased from 0.05 ($\mu\text{mol photons } \mu\text{mol CO}_2^{-1}$) to 0.09 ($\mu\text{mol photons } \mu\text{mol CO}_2^{-1}$) and light compensation point decreased from 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to 27 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 330 $\mu\text{l l}^{-1}$ and 660 $\mu\text{l l}^{-1}$ CO₂, respectively.

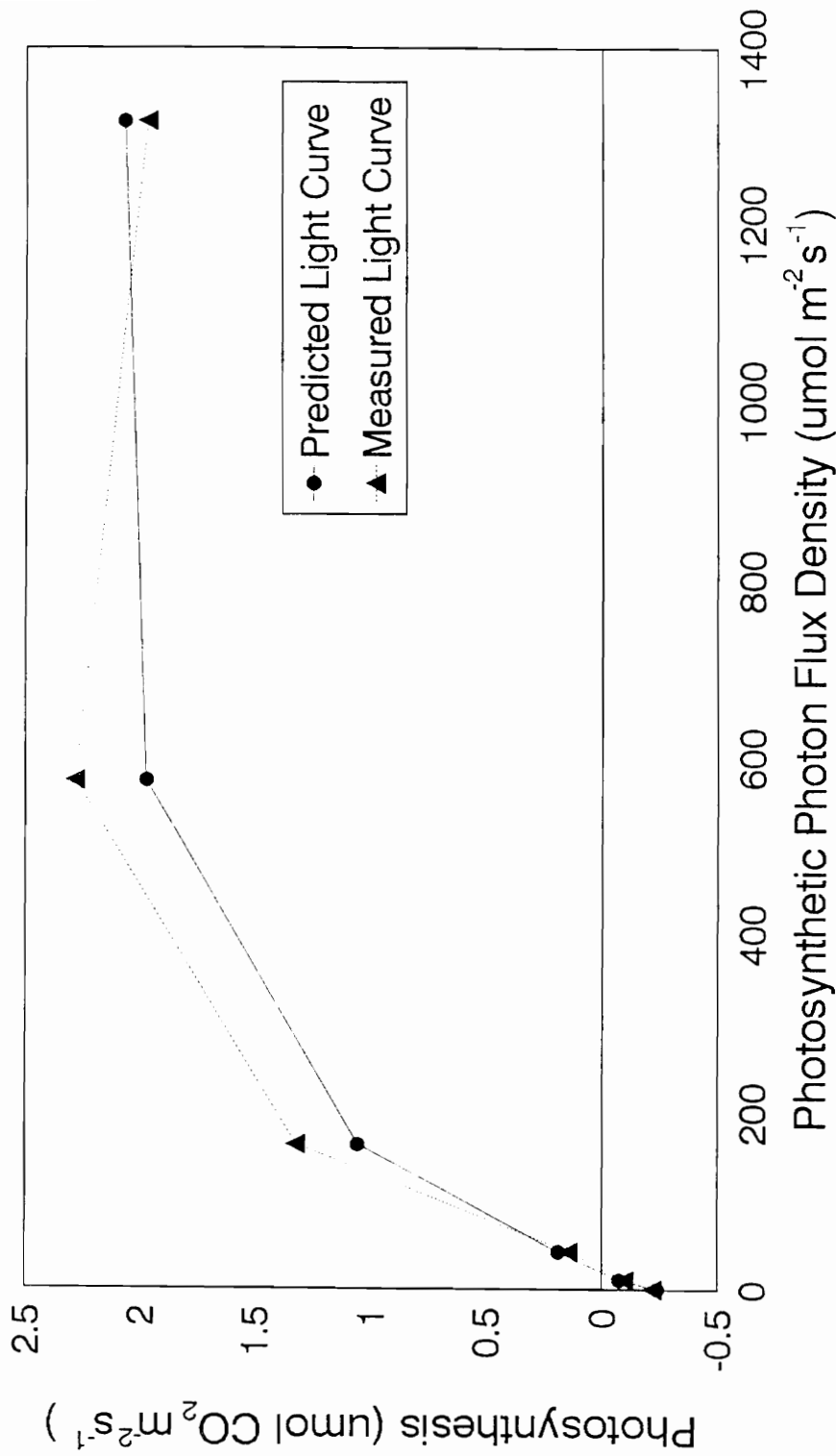


Figure 3.1: A representative light curve, constructed at ambient CO_2 , using a 12-month-old loblolly pine grown in $379 \mu\text{l l}^{-1} \text{CO}_2$ and the predicted fit from the nonlinear regression equation.

Table 3.1: Quantum yield, light-compensation point, and light-saturated photosynthetic rate obtained from light curves, constructed under ambient CO₂ levels on 16 September 1993 (loblolly pine) 20 December 1993 (sweetgum) and 4 January 1993 (red maple) following 12 (loblolly pine), 5 (sweetgum), or 4 (red maple) months at a CO₂ level.

CO ₂ Treatment ($\mu\text{l l}^{-1} \text{CO}_2$)	Quantum Yield ($\mu\text{mol photons } \mu\text{mol CO}_2^{-1}$)	Light-Compensation Point ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Light-Saturated Photosynthetic Rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
<u>Loblolly Pine</u>			
746	.01387 (0.48) ¹	21.009 (0.38)	1.9150 (0.43)
379	.01519	18.631	2.2546
<u>Sweetgum</u>			
746	.04058 (0.70) ¹	10.350 (0.74)	2.4937 (0.25)
379	.03677	9.362	1.7122
<u>Red Maple</u>			
746	.05729 (0.73) ¹	8.757 (0.16)	2.1952 (0.004)
379	.05015	2.460	3.9854

¹ p-value for test of significance between treatments

Contrary to the experiments by Long and Drake (1991), Valle et.al. (1985) and others completed by Long (1991) and Wullschleger et al. (1992) no significant changes were found in the quantum yields and light compensation points of loblolly pine, sweetgum, or red maple seedlings grown in elevated CO₂ (Table 3.1). In fact, the light-compensation point for red maple increased substantially in elevated CO₂ ($p = 0.16$). No change in the quantum yields and light-compensation points agrees with Curtis and Teeri (1992) who found no difference in light-compensation points and quantum yields between bigtooth aspen grown in ambient or elevated CO₂. The results from this experiment with loblolly pine, sweetgum, and red maple suggest, that with increasing atmospheric CO₂, these species will not change their light harvesting capabilities or their ability to compete with each other under low light conditions.

No change in the quantum yield of these species demonstrates that these species will not become more efficient at the capture of light under light-limiting conditions. The numbers obtained for quantum yield for loblolly pine were lower than others previously reported by Long and Drake (1991) for *Scirpus olneyi*, by Valle et. al (1986) for soybean, by Curtis and Teeri (1992) for bigtooth aspen, and by Day et al. (1991) for loblolly pine. Quantum yields for red maple and sweetgum were similar to those in the literature. The low quantum yields for loblolly pine could have been due to the fact that the seedlings were pot bound and less responsive.

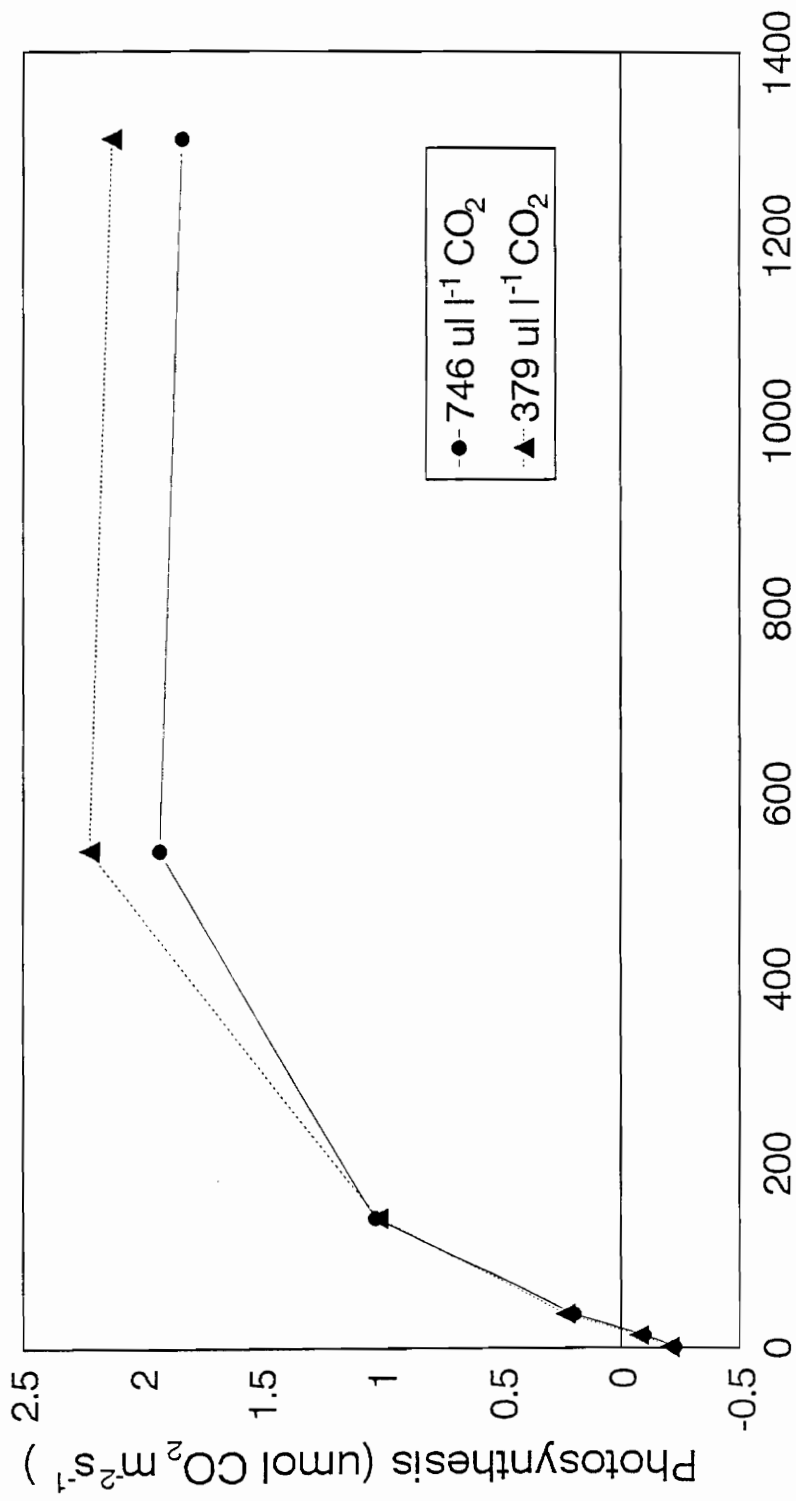
No change in the light-compensation point of these species indicates that none of the species have changed their tolerance to shade. By observing the light-compensation point, the least shade tolerant species was loblolly pine followed by sweetgum and red maple (Table 3.1). This is in agreement to the generally accepted shade tolerances of these species (Baker and Langdon 1990, Kormanik 1990, Walters and Yawney 1990). Based on these results, it appears that red maple and sweetgum will be as much of a competitor

for light and a continued competitor in the understory of pine forests in the future as they are presently.

The light-saturated photosynthetic rate of red maple was lower for seedlings grown in elevated CO₂ ($p=0.004$), which is in agreement with reports by Farrar and Williams (1991) and Tissue et al. (1993) who found that light-saturated photosynthetic rates declined for plants grown in elevated CO₂. Unlike those reports but similar to reports by Gunderson et al. (1993) and Wullschleger et al. (1992), who found the increased light saturated photosynthetic rate was maintained for plants grown in elevated CO₂, the light saturated photosynthetic rates for loblolly pine and sweetgum grown in elevated CO₂ were found not to differ significantly ($p=0.43$, $p=0.25$).

Average photosynthetic rates at each light level (Figure 3.2, 3.3, 3.4) confirm the modeled values obtained using the nonlinear regression model. No significant changes in any of the parameters (quantum yield, light compensation point, and light saturated photosynthetic rate) are evident for either loblolly pine or sweetgum. However, consistent with the modeled estimates, red maple had higher light saturated photosynthetic rates when grown in ambient CO₂.

The lower light-saturated photosynthetic rate of the red maple grown in the elevated CO₂ atmosphere could mean that red maple may not be as much of a nuisance at seedling establishment in the future CO₂ environment and that their initial increase in growth will not be maintained. If the maximum photosynthetic rate of the red maple is lower in the future CO₂ environment, then the species will not grow as quickly, which could save money on the suppression of red maple either through burning or chemicals. This decrease in the maximum light-saturated photosynthetic rate of red maple could also mean that suppression may not have to occur as often. Since loblolly pine did not have any of the measured photosynthetic responses to light significantly changed they may be able to grow up and



Photosynthetic Photon Flux Density (umol m⁻² s⁻¹)

Figure 3.2: Mean photosynthetic rates at different light levels, constructed at ambient CO₂, for 12-month-old loblolly pine seedlings grown in 379 ul l⁻¹ CO₂ or 746 ul l⁻¹ CO₂. Means at the different light levels do not differ significantly.

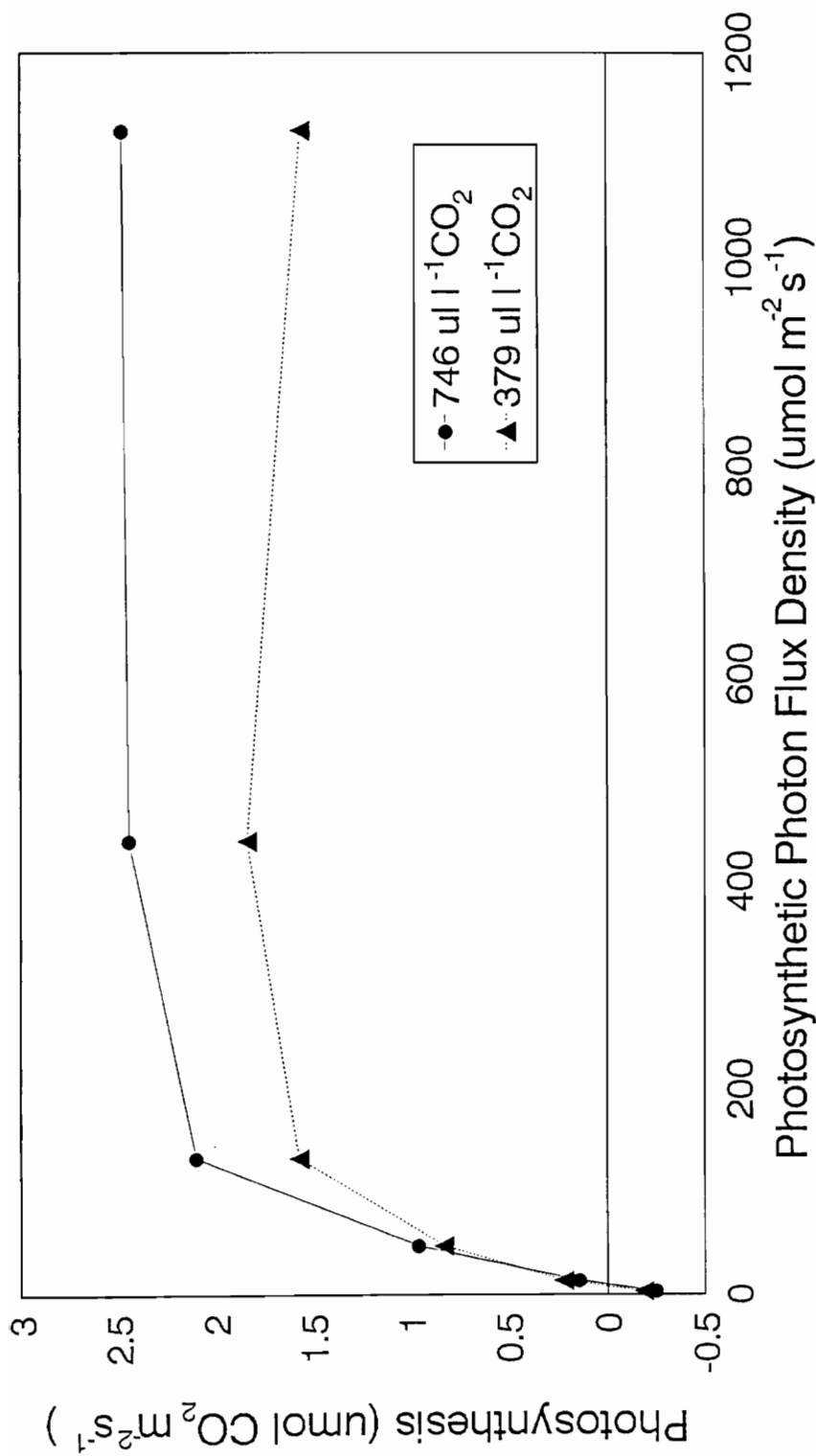


Figure 3.3: Mean photosynthetic rates at different light levels, constructed at ambient CO_2 , for 5-month-old sweetgum seedlings grown in 379 $\mu\text{l l}^{-1} \text{CO}_2$ or 746 $\mu\text{l l}^{-1} \text{CO}_2$. Means at the different light levels do not differ significantly.

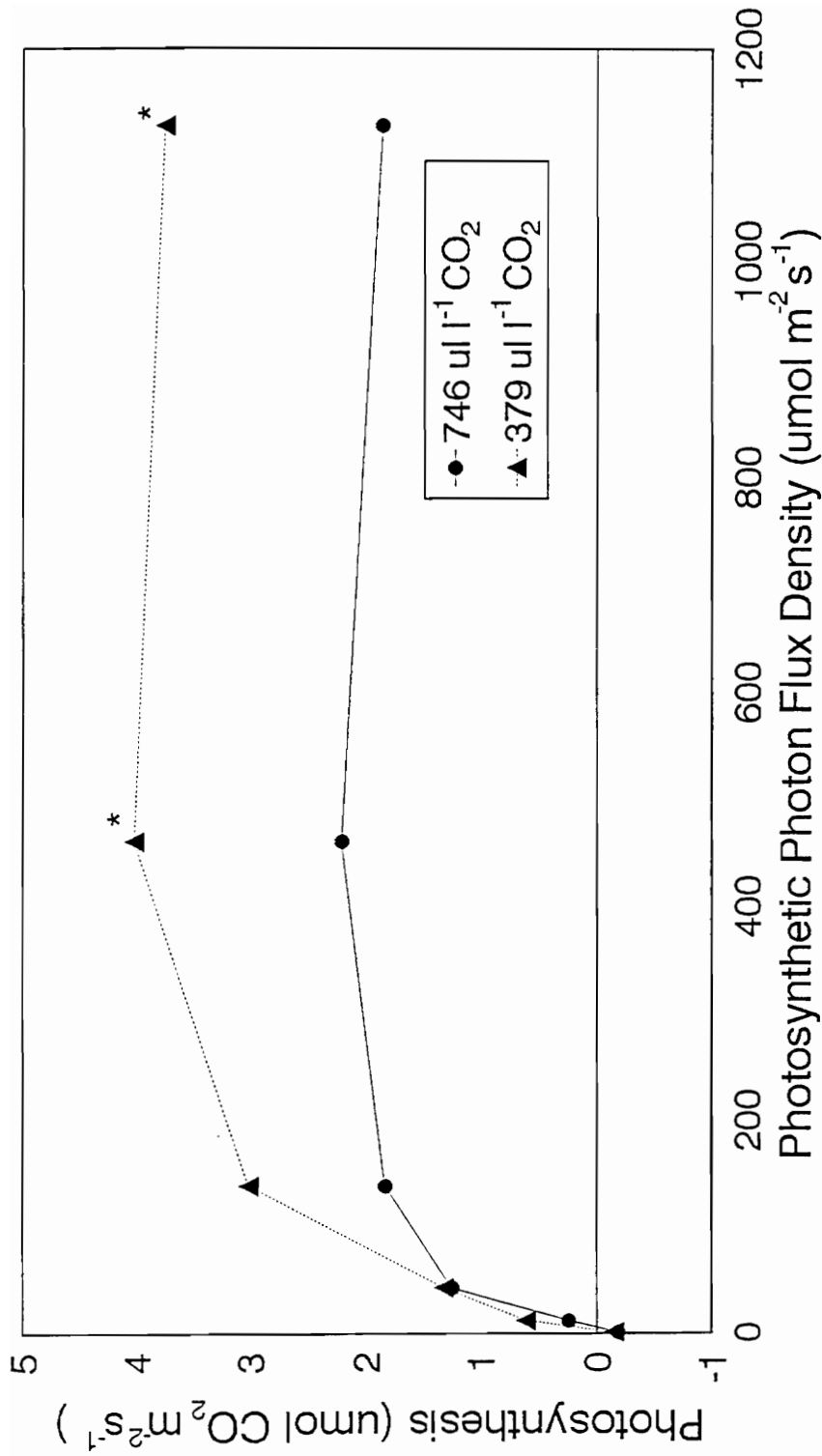


Figure 3.4: Mean photosynthetic rates at different light levels, constructed at ambient CO₂, for 4-month-old red maple seedlings grown in 379 ul l⁻¹ CO₂ or 746 ul l⁻¹ CO₂.

* Means were significantly different at the 0.05 level of significance.

away from red maple faster, which could again save money in suppression and perhaps enhance seedling survival. This could save planting costs, because it may be possible to plant fewer seedlings. However, it appears that sweetgum will stay a major competitor in a future CO₂ environment, because none of its photosynthetic responses to light were altered by growth in elevated CO₂.

CONCLUSION

Growth in elevated CO₂ did not significantly change any photosynthetic characteristics of loblolly pine or sweetgum when measured at ambient CO₂. This suggests that their competitive relationship will not be influenced by changes in light use efficiency in a future CO₂ environment. Quantum yield and light compensation point did not differ for red maple grown in elevated CO₂ but, the maximum photosynthetic rate for red maple, measured at ambient CO₂, was significantly lower for seedlings grown in elevated CO₂. This suggests that the initial increase in growth of red maple due to the increase in atmospheric CO₂ may not be maintained. Therefore, red maple may lose some of its competitiveness in a future CO₂ environment.

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CHAPTER 4

The Influence of Water Stress and Elevated CO₂ Concentrations on Gas Exchange and the Photochemical Efficiency of PSII of Loblolly Pine, Sweetgum, and Red Maple

INTRODUCTION

Changes in gas exchange in response to water stress and elevated CO₂ can alter the competitive outcomes between species. It would clearly be advantageous to a species to maintain photosynthesis under water stress. Changes in gas exchange could also alter seedling survival or species interference. Three important species in the southern forest ecosystem are loblolly pine (*Pinus taeda*), sweetgum (*Liquidambar styraciflua*), and red maple (*Acer rubrum*) because of their significance as crop trees and regional competitors. Loblolly pine is used commercially for both sawtimber and pulpwood, dominant on about 11.7 million hectares, and makes up over one-half of the standing pine volume (Baker and Langdon 1990). Red maple is one of the most widespread tree species in North America. Both sweetgum and red maple are often present in the southeastern pine forest understory (Kormanik 1990 and Walters and Yawney 1990). Growth of loblolly pine can be hindered by competing vegetation such as sweetgum and red maple in the southern and northern range of loblolly pine respectively.

Water deficits have a negative effect on the photosynthesis. Two explanations for this impact on photosynthesis are stomatal (Chaves and Pereira 1992, Cornic et al. 1989) and non-stomatal inhibition (Ögren and Öquist 1985, Björkman and Powles 1984). Stomatal limitations result from stomatal closure which prohibits CO₂ uptake into the leaf. Non-stomatal limitations occur inside the leaf and are metabolically and biochemically related. One way to determine whether a decline in photosynthesis by water stress is due to stomatal or non-stomatal inhibitions is to analyze the chlorophyll fluorescence of a plant. Chlorophyll fluorescence is a more recent tool in understanding plant reaction to water

stress. Chlorophyll fluorescence, which is primarily connected with Photosystem II (PSII), is an efficient way to determine if environmental factors modify photosynthetic capacity (Bolhàr-Nordenkampf et al. 1989). With the recent development of chlorophyll fluorescence measuring instruments, it can now be measured rapidly and non-destructively. The impact of water stress on chlorophyll fluorescence in loblolly pine, sweetgum, and red maple is not known. An increase in chlorophyll fluorescence during water stress would suggest a direct impact on the internal photosynthetic apparatus. Alternatively, no change would leave open the hypothesis that water stress impacts on photosynthesis are largely stomatal in nature. In addition, how growth in elevated CO₂ may change these responses is not known.

Many studies have focused on the influence of drought stress on the photosynthetic processes of plants. Jones and Fanjul (1983), in their work with apple, found that all components of the photosynthetic system were affected under mild or moderate water stress. Ögren and Öquist (1985) found that the maximum photosynthetic rate in willow leaves became less as water stress increased at high and low intercellular CO₂ levels. The decrease in photosynthesis at leaf water potentials of -1 MPa was equally attributable to stomatal or non-stomatal factors but decreases in photosynthesis below -1 MPa were only attributable to non-stomatal factors (Ögren and Öquist 1985).

Tolley and Strain (1985) found that water-stressed loblolly pine and sweetgum had lower photosynthetic rates than their well watered counterparts. However, sweetgum seedlings grown in elevated CO₂ maintained higher photosynthetic rates throughout the water stress cycle than sweetgum seedlings grown in ambient CO₂ and the loblolly pine grown in either elevated or ambient CO₂. The sweetgum also showed an increase in water use efficiency. Therefore, Tolley and Strain concluded that sweetgum should tolerate longer exposure to water stress and have greater survival on drier sites in an elevated CO₂

environment. Similarly, Hollinger (1987) concluded from water-stressed *Pinus radiata* and *Pseudotsuga menziesii* that elevated CO₂ may moderate the effects of atmospheric water stress.

In 1984, Björkman and Powles used chlorophyll fluorescence to analyze the influence of water stress on photosynthetic reactions of the evergreen C₃ shrub *Nerium oleander*, which is native to arid regions of Southwest Asia. They found both stomatal and non-stomatal limitations to photosynthesis and inactivation of light reactions associated with PSII under water stress (Björkman and Powles 1984). Ögren and Öquist (1985) found that, at severe water stress, chlorophyll fluorescence oscillations were changed, which supports findings by Conroy et al. (1986). Conroy et al. (1986) found that the maximal rate of quenching of fluorescence and induced fluorescence were reduced in droughted plants grown at ambient CO₂ but constant yield fluorescence was not changed. From their results they concluded that PSII was directly impacted by drought stress. When plants grown at elevated CO₂ were studied, the maximal rate of quenching of fluorescence did not decrease. This suggests that plants grown in elevated CO₂ may have an approved ability to fight drought.

Not all researchers agree that PSII is influenced by water stress. Sharkey and Badger (1982) found that photophosphorylation of *Xanthium strumarium* mesophyll cells was sensitive to water stress but that electron transport was unaffected by water stress even at very low water potentials. This is in agreement with Genty et al. (1987), Epron and Dreyer (1990), and Havaux (1992) who reported that PSII is not influenced by drought stress. Jefferies (1992) found some aspects of fluorescence to decline; but the yield of variable fluorescence was unaffected by drought stress, and changes in chlorophyll fluorescence were not closely related to changes in leaf water potential in potato (*Solanum tuberosum* L.).

Downton et al. (1988) concluded that the drought-induced decline in photosynthesis was due to stomatal closure and not non-stomatal limitations. They further suggested that much of the apparent non-stomatal water stress effects reported in plants could be due to non-uniform stomatal closure during water stress. Cornic and Briantais (1991) suggested that under a mild stress the decline in photosynthesis is due to closing stomata. They concluded that, although photosynthesis declined and the internal CO₂ concentration declined, the allocation of electrons to O₂ inferred from chlorophyll fluorescence increased during the desiccation of the leaf. Another experiment by Cornic et al. (1989) with fluorescence in *Phaseolus vulgaris* L. and *Elatostema repens* (Lour.) Hall f. indicated that the functioning of the PSII reaction center was hardly modified by water stress.

The photochemical efficiency of PSII (estimated by Fv/Fm) is an ideal measurement for determining the influence of environmental conditions on fluorescence. Fv/Fm is the ratio of variable fluorescence (Fv) to maximal fluorescence (Fm). Variable fluorescence originates from the pigments of PSII and arises due to the rapid reduction of electron accepting molecules. Maximal fluorescence is the point at which Fv is at a maximum and all electron acceptors are fully reduced. Alternatively, Fm can be determined by summing Fv and the initial rise in chlorophyll fluorescence (Fo). Fo is the fluorescence of the light harvesting pigments in the oxidized state (Greaves et al. 1991). These measurements provide information on inhibition or damage to transfer of light energy to electrons in PSII and are a sensitive indicator of photoinhibition (Bolhàr-Nordenkamp et al. 1989).

With past experiments and possible competitive alterations between species in mind, the influence of water stress on loblolly pine, sweetgum, and red maple was analyzed to determine if:

1. Chlorophyll fluorescence correlates with gas exchange during water stress in loblolly pine, red maple, and sweetgum.

2. Growth in elevated CO₂ concentrations changes the relationship between chlorophyll fluorescence and gas exchange during water stress.

MATERIALS AND METHODS

Seedling Culture

Seed from loblolly pine (Virginia Department of Forestry, mixed stock), sweetgum (Georgia, mixed stock), and red maple (Erie County, PA, mixed stock) were germinated in a greenhouse under natural light using covered flats which contained Pro-Mix®. Prior to germination, the seeds were first stratified by soaking overnight in water, draining, and chilling (2°C) for 4 weeks (loblolly pine) and 8 weeks (red maple). The sweetgum seed was not stratified. After the seeds germinated and seedlings reached a height of approximately 5 cm, twenty seedlings of each species were transplanted into 1-L pots containing a 2:1:1 mixture (V/V) of peat moss (Fisons Horticulture Inc., Bellevue, WA), perlite (Silbrico Corporation, Silbrico, IL), and vermiculite (Palmetto Co. Inc.). The loblolly pine were transplanted into the 1-L pots on 1 September 1992, the sweetgum on 21 July 1993, and the red maple on 27 August 1993. The loblolly pine were placed into the chambers on 1 October 1992, the sweetgum on 9 August 1993, and the red maple on 10 September 1993. CO₂ chambers located at Virginia Tech were used for this study as described by Samuelson (1992). The two chambers measured 1.5m x 0.91m x 0.91 m and were covered with teflon film plastic. Light transmittance for each chamber was over 90% of greenhouse light and air-conditioners in each chamber controlled temperature and facilitated air mixing (Samuelson 1992). The ambient CO₂ chamber averaged $360 \pm 41 \mu\text{l l}^{-1}$ CO₂ during the day, $395 \pm 44 \mu\text{l l}^{-1}$ CO₂ during the night, and $379 \pm 47 \mu\text{l l}^{-1}$ CO₂ overall. The elevated CO₂ chamber averaged $725 \pm 74 \mu\text{l l}^{-1}$ CO₂ during the day, $774 \pm 80 \mu\text{l l}^{-1}$ CO₂ during the night, and $746 \pm 87 \mu\text{l l}^{-1}$ CO₂ overall. Temperature in the ambient

CO₂ chamber averaged $25 \pm 2^\circ\text{C}$ during the day and $23 \pm 3^\circ\text{C}$ during the night and relative humidity averaged $67 \pm 12\%$ during the day and $76 \pm 13\%$ during the night. Temperature in the elevated CO₂ chamber averaged $25 \pm 2^\circ\text{C}$ during the day and $23 \pm 1^\circ\text{C}$ during the night and relative humidity averaged $68 \pm 9\%$ during the day and $80 \pm 17\%$ during the night. Once in the chambers, the seedlings were rotated one position clockwise every other day, and the CO₂ treatment and the seedlings were moved between chambers once a week. This was compensate for possible variations within and between the chambers. The seedlings were kept well-watered and, every week, received 20 ml of Peters general purpose 20-20-20 fertilizer at a concentration of 200 ppm N, 87 ppm P, and 166 ppm K. (Peters, Fogelsville, PA).

Measurements

Six seedlings of each species from each CO₂ treatment that had a suitable number of leaves were selected to complete all measurements. These seedlings were then subjected to water stress by withholding water. As the seedlings became progressively stressed, gas exchange, chlorophyll fluorescence, and water potential or percent water content were measured. Measurements continued until the seedlings reached zero photosynthesis.

Measurements for loblolly pine began on 27 September 1993 and were completed on 12 October 1993. Measurements for sweetgum and red maple began on 11 January 1994 and were completed on 31 January 1994. Gas exchange was measured using one fully expanded fascicle or leaf with the LI-6200 Portable Photosynthesis System (LI-COR, Inc. Lincoln, NB) under ambient CO₂ concentrations. The same fascicle or leaf was used for each measurement and, to ensure that the same needle or leaf area entered the cuvette each time, a line was marked on the fascicle or leaf. The source of light was a 400 watt sodium vapor lamp. During the measurement period for loblolly pine, the CO₂ concentration averaged $374 \pm 3 \mu\text{l l}^{-1}$, photosynthetic photon flux density (PPFD) averaged

$1132 \pm 85 \mu\text{mol m}^{-2} \text{sec}^{-1}$, relative humidity averaged $47 \pm 8 \%$, and the temperature averaged $22 \pm 2^\circ\text{C}$. During the measurement period for sweetgum and red maple, the CO_2 concentration averaged $364 \pm 8 \mu\text{l l}^{-1}$, photosynthetic photon flux density (PPFD) averaged $1121 \pm 71 \mu\text{mol m}^{-2} \text{sec}^{-1}$, relative humidity averaged $26 \pm 6 \%$, and the temperature averaged $25 \pm 2^\circ\text{C}$.

For final analysis, photosynthesis was determined on an area basis for all species. Leaf area for loblolly pine was determined using the formula

$$\text{LA} = d l (3.142 + n)$$

where d equals the fascicle diameter, l equals the needle length, and n equals the number of needles in the fascicle (Nowak 1991). Leaf area for red maple and sweetgum was determined using the LI-3000 Portable Area Meter (LI-COR, Inc. Lincoln, NB).

Chlorophyll fluorescence was measured with the CF-1000 Chlorophyll Fluorescence Measurement System (P.K. Morgan Instruments, Inc. Andover, MA) on a separate fully expanded fascicle or leaf. The fascicle or leaf was dark acclimated for 15 minutes. After 15 minutes, the fascicle or leaf was measured with the CF-1000 at a light level of $1000 \mu\text{mol m}^{-2} \text{sec}^{-1}$ and a sample time of 10 sec. The fascicle or leaf was marked to ensure that the same fascicle was used throughout the measurements.

The leaf water potential for loblolly pine was monitored using a pressure chamber (PMS Instrument Co., Corvallis, OR) on a fully expanded fascicle. Water potential was not measured for sweetgum and red maple because of the small numbers of leaves on the seedlings. Phloem bubbling in red maple also makes an accurate measurement impossible (Bahari et al. 1985). Therefore, the level of water stress for sweetgum and red maple was monitored using percent water content which was measured by taking three leaf punches from three separate leaves. The wet weight was taken and, after 24 hrs at 60°C , the dry weight was taken and the percent water content was determined using the following

formula:

$$(\text{wet weight} - \text{dry weight})/\text{dry weight}$$

After the dry-down measurements of gas exchange and fluorescence, all seedlings were harvested for biomass estimation. The seedlings were oven-dried at 60°C for 48 hours after which shoot, root, and leaf weights were measured. Since seedlings were not selected randomly for gas exchange measurements, estimates of biomass were only collected to aid in the interpretation of dry down responses.

Statistical Design and Analysis

The experiment was arranged in a completely randomized design with six replicates of each species and two CO₂ treatments. The influence of CO₂ treatment and water stress on photosynthesis and fluorescence was analyzed using analysis of variance. Photosynthesis and fluorescence were compared using simple linear regression. Each species was analyzed separately and not compared statistically.

RESULTS AND DISCUSSION

Percent water content of foliage was not a sensitive measure of water stress in sweetgum and red maple seedlings. Percent water content, as measured by leaf disks, was found not to change until the plants were wilted and near death. Photosynthesis showed no relationship to percent water content for either species and is shown for sweetgum in Figure 4.1. This is not totally surprising, since water content changes little over a wide range of water potentials. A plant with a water potential decreasing from -.5 MPa to -1 MPa may show little or no change in percent water content, since a given water deficit does not represent the same level of water potential (Kramer 1983). It is not until near or after the turgor loss point that leaf water content begins to change rapidly. Therefore, for sweetgum and red maple, photosynthesis and fluorescence were analyzed over time rather

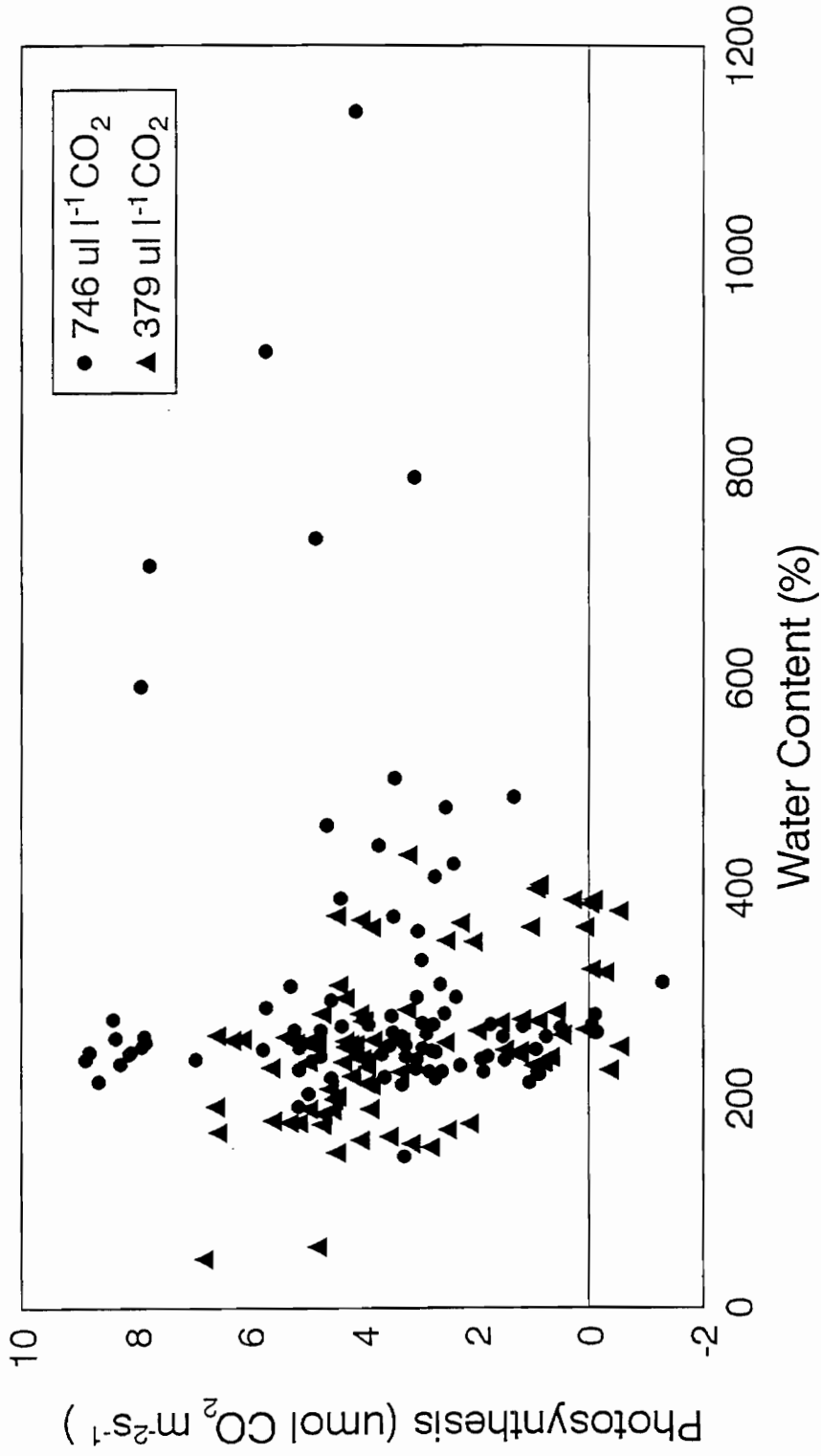


Figure 4.1: Photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), measured at ambient CO_2 , for 5-month-old sweetgum seedlings grown in 379 or 746 $\mu\text{l l}^{-1}$ CO_2 as influenced by increasing water content (%).

than using percent water content.

Gas Exchange

Photosynthesis was significantly decreased by withholding in all three species grown in ambient and elevated CO₂ (Table 4.1; Figure 4.2, 4.3, 4.4). This agrees with findings by Jones and Fanjul (1983) and Ögren and Öquist (1985) among others. Photosynthesis was maintained longer in the seedlings grown in elevated CO₂ for all three species (Figure 4.2, 4.3, 4.4). The slopes and intercepts of the lines for loblolly pine and red maple seedlings grown in elevated CO₂ were significantly lower than those grown in ambient CO₂ (Table 4.1). However, the slope of the line for sweetgum seedlings grown in elevated CO₂ was only slightly lower than those grown in ambient CO₂ ($p = 0.14$) and the intercept was not significantly different (Table 4.1).

The lower slopes of the lines in elevated CO₂ for loblolly pine, sweetgum, and red maple agree with research by Tolley and Strain (1985), Conroy et al. (1986), and Hollinger (1987) and reviews by Bowes (1993) and Kimball et al. (1993) who concluded that growth in elevated CO₂ may mediate the influence of water stress. This is particularly significant for the red maple and sweetgum, since the plants grown in elevated CO₂ had significantly more foliage (4.5 -vs 2.7 and 1.9 -vs 1.7g in elevated versus ambient CO₂ for sweetgum and red maple, respectively) and they still maintained photosynthesis longer than plants grown in ambient CO₂. Possible explanations for this greater tolerance of drought are greater water use efficiency (WUE), through decreased stomatal conductance, and possible osmotic adjustment within the leaf. A greater WUE would allow the plant to carry on normal physiological functions while using less water. A decrease in stomatal conductance could improve water potentials and leaf expansion, because more water would be retained in the leaf. An osmotic adjustment could result in the plant improving its ability to draw water into the leaf.

Table 4.1: Coefficient of determination values (r^2), slopes, intercepts, and p-values describing the relationship between water potential (Independent Variable) and photosynthesis (Dependent Variable) for loblolly pine and day (Independent Variable) and Ps for sweetgum and red maple.

Treatment ($\mu\text{l l}^{-1} \text{CO}_2$)	Intercept ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Slope	r^2	p-value
<u>Loblolly Pine</u>				
746	1.9473(0.04) ¹	-0.1014(0.04)	0.34	0.0001
379	2.5428	-0.1536	0.51	0.0001
<u>Sweetgum</u>				
746	5.2823(0.86) ¹	-0.1753(0.14)	0.11	0.0011
379	5.3884	-0.2706	0.38	0.0001
<u>Red Maple</u>				
746	2.4973(0.01) ¹	-0.0998(0.01)	0.07	0.0257
379	5.8771	-0.4228	0.61	0.0001

¹ p-value for test of significance between treatments

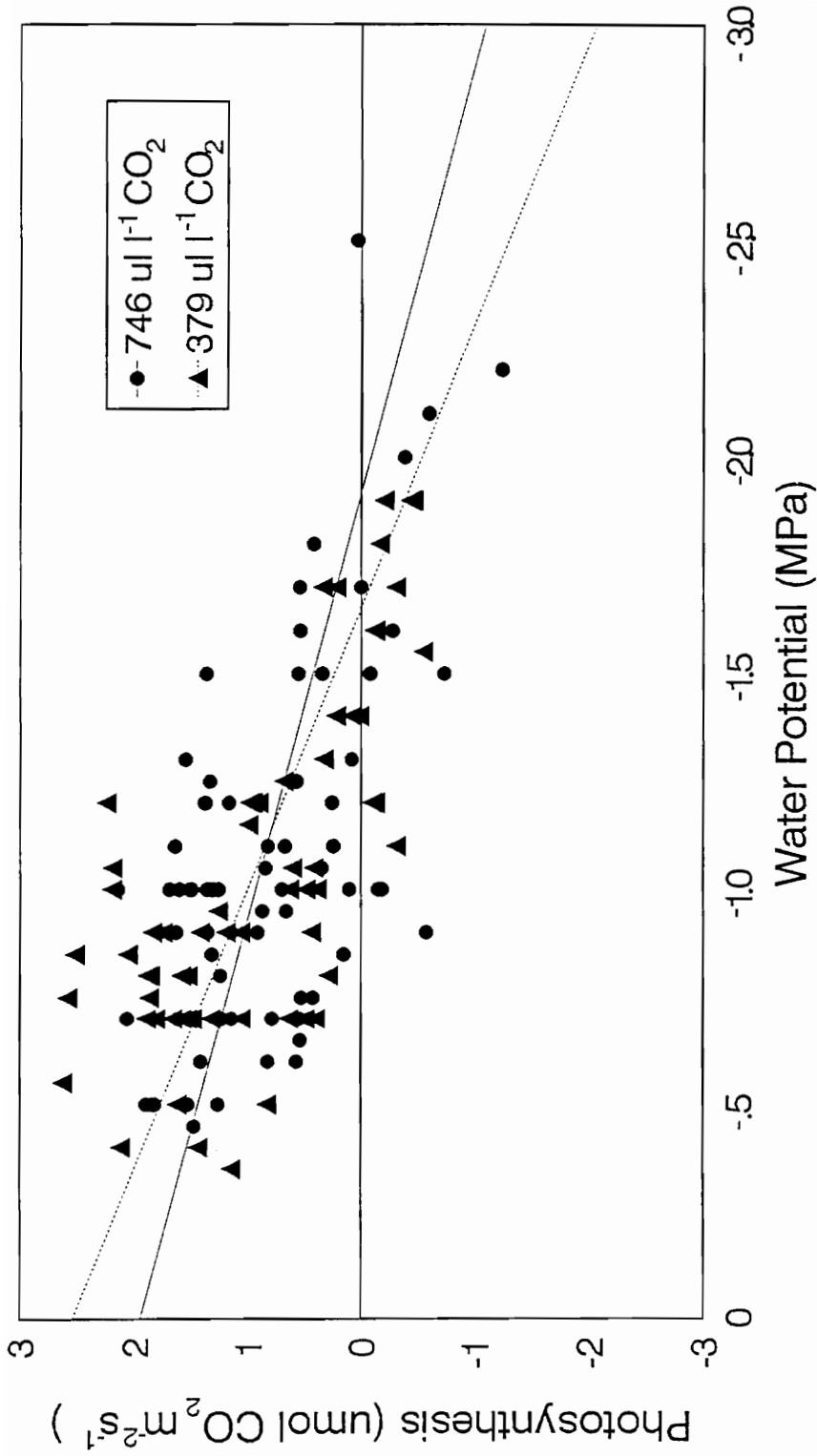


Figure 4.2: Photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), measured at ambient CO_2 , for 12-month-old loblolly pine seedlings grown in 379 or $746 \mu\text{l l}^{-1} \text{CO}_2$ as influenced by decreasing water potential (MPa).

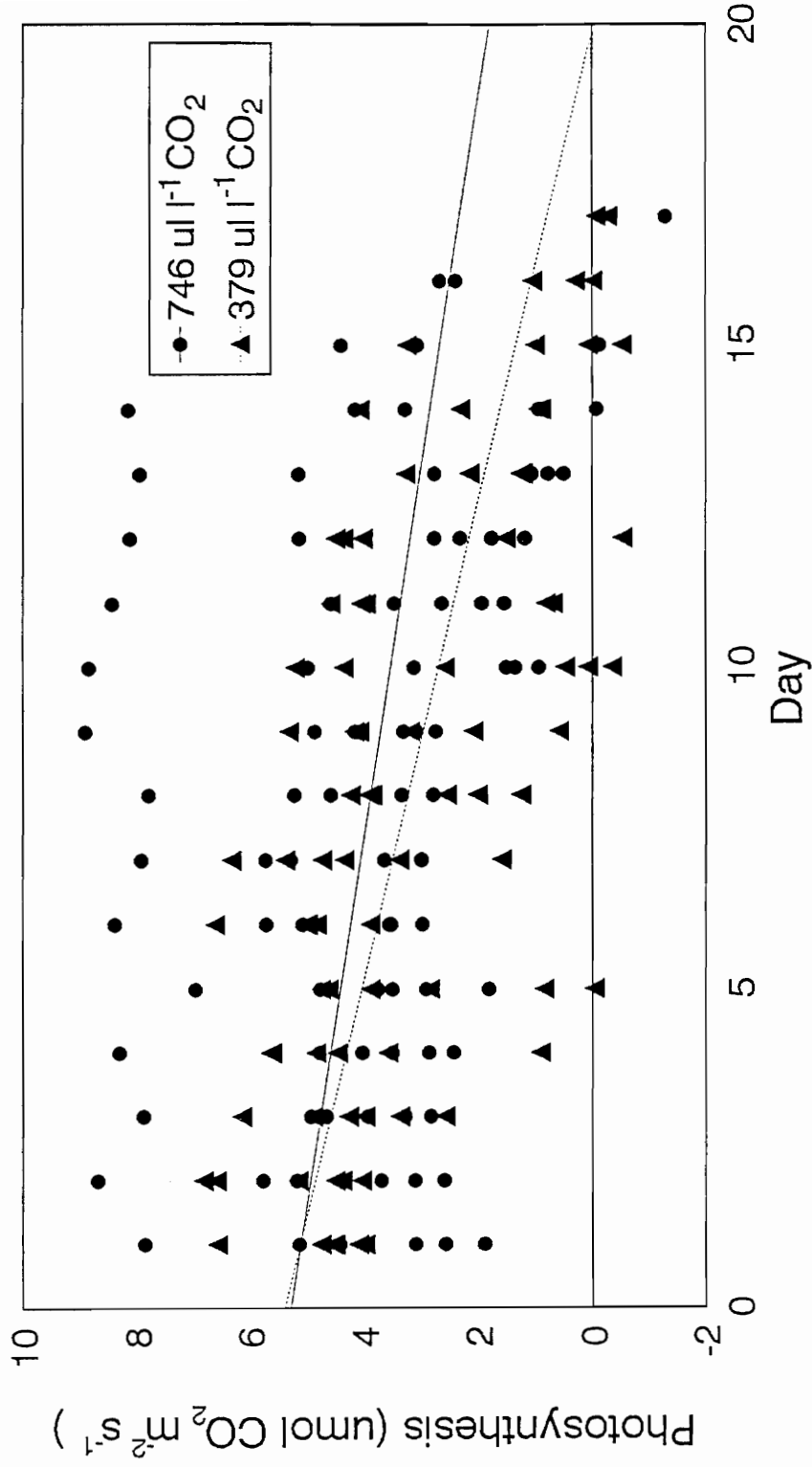


Figure 4.3: Photosynthesis ($\text{umol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), measured at ambient CO_2 , for 5-month-old sweetgum seedlings grown in 379 or 746 $\text{ul l}^{-1} \text{ CO}_2$ as influenced by increasing time (Day) with water withheld.

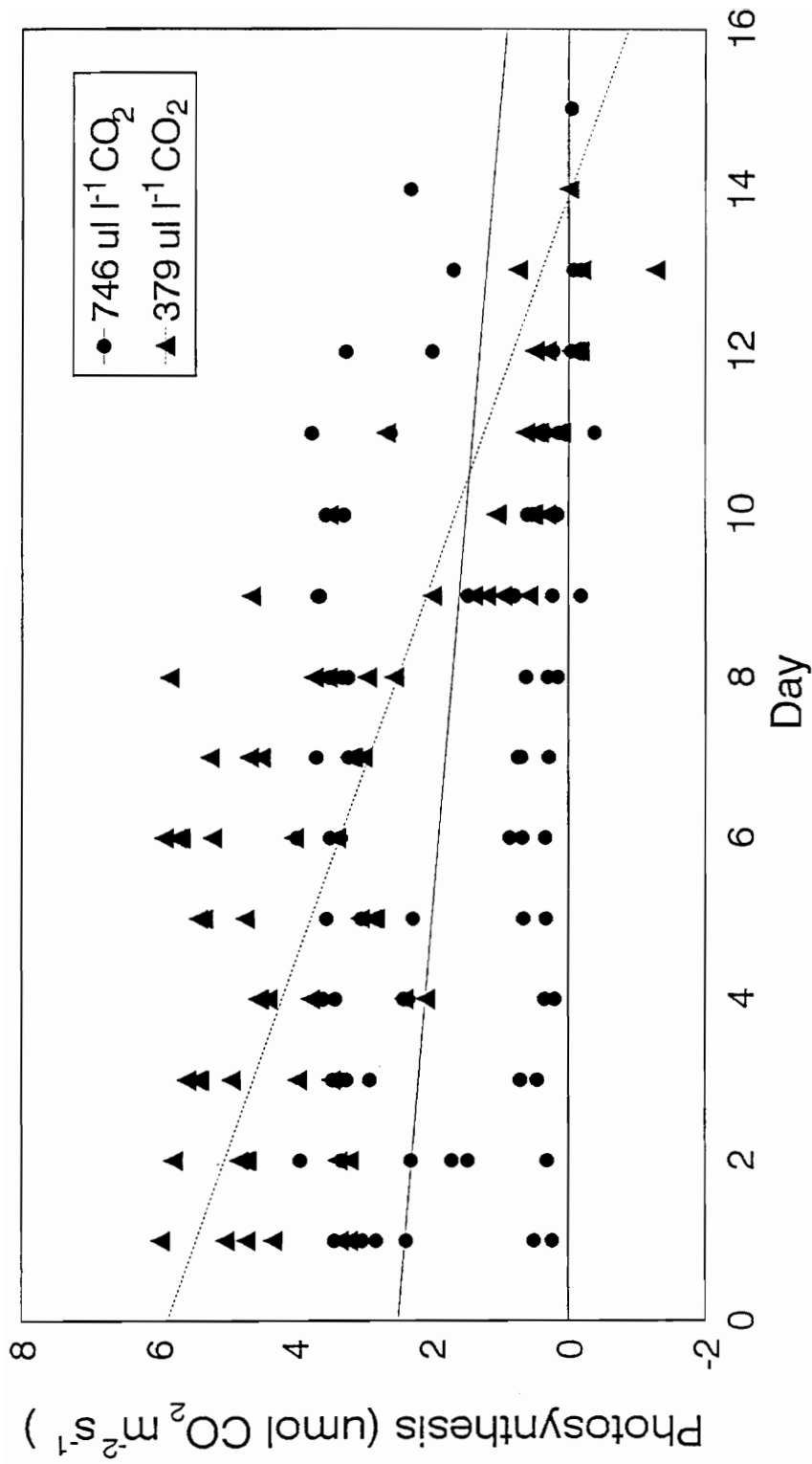


Figure 4.4: Photosynthesis ($\text{umol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), measured at ambient CO_2 , for 4-month-old red maple seedlings grown in 379 or 746 $\text{ul l}^{-1} \text{CO}_2$ as influenced by increasing time (Day) with water withheld.

The increased tolerance to drought observed in the three species could change the competitive outcomes of these species in a future elevated CO₂ environment. Sweetgum and red maple may become more competitive on drier sites where loblolly pine is normally advantaged. On the positive side, loblolly pine may tolerate an even drier site and have increased seedling survival thereby saving planting costs through planting less trees per hectare.

The lower intercepts for the loblolly pine and red maple seedlings support the reports of acclimation made by Farrar and Williams (1991) and Peet et al. (1986). The lower photosynthetic rates for plants grown in elevated CO₂ concentrations, or lack of it as found in the sweetgum seedlings, could also change the competitive outcomes of these species. Red maple may become less of a competitor, while sweetgum may maintain or increase its competitive ability in a future CO₂-enriched atmosphere.

Photochemical Efficiency of PSII

The relationship between photochemical efficiency of PSII (expressed as Fv/Fm) and water potential or time was not strong (Table 4.2). Photochemical efficiency of PSII significantly decreased with decreasing water potential in loblolly pines grown at ambient and elevated CO₂. However for sweetgum, although not strongly related, photochemical efficiency of PSII increased over time for seedlings at both CO₂ concentrations (Table 4.2). For red maple, the models were not significant (Table 4.2). The findings with loblolly pine support Björkman and Powles (1984), Ögren and Öquist (1985), and Conroy et al. (1986) who found that PSII was influenced by drought stress. Others, such as Genty et al. (1987) and Epron and Dreyer (1990), found the PSII reaction center to be highly drought resistant which supports the findings with sweetgum.

Table 4.2: Coefficient of determination values (r^2), slopes, intercepts, and p-values describing the relationship between water potential (Independent Variable) and photochemical efficiency of PSII (Dependent Variable) for loblolly pine and time (Independent Variable) and Fv/Fm for sweetgum and red maple.

Treatment ($\mu\text{l l}^{-1} \text{CO}_2$)	Intercept (unitless)	Slope	r^2	p-value
<u>Loblolly pine</u>				
746	0.5347(0.41) ¹	-0.0092(0.68)	0.33	0.0001
379	0.5593	-0.0081	0.17	0.0008
<u>Sweetgum</u>				
746	0.6038(0.01) ¹	0.0053(0.01)	0.15	0.0001
379	0.6384	0.0011	0.03	0.1170
<u>Red Maple</u>				
746	0.4840(0.03) ¹	0.0015(0.42)	0.002	0.7273
379	0.5621	-0.0022	0.02	0.2326

¹ p-value for test of significance between treatments

The slopes and intercepts for the photochemical efficiency of PSII for the two levels of CO₂ loblolly pine seedlings (Figure 4.5) do not differ significantly. Slopes and intercepts for sweetgum seedlings (Figure 4.6) grown in elevated CO₂ increase and decrease respectively (Table 4.2). These results suggest that growth in elevated CO₂ will not change the response of the photochemical efficiency of PSII for loblolly pine under water stress but may change the response of sweetgum. An increase in the photochemical efficiency of PSII in sweetgum could allow for increased competitiveness, because the tree would be more efficient at passing along electrons and energy produced in the light reactions of photosynthesis to the dark reactions of photosynthesis. Alternatively, a decrease in the photochemical efficiency of PSII in loblolly pine could cause it to be less competitive with sweetgum and red maple, because it would not be as efficient in its use of light energy.

Correlation of Photosynthesis and Photochemical Efficiency of PSII

Although weakly related, photochemical efficiency of PSII and photosynthesis were positively correlated in loblolly pine and red maple under both CO₂ levels (Table 4.3; Figure 4.7, 4.8). For sweetgum, photochemical efficiency of PSII and photosynthesis were positively correlated in seedlings grown in elevated CO₂ but had no relationship in seedlings grown in ambient CO₂ (Table 4.3; Figure 4.9). Again, with the exception of the red maple grown in elevated CO₂, the r^2 values were quite low, but a general trend can be seen in the data. This suggests that the drop in photosynthesis under water stress is partly due to a decline in the photochemical efficiency of PSII, meaning that a decline in photosynthesis is non-stomatal in nature. This would support findings by Björkman and Powles (1984) and Ögren and Öquist (1985), who found PSII to be influenced by water stress.

Prior exposure to elevated CO₂ did not significantly alter the intercepts or slopes of the correlation lines in sweetgum, but the intercepts and slopes of the correlation for red maple

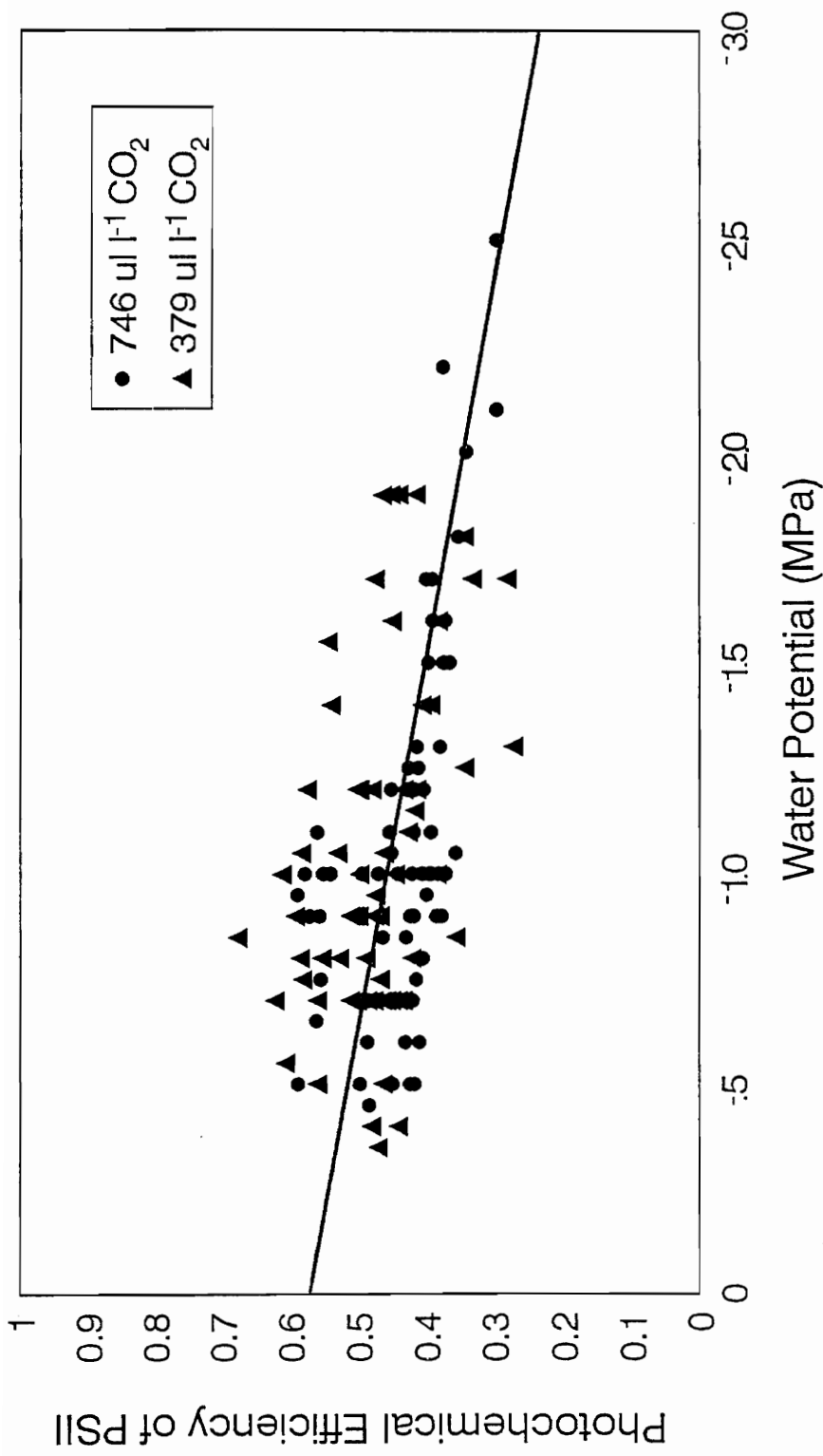


Figure 4.5: Photochemical efficiency of PSII (unitless) for 12-month-old loblolly pine seedlings grown in 379 or 746 $\text{ul l}^{-1} \text{CO}_2$ as influenced by decreasing water potential (MPa).

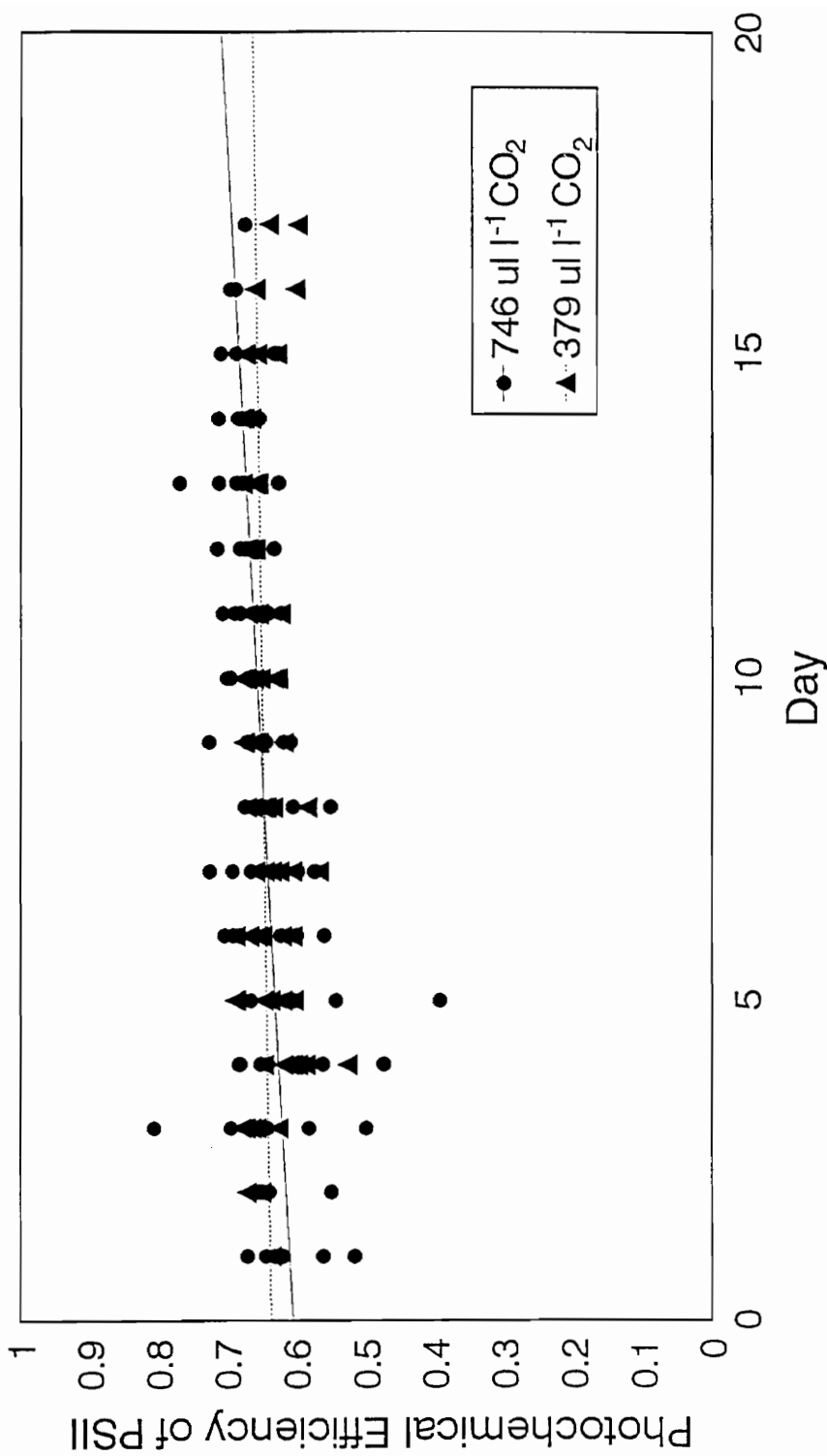


Figure 4.6: Photochemical efficiency of PSII (unitless) for 5-month-old sweetgum seedlings grown in 379 or 746 $\text{ul l}^{-1} \text{CO}_2$ as influenced by increasing time (Day).

Table 4.3: Coefficient of determination values (r^2), slopes, intercepts, and p-values describing the relationship between photosynthesis (Independent Variable) and photochemical efficiency of PSII (Dependent Variable) for loblolly pine, sweetgum, and red maple.

Treatment ($\mu\text{l l}^{-1} \text{CO}_2$)	Intercept (unitless)	Slope	r^2	p-value
<u>Loblolly pine</u>				
746	0.4192(0.17) ¹	0.0214(0.44)	0.05	0.0435
379	0.4442	0.0330	0.13	0.0033
<u>Sweetgum</u>				
746	0.6282(0.26) ¹	0.0048(0.26)	0.04	0.0723
379	0.6436	0.0011	0.005	0.4981
<u>Red Maple</u>				
746	0.4020(0.01) ¹	0.0504(0.01)	0.29	0.0001
379	0.5270	0.0067	0.05	0.0446

¹ p-value for test of significance between treatments

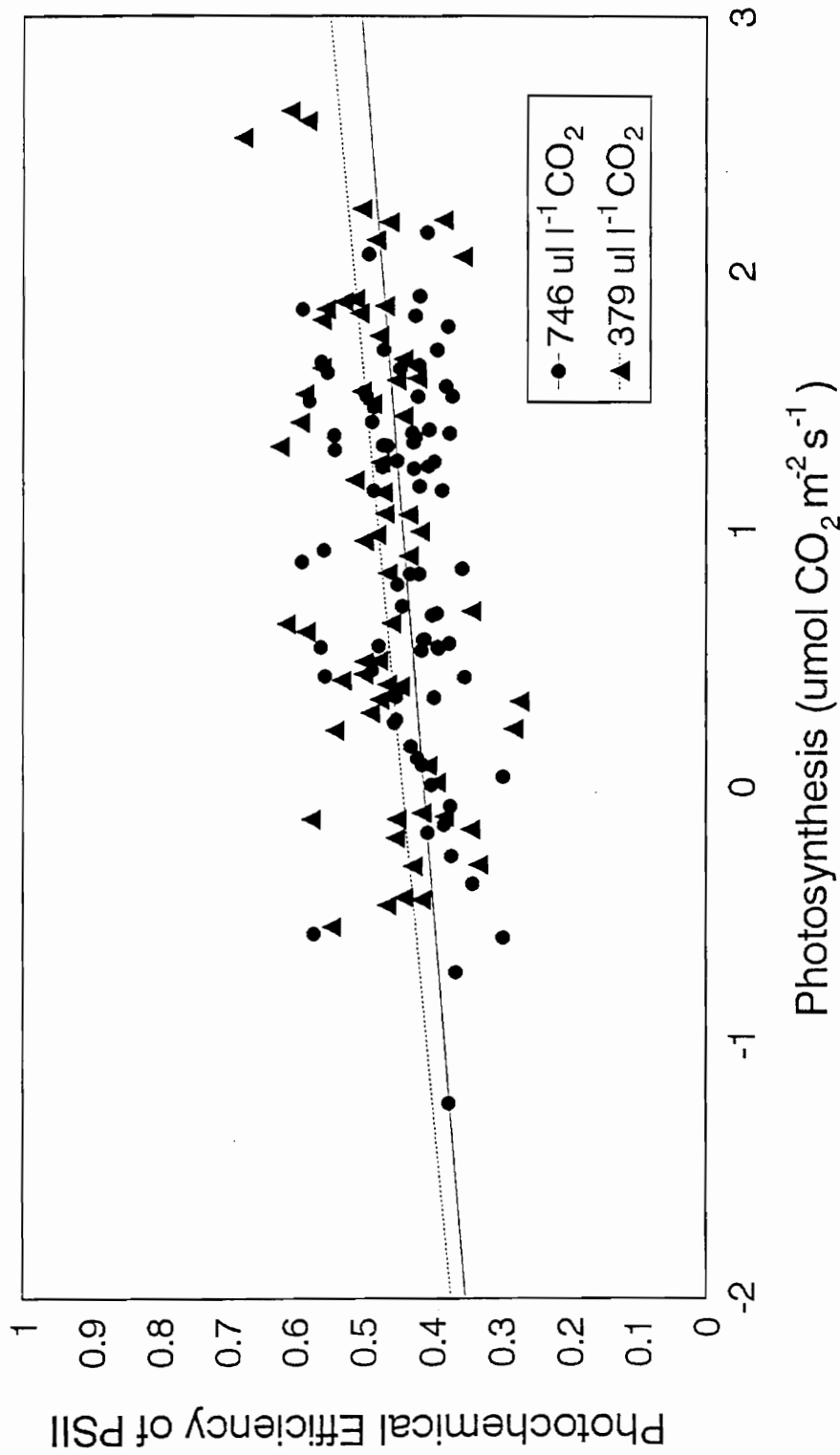


Figure 4.7: Photochemical efficiency of PSII (unitless) for 12-month-old loblolly pine seedlings grown in 379 or 746 $\mu\text{l l}^{-1} \text{ CO}_2$ correlated with increasing photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).

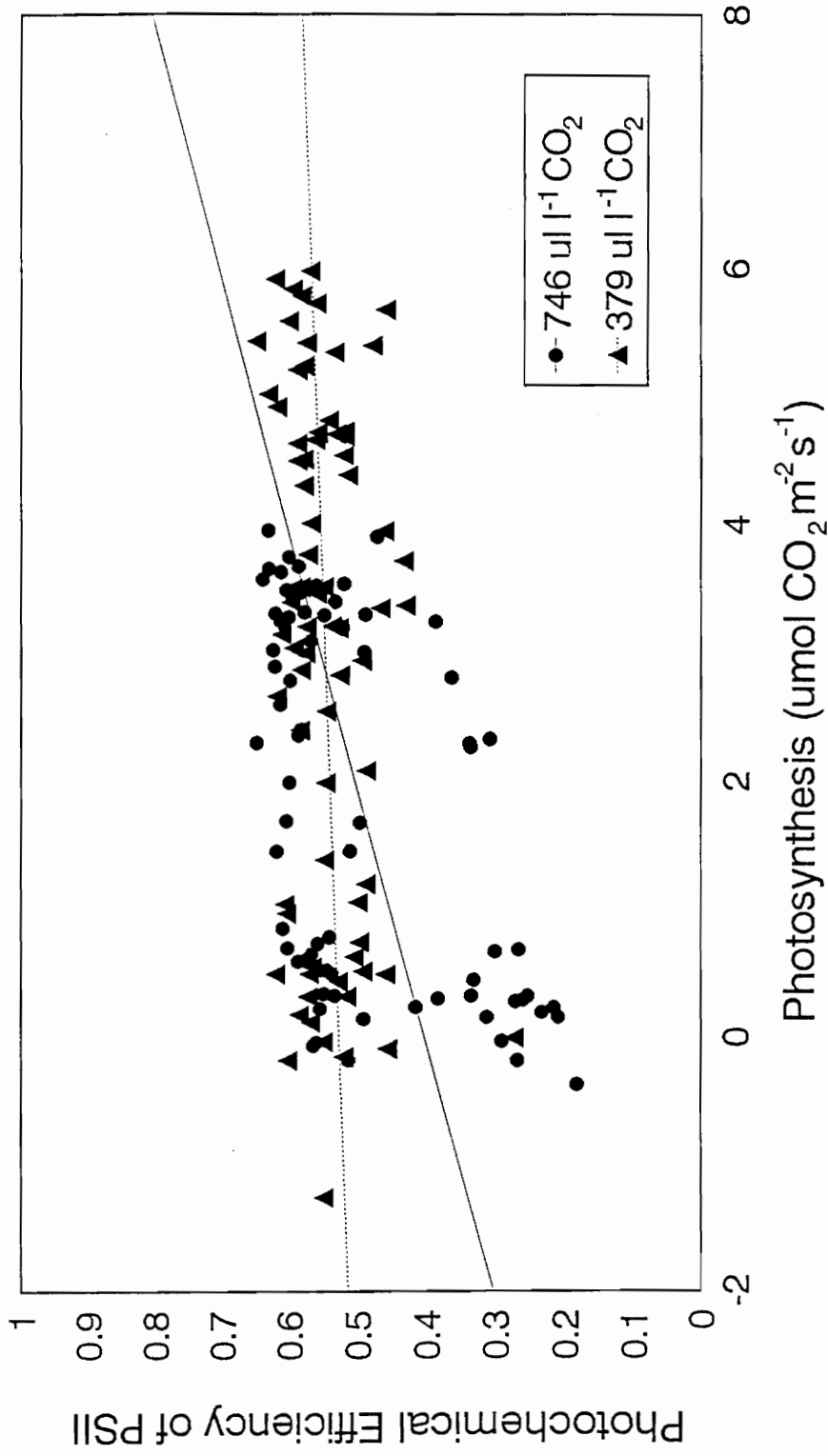


Figure 4.8: Photochemical efficiency of PSII for 4-month-old red maple seedlings grown in 379 or 746 $\mu\text{l l}^{-1} \text{ CO}_2$ correlated with increasing photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).

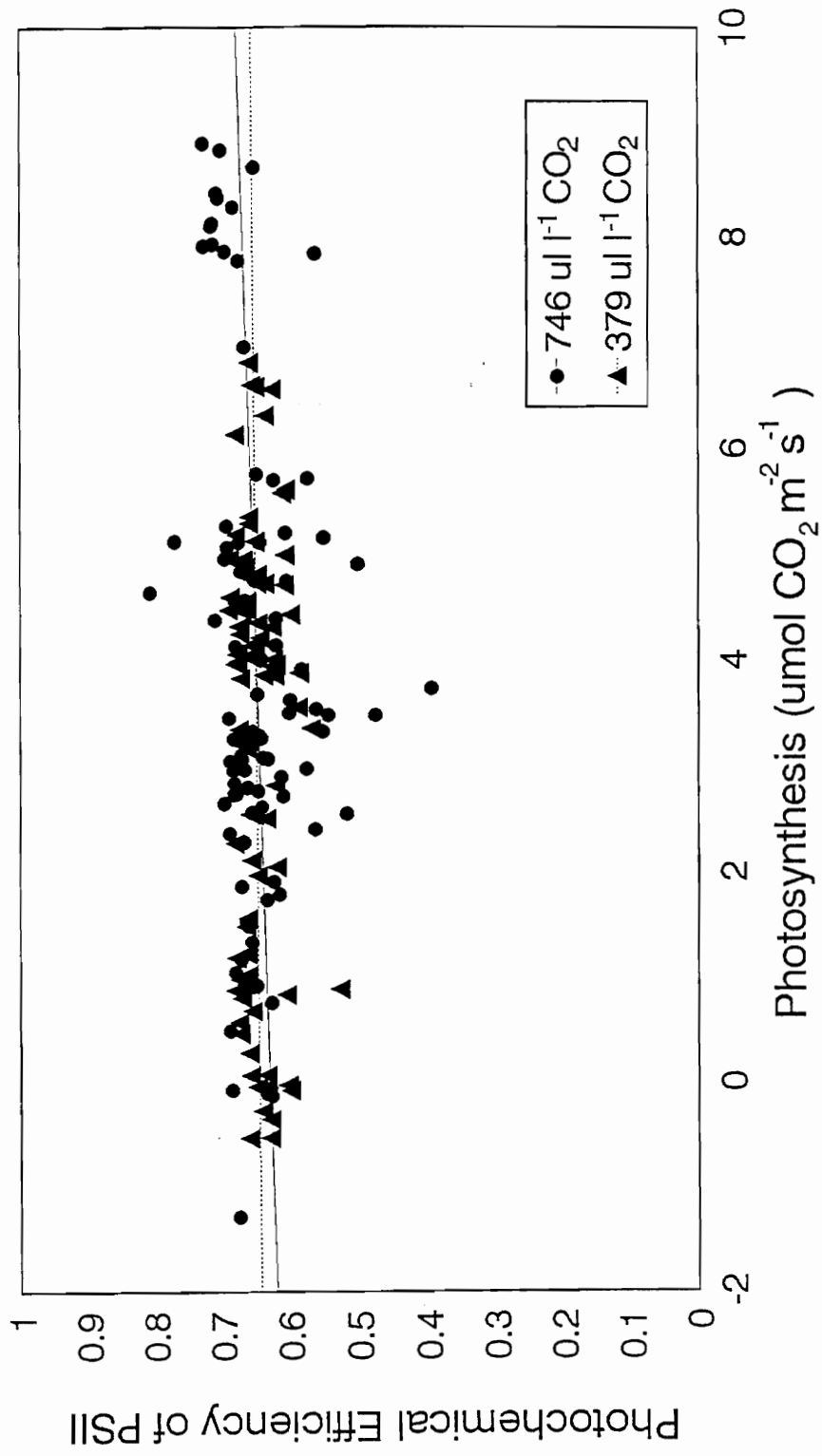


Figure 4.9: Photochemical efficiency of PSII (unitless) for 5-month-old sweetgum seedlings grown in 379 or 746 $\mu\text{l l}^{-1} \text{ CO}_2$ correlated with increasing photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).

grown in elevated CO₂ were significantly lowered and increased, respectively (Table 4.3). The intercept for loblolly pine was slightly lower (Table 4.3). However, with the exception of the r² for the red maple grown in elevated CO₂, the low r² values make it impossible to state with confidence whether the two parameters are truly correlated and if growth in elevated CO₂ will influence the relationship between photosynthesis and photochemical efficiency of PSII.

CONCLUSION

Photosynthesis declined with decreasing water potential and increasing time for loblolly pine, sweetgum, and red maple. Photosynthesis, measured at ambient CO₂, for all three species grown in elevated CO₂ was maintained longer under water stress. This suggests that growth in elevated CO₂ may increase the drought tolerance of loblolly pine, sweetgum, and red maple. Photochemical efficiency of PSII declined in loblolly pine and remained unchanged in red maple. The photochemical efficiency of PSII was slightly increased for sweetgum seedlings grown in elevated CO₂, which suggests that they will become more efficient at harvesting light energy, which may enhance their competitive ability.

Although weakly related, a positive correlation between photosynthesis and photochemical efficiency of PSII was found at both CO₂ concentrations for loblolly pine and red maple but only at elevated CO₂ for sweetgum, suggesting that the decline in photosynthesis is partly due to the decline in the photochemical efficiency of PSII. Growth in elevated CO₂ did not greatly alter this relationship in loblolly pine, but in red maple the relationship was increased.

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Summary and Conclusions

The overall objective of this research was to determine how future changes in atmospheric CO₂ concentrations may influence light responses and physiological responses under water stress of loblolly pine, sweetgum, and red maple. Also, how these changes may impact the competitive relationships of these three species. Seedlings of the three species were grown in an ambient (379 $\mu\text{l l}^{-1}$) or an elevated (746 $\mu\text{l l}^{-1}$) CO₂ chamber for 1 year (loblolly pine), 5 months (sweetgum), and 4 months (red maple). The first experiment involved constructing light response curves for the three species. No changes were found in the quantum yields, light-compensation points, or maximum photosynthetic rates of the three species except that the maximum photosynthetic rate for red maple grown in elevated CO₂ was significantly lower. This suggests that the competitive relationship of loblolly pine and sweetgum will not be influenced by changes in light use efficiency in a future CO₂ environment. The decrease in the maximum photosynthetic rate of red maple suggests that the initial increase in growth due to the increase in atmospheric CO₂ may not be maintained over time and therefore, red maple may lose some of its competitiveness to loblolly pine.

The second experiment involved studying the influence of water stress on gas exchange and chlorophyll fluorescence of the three species and to determine if the two parameters are correlated. Photosynthesis was lowered by water stress in loblolly pine, sweetgum, and red maple; However, this effect was mitigated by growth in elevated CO₂ suggesting that increased atmospheric CO₂ may increase the drought tolerance of all three species. This may change the competitive relationships of the three species on dry sites but may also increase seedling survival and growth of loblolly pine on poorer sites. Photochemical efficiency of PSII declined as a result of water stress in both treatments of loblolly pine

and, although weakly related, increased for sweetgum seedlings. This suggests that sweetgum may become more efficient at harvesting light energy in a future increased CO₂ atmosphere which may give it a competitive advantage over loblolly pine. A positive correlation between the two parameters was found at both CO₂ concentrations for loblolly pine and red maple but only at elevated CO₂ for sweetgum implying that the observed decline in photosynthesis is partly due to a decline in the photochemical efficiency of PSII. Growth in elevated CO₂ greatly increased this relationship in red maple.

From this research, it appears that these species will be influenced by an increase in the atmospheric CO₂ concentration which is expected to occur. Their competitive relationships could change both positively or negatively and this research provides some insight into explaining these possible changes.

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Vita

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A handwritten signature in black ink that reads "Philip Jason Lenham". The signature is written in a cursive style with a large initial 'P' and 'L'.