RESPONSE OF GROWTH AND GAS EXCHANGE OF TULIP POPLAR (LIRIODENDRON TULIPIFERA L.) SEEDLINGS TO OZONE EXPOSURE

A Thesis

by

SONGQIAO HUANG

Submitted to the Graduate School

Appalachian State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 1992

Major Department: Biology

RESPONSE OF GROWTH AND GAS EXCHANGE OF TULIP POPLAR (LIRIODENDRON TULIPIFERA L.) SEEDLINGS TO OZONE EXPOSURE

A Thesis

by

SONGQIAO HUANG

August 1992

APPROVED BY:

Howard S. Neufeld
Howard S. Neufeld
Chairperson, Thesis Committee

Kelly P. Steele
Kelly P. Steele
Member, Thesis Committee

Gary L. Walker
Gary, L. Walker
Member, Thesis Committee

Jeffrey A. Butts
Chairperson, Department of Biology

Joyce V. Lawrence
Joyce V. Lawrence
Dean of Graduate Studies and Research

ABSTRACT

RESPONSE OF GROWTH AND GAS EXCHANGE OF TULIP POPLAR (LIRIODENDRON TULIPIFERA L.) SEEDLINGS TO OZONE EXPOSURE

(August 1992)

Songqiao Huang, B.S., Beijing Teachers' College

M.S., Appalachian State University

Thesis Chairperson: Howard S. Neufeld

Tulip poplar (*Liriodendron tulipifera* L.) seedlings were exposed to a gradient of ozone for two seasons in open-top chambers at the Uplands Field Research Lab, Great Smoky Mountains National Park. There were six ozone treatments: open plot, charcoal-filtered, 0.5 X, 1.0 X, 1.5 X and 2.0 X ambient treatments. The seedlings were exposed to ozone 24 hours per day, seven days per week from June 30 to September 29, 1990 and May 3 to October 8, 1991. The total mean ozone exposure for 1.0 X ambient was 40 ppm-hr in 1990, and 45 ppm-hr in 1991. Significant linear reductions were found for height and diameter as ozone increased during each season. Linear decreases were also found for leaf count and weight, root weight, and total dry weight in 1990. Leaf number, weight, and area were significantly reduced in 1991. There were no significant effects detected for total dry weight after the second season of ozone exposure. This absence of an ozone effect on biomass accumulation in second season trees might have been the result of root binding and its deleterious effects on growth

of larger trees. Diurnal rates of photosynthesis were significantly lower in 2.0 X ambient in September of 1991, due mainly to reduced rates in older leaves. Few treatment effects were found in the light or CO_2 curves, with the exception of photosynthetic capacity and intercellular CO_2 concentration at light saturation, which were reduced by ozone in June of 1991. Later in the season, older leaves in 2.0 X ambient had lower values for photosynthetic capacity, and both quantum and carboxylation efficiencies. Stomatal conductances were not affected by ozone, suggesting that decreases in gas exchange were biochemical in origin, and not diffusional. Results from the gas exchange experiments suggest impaired electron transport and lowered activity of the tricarboxylic acid cycle as the primary causes for reductions in photosynthesis in the older leaves. Reductions in growth appear to result from loss of foliage through early leaf senescence, and lower rates of gas exchange in older leaves.

ACKNOWLEDGEMENTS

I would like to thank Dr. Howard Neufeld, the chair of my committee, who has put a massive amount of time and energy into this project. He provided some original ideas for the thesis and guidance for my research. Many thanks to Drs. Kelly Steele and Gary Walker, my committee members, Dr. W. David Hacker, and Mr. Jim Renfro at the Uplands Field Research Lab for their tremendous help and concern with this project. I also appreciate very much the help offered by the seasonal technicians, in particular, Laura Gilreath, Lisa Stansfield, Paul Durr, Monia Bond, Greg Salansky, Ulrike Boesser, Marie-Teresa Engles, Dan Stratton, Michelle Rymond, Stefan Scherzer, and Jimmy Hecht. Also thanks to my fellow graduate students: James Hutcherson, Mack Miller, Todd Radenbaugh, and Michael Rohall for helping with the harvesting of the plants. Thanks are also extended to Dr. Steven Williams and Dr. Chuck Parker for letting me use their spectrophotometers. I also appreciate the assistance of Dr. E. Henry Lee and Mr. Michael Zelasky from the EPA Lab in Corvallis, Oregon, Ms. Karen Callahan and Dr. Edgar Green for their assistance with statistical analyses.

I would like to thank the Graduate School, and especially Dean Joyce Lawrence, and Dr. Jeffrey Butts of the Department of Biology, for providing financial support. I also sincerely appreciate the encouragement and help that I

received from all faculty members, staff and graduate students in the Biology Department during the past three years.

This research was sponsored by U. S. Environmental Protection Agency (EPA) grant OMB-2000-0066 to Dr. Howard S. Neufeld.

DEDICATION

This paper is dedicated to my father and my mother, Dazhi Huang and Zhiyun Liu, and my American parents, Dr. and Mrs. William Knight, for their love, guidance and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF SYMBOLS

Symbols	Definition
A	Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})
A_a	Photosynthesis at ambient CO_2 concentration (μmol CO_2 m^{-2} s^{-1})
A_{max}	Light saturated rate of photosynthesis (μmol CO_2 m^{-2} s^{-1})
A∘	Photosynthesis at a particular internal CO_2 concentration (μmol CO_2 m^{-2} s^{-1})
a:b	Chlorophyll a to chlorophyll b ratio
C_a	Ambient CO_2 concentration (ppm)
C_i	Internal CO_2 concentration (ppm)
CF	Charcoal-filtered
CV	Coefficient of variation (%)
Chl a	Chlorophyll a (μg/g dry weight)
Chl b	Chlorophyll b (μg/g dry weight)
dA/dC_i	Carboxylation efficiency (mol m^{-2} s^{-1})
E	Transpiration rate (mmol H_2O m^{-2} s^{-1})
g_a	Stomatal conductance to CO_2 (mol CO_2 m^{-2} s^{-1})
g_s	Stomatal conductance to water vapor (mol H_2O m^{-2} s^{-1})
J_{Total}	Total ozone flux (μmol O_3 m^{-2} s^{-1})
$J_{Surface}$	Leaf surface ozone flux (μmol O_3 m^{-2} s^{-1})
$J_{Internal}$	Internal ozone flux (μmol O_3 m^{-2} s^{-1})

J_{max}	CO_2 saturated photosynthetic rate (μmol CO_2 m^{-2} s^{-1})
L	Stomatal limitation (%)
LAR	Leaf area ratio (cm^2/g dry weight)
NO_x	Nitrogen oxides
O_3	Ozone
PAR	Photosynthetically active radiation (μmol photons m^{-2} s^{-1})
PBL	Planetary boundary layer
Q	Photosynthetically active radiation ((μmol photons m^{-2} s^{-1})
ϕ	Apparent quantum yield (μmol photons/μmol CO_2)
SLM	Specific leaf mass (mg/cm^2)
SO_2	Sulfur dioxide
TCA cycle	Tricarboxylic Acid Cycle
TotChl	Total chlorophyll content (μg/g dry weight)
UV	Ultraviolet

INTRODUCTION

The impact of air pollutants on forest ecosystems has been observed for over 100 years (Krause, 1988b). Of the several air pollutants common to various regions of the USA, ozone (O_3) is believed to have the most adverse impact and therefore has received increasing recognition as an ecological problem. During the past 30 years, hundreds of reports have been published on the effects of ozone on vegetation. Ozone is known to reduce crop yields and has been implicated as a contributing stress factor in the forest declines in North America and Europe (McLaughlin, 1985; Krause et al., 1986).

Ozone not only causes foliar injury, but also may affect growth and biomass partitioning in plants without inducing visible injury. Exposure of trees and crops to ambient levels of ozone often reduces net photosynthesis (Reich and Amundson, 1985) and increases the rate of respiration (Barnes, 1972). In addition to inhibiting carbon assimilation and plant growth, ozone stress alters carbon partitioning among structures, often reducing the root:shoot ratio (Hogsett et al., 1985; Chappelka and Chevone, 1986; Winner and Atkinson, 1986; Pye, 1988).

Despite the studies that have been done on tree seedlings, it is still difficult to develop a growth model which includes ozone as an explicit parameter, partly because such investigations have not employed comparable methodologies.

Ozone exposure methodologies, ozone exposure profiles, ozone doses, exposure durations, and plant growing conditions vary considerable among individual studies (Reich, 1987; Pye, 1988). The purpose of this study was to investigate the dynamic responses of tulip poplar (*Liriodendron tulipifera* L.) seedlings to ozone in the southern Appalachians and to provide data for the U.S. Environmental Protection Agency (EPA) to develop a growth model that includes ozone as an explicit parameter.

Gas Exchange

Gas exchange is the process by which plants emit and absorb gases. Most gas exchange takes place in the foliage and involves the uptake and release of CO_2, H_2O and O_2 which are involved in photosynthesis, transpiration and respiration. Air pollutants such as O_3 and SO_2 enter plants through the open stomata, and therefore stomatal conductance plays an important role in air pollutant uptake and subsequent plant responses.

Changes in photosynthesis are very important indicators of pollutant stress because of the central role of photosynthesis in energy capture, and ultimately, in plant growth (Coyne and Bingham, 1982). Decreased rates of biomass production in trees and crops from exposure to elevated levels of ozone have been related to decreased rates of net photosynthesis (Reich and Amundson, 1985). These reductions in net photosynthetic rates are probably due to decreased stomatal conductance (Reich, 1987), oxidant damage to the

biochemical processes of light harvesting and dark reactions (Richardson et al., 1990), increased respiration rate and accelerated leaf aging (Reich, 1983).

Diagnostic Gas Exchange

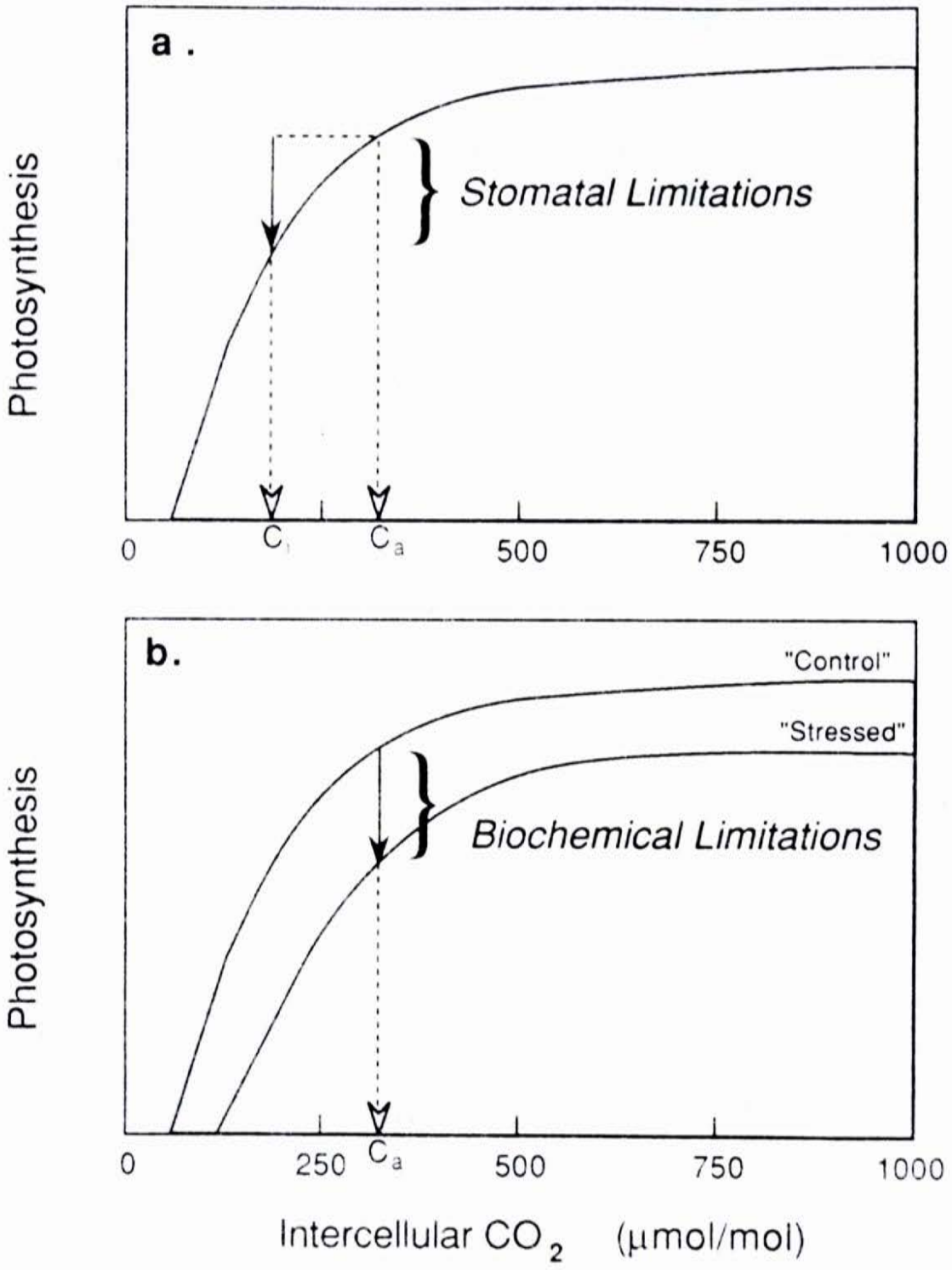
Short-term or instantaneous measurements of photosynthesis and transpiration are commonly used as indicators of the physiological status of plants. Data obtained from these measurements are useful for comparing physiological differences among trees or branches but they do not provide information for understanding the mechanisms that control photosynthetic capacity and water flux, especially under pollution stress (Richardson et al., 1990).

Diagnostic gas exchange analysis addresses the problems of determining the interactions between biological and environmental factors and can be used to establish cause and effect relationships (Richardson et al., 1990). Light and CO_2 response relationships provide clues to biochemical changes in the photosynthetic processes of plants resulting from stress. In addition, the relative importance of stomatal and biochemical limitations to photosynthesis can be estimated (Fig. 1) (Richardson et al., 1990).

Light Response Curves

Light response curves, which describe the relationship between irradiance and photosynthesis, can be used to determine the effects of ozone on

Figure 1. Theoretical response of photosynthesis to intercellular CO_2 concentration. (a) Stomatal limitations to photosynthesis are calculated from the ambient CO_2 concentration. (b) Biochemical limitations to photosynthesis are calculated as the difference between the curves at a specific CO_2 concentration, such as ambient CO_2. From Richardson et al. (1990). Used with permission of American Society for Testing and Materials.


a .
Stomatal Limitations
Photosynthesis
0
C_i
C_a
500
750
1000
b.
"Control"
"Stressed"
Biochemical Limitations
Photosynthesis
0
250
C_a
500
750
1000
Intercellular CO_2 (µmol/mol)

photosynthetic capacity and photochemical efficiency (Richardson et al., 1990). The response of photosynthetic rate (**A**) to photon flux (**Q**) describes a curve which contains two phases: an initial linear phase of increase in **A** with **Q** through the light compensation point, and a progressive decrease in the slope of the curve with the increase in **Q** to a plateau, the light saturated assimilation rate ($\mathbf{A_{max}}$). It is known that at low irradiances, net photosynthetic rate is linearly dependent on **Q** since the speed of the photochemical reactions is the limiting factor. The apparent quantum yield (ø) is the initial slope of this linear portion of the light response curve (Long and Hällgren, 1985) and represents the efficiency of light utilization by photosynthesis. As a result, any changes in the apparent quantum yield of photosynthesis are correlated to changes in the light harvesting system and to the efficiency of the light reactions. At saturation irradiances, **A** is limited by the rate of enzymatic reactions and the CO_2 supply and $\mathbf{A_{max}}$ is called the photosynthetic capacity. Therefore changes in $\mathbf{A_{max}}$ due to stress can be correlated to these biochemical processes. ø and $\mathbf{A_{max}}$ depend on long term adaptation to environmental conditions such as light ("sun" and "shade") and water. Environmental stressors can change the quantum yield and photosynthetic capacity. For example, damage or inactivation of chlorophyll or accessory pigments may reduce light capture (Coulson and Heath, 1974).

Carbon Dioxide Response Curves

Carbon dioxide response curves illustrate the response of assimilation rate to

ambient CO_2 (C_a) or internal CO_2 (C_i). At low C_i, **A** is determined by the kinetics of ribulose bisphosphate-1,5 carboxylase/oxygenase (RUBISCO) since the substrate, RuBP, is present in saturating amounts. The initial slope of the A/C_i curve, which is termed the carboxylation efficiency (dA/dC_i), is initially linearly dependent on C_i concentration but affected by irradiance. At high C_i concentrations, when the curve begins to plateau (dA/dC_i approaches zero), the rate of RuBP regeneration becomes limiting. Regeneration of RuBP depends on the production of ATP and NADPH, and is therefore dependent on the photochemical reactions. At CO_2 saturation, **A** is dependent primarily on irradiance and temperature, rather than C_i concentration. The saturated rate is also called the RuBP regeneration rate (J_{max}). By comparing A/C_i curves from plants exposed to different ozone treatments, it is possible to determine if ozone induces biochemical effects on photosynthetic processes. But because it is known that ozone and other environmental factors can affect stomatal behavior, it is important to consider differences in stomatal limitations among treatments before calculating biochemical limitations due to air pollutants. CO_2 curves can be used (1) as an alternative method of separating stomatal and mesophyll conductance limitations, and, (2) to separate *in vivo* carboxylation from photochemical limitations.

According to Farquhar and Sharkey (1982), stomatal and mesophyll limitations can be analyzed by using CO_2 response curves. C_i can be estimated from C_a, **A**, and the stomatal conductance to CO_2 (g_a). g_a is calculated from the

simultaneous measurement of transpiration (**E**) and photosynthesis. Some of the CO_2 diffusing into the leaf is carried back out by the evaporation of water, which is partly a mass flow process. The equation developed by von Caemmerer and Farquhar (1981) relates photosynthesis to the CO_2 gradient and the mass flow loss of CO_2:

$$A = g_a(C_a - C_i) - \frac{E(C_i - C_a)}{2} \qquad (1)$$

This equation can be rearranged to calculate C_i:

$$C_i = \frac{(g_a - \frac{E}{2})\,C_a - A}{g_a + \frac{E}{2}} \qquad (2)$$

(1) Stomatal Limitations to Photosynthesis

If g_a is assumed to be infinite, C_i would be equal to C_a. As a result, the difference between the potential rate of photosynthesis at C_a and the actual rate of photosynthesis at C_i is due to stomatal limitations (L) (Fig. 1). The relative limitations which the stomata impose, may then be calculated as a percentage by using equation (3):

$$L = \frac{(A_a - A_o) * 100}{A_a} \qquad (3)$$

where A_o is photosynthesis at C_i equal to C_a and A_a is photosynthesis at C_a.

(2) Biochemical Limitations

According to Farquhar et al. (1980), when C_i approaches zero, the initial slope of the curve relating carboxylation efficiency to C_i is:

$$dA/dc_i = v_{RUBISCO} \quad (4)$$

where $v_{RUBISCO}$ is the velocity of RuBP carboxylation (mol m^{-2} s^{-1}).

When C_i is raised to the point where the photosynthetic rate is a maximum, then:

$$A = J_{max} \quad (5)$$

where J_{max} = maximum rate of RuBP regeneration, which is assumed to equal the maximum rate of coupled photosynthetic electron transport.

If CO_2 curves differ for control and stressed plants, the differences can be compared by calculating biochemical limitations at a common CO_2 concentration, for example, ambient CO_2 conditions (Fig. 1) (Richardson et al., 1990).

Leaf Growth

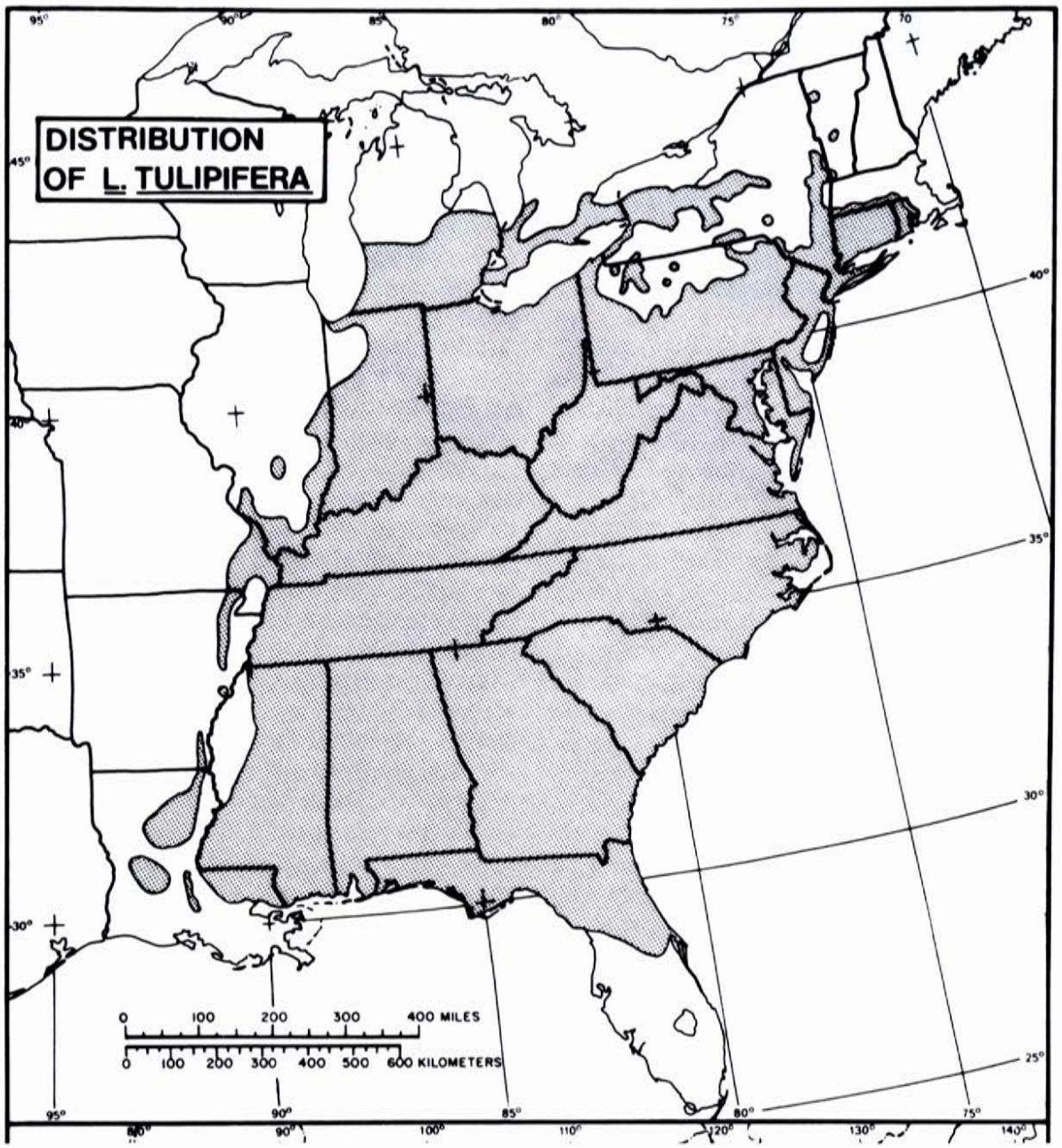
In most actively growing plants, growth is the product of net assimilation rates and leaf area (Jackson, 1963; Potter and Jones, 1977). Therefore, leaf area is a very important growth parameter (Jensen, 1985). Total plant leaf area depends on the following components (1) leaf production rate (number of leaves produced per unit time); (2) maximum leaf area per leaf; and (3) leaf area duration, a measure of the persistence of the assimilatory surfaces. Previous studies of poplar (*Populus tremuloides* Michx.) indicated that the rate of leaf

initiation and leaf expansion were directly proportional to productivity (Ridge et al., 1986). Investigations of the effects of chronic air pollution stress on white pine trees (*Pinus strobus* L.) and ponderosa pine (*Pinus ponderosa*) concluded that one of the important factors causing a decline in the vigor of sensitive trees was a reduction in needle longevity (Coyne and Bingham, 1982; McLaughlin et al. 1982; Reich, 1983). Therefore, components of leaf area may be very important criteria for evaluating effects of ozone on plant growth.

Tulip poplar (*Liriodendron tulipifera* L.) ranges from the Canadian border in the north to the Gulf of Mexico in the south, and from the east coast to the midwest (Fig. 2) (Parks and Wendel, 1990). It is one of the most important deciduous trees in the southeast and shows some evidence of ozone sensitivity (Chappelka et al., 1988). Krause (1988a) stated that this species is very sensitive to ozone. Visible injury, expressed as dark stippling of leaves, has been observed by several researchers (Skelly et al., 1982; Renfro, 1989) and also in field surveys in the Great Smoky Mountains National Park (Neufeld et al., 1992). However, there are conflicting reports about the sensitivity of this species to ozone in terms of biomass and growth (Pye, 1988). In addition, previous studies have been short-term, and therefore, the responses of tulip poplar to long-term ozone exposure and to a gradient of ozone levels is still poorly understood.

The specific objectives of this study were to characterize the effects of ozone on (1) height, diameter, and leaf growth responses of tulip poplar tree seedlings over the course of two growing seasons, (2) to evaluate the alteration in biomass

Figure 2. Geographic distribution of tulip poplar. From Parks and Wendel (1990). Used with permission of Botanical Society of America.


DISTRIBUTION
OF L. TULIPIFERA
0 100 200 300 400 MILES
0 100 200 300 400 500 600 KILOMETERS

accumulation and allocation under long-term ozone exposure conditions, and (3) to determine the effects of ozone on gas exchange responses of tulip poplar seedlings.

REVIEW OF THE LITERATURE

Ozone in the Troposphere

Ozone (O_3) is present both in the stratosphere and troposphere. The stratosphere is located approximately between altitudes of 10 and 50 km where temperature increases with altitude (Krupa and Manning, 1988). This region contains 90% of the vertical ozone column above the earth's surface. Since ozone strongly absorbs solar radiation between wavelengths of approximately 210 nm to 290 nm, the stratospheric ozone layer serves as a shield against biologically harmful solar ultraviolet (UV) radiation.

The troposphere extends outward from 7 to 10 km above the earth's surface. Tropospheric ozone is a secondary air pollutant formed under the influence of UV-light by photochemical reactions. These photochemical reactions involve the oxidation of hydrocarbons and carbon monoxide in the presence of nitrogen oxides (NO_x) and sunlight (Seinfeld, 1989). The tropospheric ozone concentration across the earth's surface is controlled by natural processes and by man's influence. According to Altshuller (1986), surface ozone concentrations measured at rural locations are influenced by several different mechanisms. These include: (a) transport of ozone formed in the stratosphere into the free troposphere and subsequent transport down into the planetary boundary layer (PBL); (b) photochemical ozone formation within the free troposphere and the

clean PBL; (c) photochemical ozone formation within the polluted PBL, especially during the passage of warm high-pressure systems; and (d) ozone formation within single or superimposed plumes. In regions directly influenced by anthropogenic emissions, the relatively high levels of NO_x catalyze ozone photochemical generation from the oxidation of man made and natural hydrocarbons. This mechanism can cause frequent air pollution episodes in the summer (Chameides and Lodge, 1992).

It is known that ozone concentrations are on the rise throughout large portions of the troposphere (Volz and Kley, 1988). Background concentrations of ozone observed in a number of places worldwide show average daily one hour maxima of about 0.02-0.06 ppm and long-term data show a yearly cycle with a maximum in the summer months (Krupa and Manning, 1988).

Tropospheric ozone occurs at harmful concentrations over wide areas of Europe and the United States, and is considered as the most important phytotoxic air pollutant in North America (Krause, 1988b; Krupa and Manning, 1988). Periodic high concentrations of ozone have been found to injure foliage of eastern white pine trees in various locations within its natural range in the northern and eastern United States, such as the ridges and valleys of the Appalachians (Duchelle et al., 1982). High concentrations of ozone near Los Angeles, California regularly injure 10% to 20% of the trees growing in the nearby San Bernardino Mountains (Miller, 1983).

Effects of Ozone on Trees

The effects of ozone on trees are diverse, ranging from overt injury such as leaf chlorosis and necrosis (Sakaki et al., 1983), to subtle modifications of cellular biochemistry (increased membrane permeability, lowered enzyme activities) and modifications of whole-plant physiology, such as photosynthesis and respiration (Heath, 1980; Tingey and Taylor, 1982). If the ozone dose is high enough to overcome the plant protective or repair mechanisms, it will eventually cause tissue death, followed by reduced plant growth and productivity.

Ozone Uptake and Stomatal Conductance

All green plants have the capacity to absorb and emit gases. During this gas exchange process, ozone and other gaseous pollutants which may be present in the ambient air will be absorbed by the foliage and moved into the plant tissue. The movement into the interior tissue is the essential prerequisite for most of the biochemical and physiological effects of exposure to ozone. Without access to the internal sites of reaction, gaseous pollutants such as O_3 and SO_2 may not do much harm to plants. As a result, uptake plays a very important role in the responses of plants to ozone.

(1) Ozone Uptake

Heath (1980) has suggested that O_3 travels with apoplastic water flow to the stomatal epidermal region. Tingey and Taylor (1982) indicated that ozone flux

(J) into the leaf results from a chemical potential gradient between the bulk air and the leaf interior. Flux is proportional to the pathway conductance and is inversely related to the resistance to mass transfer as ozone diffuses through the boundary layer, stomatal and intercellular spaces and into the cytoplasm. The relationship between stomatal regulation and ozone flux is analogous to Ohm's Law (Tingey and Taylor, 1982). Total ozone flux (J_{Total}) consists of leaf-surface ($J_{Surface}$) and internal ($J_{Internal}$) fractions. $J_{Internal}$ results in injury to plants.

(2) Ozone Uptake and Stomatal Conductance

Many studies have focused on the relationship between gas-phase conductances and foliar injury, and most have concluded that stomatal conductance is the principal or sole factor underlying response differences (Olszyk and Tibbitts, 1981a; Tingey and Taylor, 1982). Stomata play a major role in controlling air pollution injury to plants. When the stomata are open, pollutants diffuse rapidly into leaf tissue and cause injury; when stomata are closed, entry is prevented and no injury occurs unless pollution levels are high (Mansfield, 1973; Temple, 1986).

Hill and Littlefield (1969) measured stomatal aperture of oats and other species during fumigation. They found that O_3 treatment closed over half of the stomata by the end of the experiment, while all stomata were open in the control chamber. The closure of stomata by O_3 appeared to protect the plants from ozone injury. Similar results have been found by other workers (Barnes, 1972;

Coyne and Bingham, 1981; Olszyk and Tibbitts, 1981a, b; Tingey and Taylor, 1982; Temple, 1986), although Evans and Ting (1974) reported that stomatal opening in response to ozone did not show any relationship between gas-phase conductance and ozone injury.

It is not clear whether ozone directly causes stomatal closure or whether closure is a secondary effect of increased C_i which results from decreased net photosynthesis or increased respiration (Reich and Amundson, 1985). It is known that stomatal conductance may be affected by plant age, leaf morphology, species ozone sensitivity, stomatal placement and morphology, and environmental factors such as relative humidity and nutrients. Therefore, any factors including physiological or environmental factors that can affect stomatal conductance will contribute to the sensitivity of plants to ozone. Stomatal conductance measurements alone may be misleading when used to investigate mechanisms of ozone resistance (Tingey and Taylor, 1982).

Effect of Ozone on Foliage

Ozone can cause visible symptoms on sensitive tree species. These visible symptoms are generally classified as either acute or chronic responses (Skelly et al., 1987). Acute injury normally involves the death of cells and is expressed as stippling, flecking, bleaching and bifacial necrosis within a few hours or days following ozone exposure. Acute injury is usually associated with relatively high ozone concentrations from a few to several hours on one or more days. Chronic

injury develops more slowly, over days or weeks, in response to long-term prolonged low concentrations of ozone. The symptoms are expressed as chlorosis, stippling, premature senescence, and necrosis. However, both types of foliage injury may occur in response to either high or low concentration exposures, depending on environmental, genetic and physiological conditions. The most common symptoms for broadleaf plants are stippling or pigmentation while on conifer species, the two most common needle symptoms are chlorotic mottle and tip burn (Skelly et al., 1987; Krupa and Manning, 1988).

In addition to causing visible injury on foliage, ozone reduces leaf longevity in many crop and tree species (Winner and Atkinson, 1986). It is believed that ozone impacts on foliage duration are the dominant factors governing its effects on growth of several species. McLaughlin et al. (1982) investigated the effects of chronic air pollution on white pine (*Pinus strobus* L.) in Oak Ridge, Tennessee and concluded that reduction in needle longevity was one of the major factors that resulted in the declining vigor of sensitive trees in this area. Reich and Amundson (1985) proposed that ozone-accelerated leaf aging appeared to account, at least partially, for the way that ozone affects hybrid poplar (*Populus deltoides* x *trichocarpa*). Allen et al. (1991) found that ozone caused premature foliage loss and indicated that ozone impacts on foliage duration appeared to be the most important factors affecting growth of loblolly pine (*Pinus taeda* L.) trees.

Effects of Ozone on Plant Growth and Yield

Plant growth, which is an irreversible gain in weight or size, is a result of the integration and coordination of the processes of photosynthesis, respiration, translocation, and biosynthesis. It is controlled by the plant's genetic makeup and modified by environmental conditions. It has been difficult to determine whether ozone significantly affects tree growth and yield in the field. Benoit et al. (1982) examined the effect of oxidant air pollution on the radial growth of native eastern white pine in the Blue Ridge Mountains of Virginia. Trees were placed into three ozone sensitivity classes (tolerant, intermediate, and sensitive) based on a foliar rating scale. Reduced radial growth was found for trees in the sensitive class during the period 1955 to 1978.

Miller (1977) compared the radial growth of ponderosa pine in air with relatively low air pollution levels (1910 to 1940) to that in air with high pollution levels (1941 to 1971) in the San Bernardino National Forest in southern California. He found that the average annual radial growth was less for trees growing in highly polluted air than for those growing in less polluted air. This growth loss was attributed primarily to ozone.

In recent years, many studies have been conducted using open-top chambers (Krupa and Manning, 1988; Chappelka and Chevone, 1992). Long-term studies (2 to 3 years) have shown reduced tree growth in ambient air chambers as compared to trees grown in charcoal-filtered air (Duchelle et al. 1982; Chevone et al., 1983; Wang et al., 1986). Studies on loblolly pine seedlings exposed to

ambient and above ambient concentrations of ozone in open-top chambers have found that seedlings exposed to the highest ozone concentrations (about 0.096 ppm) exhibited reduced growth (Shafer et al., 1987; Kress et al., 1988; Edwards et al., 1990).

Studies have been conducted on biomass allocation growth responses to different parts of the plant, including stems (Pye, 1988), all aboveground parts (Jensen, 1983; Reich et al., 1984), and both above and belowground portions (Kress and Skelly, 1982; Mahoney et al., 1984; Chappelka and Chevone, 1986). It has been found that aboveground growth is usually less affected than belowground growth. This is because when photosynthesis is reduced by environmental stresses, less of the carbohydrate pool is allocated to the roots than to growing shoots (Kozlowski et al., 1991).

Pye (1988) summarized the results of 15 studies on 42 species or hybrids and concluded that responses to ozone ranged from a growth reduction of 69% in sycamore (*Platanus occidentalis* L.) to a growth stimulation of 41% in tulip poplar. The stimulation in tulip poplar was unusual, and not easily explainable. More significantly though, out of the 42 species cited, 28 exhibited significant reductions in growth.

The ozone concentration range of 0.04 to 0.06 ppm is representative of the seasonal means prevalent in many forested regions in the eastern United States. Within this range, Pye (1988) found that of the 22 studies that reported at least nominal growth reductions, only five proved statistically different from controls,

and all of them occurred with poplar (*Populus*) or pine (*Pinus*).

Effect of Ozone on Photosynthesis

The negative impact of ozone on tree growth is also due to the inhibitory effect of ozone on photosynthesis (Reich, 1983; Reich and Amundson, 1985; Pye, 1988). Ozone inhibits photosynthesis and increases respiration (Barnes, 1972; Yang et al., 1983a, b) in a variety of conifer and hardwood tree species (Reich, 1987; Krupa and Manning, 1988; Pye, 1988).

Both short-term and long-term studies have investigated photosynthetic responses of tree species as affected by ozone. In some short-term studies, photosynthetic responses to peak ozone concentrations, similar in magnitude to those that occur during a typical diurnal cycle, have been measured. Short-term experiments provide insights into the initial events disrupting the photosynthetic process. Yang et al. (1983a, b) conducted a time-course study characterizing the inhibition by ozone of net photosynthesis in eastern white pine. They found that net photosynthesis declined during the first hour, but remained stable for the remaining three hours of fumigation. The inhibition of net photosynthesis was found to be concentration dependent and became more severe as ozone concentrations increased from 0.1 to 0.3 ppm. This inhibition was relieved within one hour after ozone exposure stopped.

Long-term or seasonal studies are important for the purposes of the determining the cellular metabolic capacity to withstand oxidative stress and

relating alterations in net CO_2 exchange to tree growth (Chappelka and Chevone, 1992). Investigations have been conducted in only a few species (Reich, 1983; Yang et al., 1983b). However, a depression in net photosynthesis was found which became more severe as ozone concentration increased (Yang et al., 1983b) and was more evident in sensitive genotypes than in tolerant genotypes (Coyne and Bingham, 1981). Retzlaff et al. (1991) fumigated nine fruit and nut tree species and found net leaf CO_2 assimilation rate decreased in a linear fashion with increasing 12-hr mean ozone partial pressures.

The light and dark reactions of photosynthesis are also individually affected by ozone. Reich (1983) found that long-term ozone exposure reduced both the incident quantum yield and light saturated rates of photosynthesis in hybrid poplar leaves. Sasek and Richardson (1989) found 21% and 27% reductions respectively, for light- and CO_2-saturated photosynthetic capacities at 2.0 X ambient ozone as compared to charcoal-filtered in loblolly pine. Leaf chlorophyll content decreased with ozone stress and was linearly correlated with the decrease in photosynthesis (Reich et al., 1986; Chappelka and Chevone, 1992). The interaction between leaf age and light response was investigated by Coyne and Bingham (1982). They found that light-saturated gross photosynthetic rates and photochemical conversion efficiencies were highest in current needles and decreased with the increasing needle age and oxidant injury in ponderosa pine.

Many studies suggest that O_3 affects photosynthesis through several mechanisms. Photosynthesis is reduced either by closure of the stomata, which

reduces CO_2 uptake, or by damage to the chloroplasts, or both. It is known that O_3 can cause ultrastructure changes in chloroplasts and reduce RUBISCO activity in both young and old leaves on rice plants (Nakamura and Saka, 1978). The quantity of RUBISCO has been shown to decrease in both symptomatic and asymptomatic alfalfa foliage exposed to ozone (Pell and Pearson, 1983). Some workers have proposed that ozone apparently interferes with chloroplast function more than with stomatal conductance. For example, Coyne and Bingham (1981, 1982) found that losses in photosynthetic capacity of ponderosa pine were greater than reductions in stomatal conductance, indicating more severe injury to mesophyll components of the CO_2 diffusion pathway than to the stomata.

In addition to inhibiting photosynthesis, ozone may increase the dark respiration rate (Barnes, 1972; Skärby et al., 1987; Amthor, 1988; Amthor and Cumming, 1988). Dark respiration is commonly considered in terms of two functional components: growth and maintenance respiration (Amthor, 1984, 1986). Growth respiration is associated with metabolism supplying energy and carbon skeletons for production of new phytomass. Maintenance respiration is associated with processes such as the turnover of labile molecules, the support of ion and metabolite gradients, and the acclimation to changing or stressful environments in existing phytomass. An increase in respiration rate is often observed as a response to ozone (Amthor, 1988), even at low ozone concentrations (Barnes, 1972; Amthor and Cumming, 1988). There are several potential reasons for this. Ozone may cause injury which is at least partially

repairable. An increase in the rate of metabolism to support repair and lesion formation may therefore occur in ozone-stressed tissue (Amthor, 1988). Sutton and Ting (1977) found that repair of O_3-induced membrane injury occurred in leaves of *P. vulgaris*. Such repair is an energy consuming process which may lead to an increased rate of respiration. This increase in respiration for repair (maintenance) results in a diversion of substrates from growth, and hence growth rate will decrease.

Metabolic damage caused by ozone may result in an uncoupling of respiration and/or an increase in engagement of the alternative oxidase as observed during mildew infection (Farrar and Rayns, 1987). Such a response would result in a decrease in respiratory efficiency.

MATERIALS AND METHODS

Plant Material

Tulip poplar seedlings were grown from seeds collected from trees growing at Twin Creeks (elevation 293 m) and Elkmont (elevation 549-763 m) in Great Smoky Mountains National Park (GRSM). These seeds were germinated in flats in sterile germination media in May, 1990 at Sunlight Gardens Inc., in Andersonville, TN. All flats were covered with plastic domes to keep high humidity within the germination chamber and watered with distilled water. Seedlings were later transplanted into 20 cm diameter by 40 cm tall PVC pipes with a screened bottom filled with commercial potting soil mix (5:1, Promix: perlite). The soil level was adjusted to a mean depth of 7 cm from the top of the pot. Seedlings were transported from the greenhouse at Sunlight Gardens to the Uplands Field Research Lab, Great Smoky Mountains National Park, Gatlinburg, TN, and put beneath 50% shade cloth for two weeks prior to placement in the fumigation chambers.

During the two weeks of acclimation, and before fumigation started, initial heights and leaf counts were measured and used to randomly assign the seedlings to different chambers or open plots. At that time, the diameters of the seedlings were less than 3 mm. Four hundred and fifty-six seedlings of similar size were randomly assigned to one of the 15 chambers or three open-plots and three different harvest dates, for a total of 24 plants in each chamber or plot. Plants

were placed in the chambers or plots for ozone exposure on June 29, 1990, at which time they were about six weeks old.

Plants were watered daily in the growing season in 1990 and twice a day later in the summer in 1991. Eighteen grams per pot of 17-6-12 (N-P-K) Osmocote (Sierra Chemical Co., Milpitas, CA) slow release fertilizer were given at the beginning of the season in 1990, and on June 10, 1991. The seedlings also received 15 ppm Mg, Epsom Salts (UPS Grade, $MgSO_4$), and 100 ppm N (18g/plant) from 20-10-20 (N-P-K) Peters fertilizer (Peat-Lite, W. R. Grace & Co., Foglsville, PA) on June 5, 1991 to alleviate general chlorosis. Plants were sprayed to control aphids (*Phyllaphis fagi*) and powdery mildew (*Sphaerotheca fuliginea*) when necessary by using Diazanon (Monsanto, St. Louis MO) and Benlate (E I. DuPont De Nemours, Wilmington, Delaware) respectively.

At the end of the 1990 season, seedlings remaining for exposure in 1991 and 1992 were moved to an area adjacent to the chambers for overwintering. These pots were wrapped with plastics bags and buried with sawdust to avoid cold damage to roots.

Ozone Fumigation

Fifteen open-top chambers (Heagle et al., 1973) were used in the field from June 30 to September 27 in 1990 and from May 3 to October 5 in 1991. Chambers were 3 m in diameter and 2.45 m high, covered with polyvinyl chloride panels. The chambers were placed approximately 3 m from each other on all

sides to avoid self-shading effects. There were six ozone treatments: ambient-no chamber (open plot), charcoal-filtered (CF), 0.5 X, 1.0 X, 1.5 X, and 2.0 X ambient.

Ozone was generated by an OREC electric discharge generator (Ozone Research and Equipment Corp., Phoenix, AZ). Liquid oxygen, instead of ambient air, was used to convert oxygen to ozone to avoid the possibility of producing nitrous oxides. Ozone was dispensed 24 hours per day, seven days per week, to chambers via rotameters under constant flow conditions. A Campbell 21X data logger (Campbell Scientific, Inc., Logan, UT) adjusted the voltage of the ozone generator to vary the amount of ozone dispensed according to the ambient profile.

Ozone concentrations were monitored by three Model 49 UV photometric O_3 analyzers (Thermo Electron Corporation, Franklin, MA). Air was pulled through a manifold system and continuously updated at the monitors. Each chamber was sampled at least five times per hour. Ozone monitors were checked weekly for zero and span, and audited by the State of Tennessee Division of Health and Environment, Air Research Specialists, Inc. (Ft. Collins, CO) and the U.S. EPA. The monitors were always within established quality control and assurance guidelines. Ozone, as well as daily meteorological data such as humidity and wind speed, were stored in the Campbell 21X data logger and dumped to a personal computer twice a day for later analysis.

Growth Analysis

Height and diameter measurements were taken on all plants, every 14 days, beginning two weeks after fumigation. Seedling heights were measured from the cotyledonary node to the base of the terminal bud in 1990 and from the top of the pot to the base of the terminal bud in 1991. Diameters were taken at a point on the stem perpendicular to a label at pot level. Leaf production was determined by counting the numbers of leaves on the main stem on a subsample of five plants per chamber (1992 harvest trees).

At the end of each of the first two seasons, on September 12, 1990 and August 19, 1991, eight randomly selected plants per chamber were harvested for biomass determinations. Leaf, stem, root (tap and secondary), and total dry weight, total leaf area and count, tap and primary root length, root:shoot ratio (total root weight/shoot weight), leaf area ratio (LAR: total leaf area/total dry weight) were determined in 1990. The bole and branch leaf weight, stem and branch weight, tap and secondary root dry weight, aboveground and total dry weight, bole and branch leaf count, bole and branch leaf area, root:shoot, and LAR were determined in 1991. Leaf area was measured by using a Li-Cor Model Li-3100 Area Meter (Li-Cor, Inc., Lincoln, NE). Dry weights were obtained after drying in an oven at 65°C for at least 36 hours, or until samples reached a constant weight.

Chlorophyll Content and Specific Leaf Mass

Chlorophyll content and specific leaf mass (SLM) were measured once at the end of the season in September 17, 1990 and three times in 1991 (May 31, July 20 and September 3) on the most recently expanded leaves on five plants (1992 harvest trees) per chamber. Leaf age effects were also investigated in mid- and late summer in 1991. The first fully matured leaf on each plant formed after fumigation was used as the old leaf age (when it was available) while the most recently matured leaf was used as the new leaf age. Main stem leaves were used most often unless they had abscised, in which case branch leaves with a similar age were used. All leaf samples were collected before noon to minimize diurnal changes in SLM. Leaf samples were obtained by using a hole punch with a sample area of 0.36 cm^2. Two punches per leaf were taken on premarked leaves, one for chlorophyll extraction and the other for SLM.

Chlorophyll was extracted using N,N-dimethylformamide (Moran and Porath, 1980; Moran, 1982). Leaf samples for chlorophyll content measurements were placed in a 20 ml polypropylene scintillation vial containing 6 ml of solution. These vials were covered and placed in a cooler for temporary storage and then transferred to the lab and put into a refrigerator (4°C) for 36 hours to extract the chlorophyll. Absorptions were measured at 664 nm and 647 nm using a Lambda Array 3840 UV/VIS (Perkin-Elmer, Oak Brook, IL 60521), or Spectronic 501 spectrophotometer (Miton Roy Company, Rochester, NY 14625). Chlorophyll contents (μg/ml) were calculated by using the following equations

(Moran, 1982):

$$\text{total chlorophyll (TotChl)} = 7.04 * A_{664} + 20.27 * A_{647} \quad (6)$$

$$\text{chlorophyll a (Chl a)} = 12.64 * A_{664} - 2.99 * A_{647} \quad (7)$$

$$\text{chlorophyll b (chl b)} = -5.60 * A_{664} + 23.26 * A_{647} \quad (8)$$

The chlorophyll a:b ratio was also calculated. Specific leaf masses were obtained after drying the leaf discs at 65°C in a forced air oven for at least 24 hours, and calculated as dry weight divided by leaf area.

Gas Exchange

In the summer of 1991, gas exchange measurements included diurnal, light and CO_2 response curves. Net photosynthetic rates and stomatal conductances were measured using a Li-6200 Portable Photosynthesis System (Li-Cor, Lincoln, NE 68504). Two Li-6200 portable systems were used simultaneously for the diagnostic gas exchange measurements. The Li-6200 systems were calibrated using a National Institute of Standards and Traceabilities CO_2 standard gas before each set of measurements. The systems were routinely re-zeroed during use. The one liter Li-Cor 6200 leaf chamber was used for all measurements.

Diurnal Curves

Diurnal patterns of net photosynthesis and stomatal conductance were measured three times during the 1991 season on both old and new leaves on each plant. The old leaf was the first or second leaf that matured after

fumigation and the new leaf was the one most recently expanded at the time when measurements were taken. Two plants (1992 harvest trees) per chamber (six trees per treatment) in the CF, 1.0 X and 2.0 X treatments were measured on July 2, August 7, and September 12, 1991 inside the chambers, except for the last measurement period. In the September measurements, seedlings were placed adjacent to the chambers to avoid self-shading effects inside the chamber, due to their large size. Measurements were taken at two hour intervals, starting early in the morning and continuing until late in the afternoon.

Diagnostic Gas Exchange

In order to estimate residual effects of ozone on gas exchange processes, light and CO_2 responses were measured twice during the season. These measurements were conducted outside the chamber in a laboratory room adjacent to the fumigation facility. The first set of measurements were conducted on five plants (1992 harvest trees) per treatment in the CF, 1.0 X and 2.0 X treatments. The second set of measurements were done on old and new leaves on six plants (1992 harvest trees) per treatment in the CF and 2.0 X treatments. All measurements were taken between 10 am and 2:30 pm (EST) and plants were well watered and set inside in the lab for at least two hours before measurement. Plants were moved back to their chambers after measurement. Irradiance was measured inside the leaf chamber using a GaASP sensor (G1118, Hamamatsu Corp., Japan), calibrated against a quantum sensor (Li-Cor 190S, Li-Cor Inc.,

Lincoln, NE). The source of irradiance was an incandescent light (GE Cool-Beam PAR Lamp Model 300PAR 56/2WFL). A water bath containing 3 cm depth of water and a 2.5 cm diameter cooling fan (Archer, Ft. Worth, TX) were used to minimize leaf heating from the lamp bulb.

(1) Light Response Curves

Light curves were measured on June 3-6 and July 30-August 4, 1991. Ambient air was pulled into the leaf chamber, thus the CO_2 concentration inside the chamber varied somewhat depending on ambient conditions. Cheese-cloth and window screens were used to vary irradiance. Aluminum foil was wrapped around the leaf chamber to achieve darkness for respiration measurements. A premarked leaf was placed inside the cuvette, and flushed with fresh air outside the lab for 10 minutes under 800 μmol m^{-2} s^{-1} light intensity before actual measurements were taken. Measurements were from high (1400 μmol m^{-2} s^{-1}) to low light intensity (darkness). At each light intensity, the leaf was allowed to equilibrate for two minutes under ambient air conditions, after which the external flow switch on the Li-6200 was closed. At least one minute was then used for the stabilization of relative humidity within the leaf chamber before logging a measurement. Repeated readings were taken at the same light intensity until successive measurements were within $\pm$10%. Environmental conditions inside the leaf chamber for the first set of light curves were as follows: CO_2 ranged from 295 ppm to 377 ppm; RH, 76% and temperature, 27.9°C. For the second set of

curves, average CO_2 concentration was around 340 ppm; RH, 76% and temperature, 27°C. Linear regressions were calculated from the original data points for the initial slope of the light curves. A_{max}, g_s at A_{max}, C_i at A_{max}, dark respiration and light compensation points were calculated as previously described.

(2) CO_2 Response Curves

CO_2 curves were measured during June 9-11 and August 13-16, 1991. The light intensity for CO_2 curves was 800 μmol m^{-2} s^{-1} (saturation light intensity) and the leaves used for the light curves were also used for the CO_2 curves. Leaves were acclimated inside the leaf chamber under high CO_2 (around 1200 ppm) for 10 minutes before actual measurements were taken. To start a curve, initial CO_2 concentrations were brought back to 1200 ppm, and the relative humidity was adjusted prior to logging the first reading. The desired CO_2 concentrations were obtained by using the scrubber on the Li-6200. The second set of curves followed a new protocol as described in Li-Cor Application Note #103 (Li-Cor, Inc., 1991). Logging started automatically whenever CO_2 dropped by 25 ppm until CO_2 reached ambient concentrations. Then, the measurements were taken every minute until a negative net photosynthetic reading was achieved. The system was stopped manually to adjusted RH when necessary. The average environmental conditions inside the leaf chambers for the first set of curves were RH, 67% and temperature, 28.8°C. For the second set of measurements RH was 74% and temperature 27.7°C.

Carboxylation efficiencies, J_{max}, g_s at J_{max}, CO_2 compensation and saturation points were calculated as previously described. The stomatal and biochemical limitations were calculated by using the equations described in the introduction (Sasek and Richardson, 1989). Initial slopes of the response of **A** to C_i were calculated by fitting a linear regression to the raw data in the initial portion of the curves.

Experimental Design

A completely randomized design, with three replicates per treatment (three chambers per treatment) was used to assign trees to treatments. Ozone treatment effects were compared among chambers and significance evaluated at the $p = 0.05$ level unless otherwise stated. All analyses were performed using Version 6.02 of the Statistical Analysis Software (SAS; 1990, SAS Institute, Cary, NC). Only trees in chambers were used in the analyses. Open-plot trees were not included.

Data Analysis

Growth

Initial height was used as the covariate for the height growth analysis. Initial diameter was used as the covariate for the diameter growth analysis. The assumptions of homogeneity of variances and normality of data were met and therefore no transformations of the data were needed. Data were analyzed using

repeated measures analysis, consisting of both multivariate and univariate techniques. Multivariate analysis of variance used the Hotelling-Lawley Trace and F-tests modified by the GreenHouse-Geisser Epsilon test. Univariate analysis of variance used F-tests based on type III sums of squares. Both treatment and time were partitioned into polynomial contrasts and if significant, modeled with growth curves and exposure-response curves. The interaction of time and treatment was partitioned into single degree of freedom contrasts to see if growth curves differed among treatments and if exposure-response curves differed among days. Regression procedures (linear and quadratic) were used to model the responses of height and diameter to time and ozone.

Biomass

All variables were analyzed in a univariate manner using orthogonal polynomial contrast analyses. Initial height and diameter were used as covariates if they contributed significantly to the variance among chambers. Treatment effects were partitioned into linear, quadratic and cubic components, and if significant, were modeled using regression techniques (Weibull if non-linear, linear otherwise). Dunnett's test was used to compare the control with other ozone levels. This test, as well as the regression modeling, was performed on the chamber means after adjustment for the covariates.

Gas Exchange, Chlorophyll and Specific Leaf Mass

Analysis of variance was used to assess for treatment effects in the diurnal curve data. Treatment and age were the main effects. Duncan's Multiple Range Test was used to determine which means were different.

For the light and CO_2 curves, a two-way analysis of variance was used, with ozone treatments and leaf age as the main effects. Duncan's Multiple Range Test was again used as the post-hoc test of main effects differences. If the interaction term was significant, the analyses were rerun separately for each main effect level.

RESULTS

Ozone Fumigation

Cumulative ozone exposures (24-hour totals) and fumigation time for the 1990 and 1991 fumigation seasons, are shown in Table 1. The CF treatment removed about 80% of the ozone in comparison to the 1.0 X ambient treatment over the two seasons of fumigation. In the summer of 1990 there were five times when the mean hourly ozone concentration exceeded 70 ppb in the open ambient plots, and no cases where it exceeded 120 ppb, the National Ambient Air Quality Standard, as set by the Clean Air Act (Table 2). The mean hourly ozone concentration exceeded 70 ppb and 120 ppb 106 and six times, respectively, in the 2.0 X ambient treatment in 1990. In 1991, there was one day with an hourly ozone concentration greater than 70 ppb and no cases where it exceeded 120 ppb in the open ambient plots (Table 2). In 2.0 X ambient, there were 467 and 13 times when ozone concentrations were greater than 70 ppb and 120 ppb, respectively.

Growth

Height and Diameter Growth

Results from the multivariate analysis showed significant linear reductions in height and diameter as ozone increased in 1990 (Tables 3 & 4). These

Table 1

Summary of fumigation duration and total ozone exposure for biomass, height and diameter measurements in 1990 and 1991

Treatment	Ozone Exposure for Biomass (ppm-hr)			Ozone Exposure for Height and Diameter (ppm-hr)		
	1990 6/30 - 9/12 74 Days	1991 5/3 - 8/19 108 Days	Total for Both Years*	1990 6/30 - 8/29 61 Days	1991 5/3 - 8/7 86 Days	Total for Both Years*
Charcoal Filtered	9.60	7.70	18.67	8.00	6.97	17.94
0.5 X Ambient	16.50	24.20	43.35	13.01	21.27	40.42
1.0 X Ambient	40.00	45.40	91.96	32.00	40.08	86.64
1.5 X Ambient	60.10	72.00	120.10	48.10	63.15	132.86
2.0 X Ambient	76.70	94.90	184.10	60.50	83.29	172.48

*Note: Total exposure is greater than sum of 1990 and 1991 exposures because plants harvested in 1991 were exposed to additional ozone in the fall of 1990, after 1990 plants were harvested.

Table 2

Summary of ozone concentrations greater than 70 and 120 ppb in 1990 and 1991

Treatment	1990				1991			
	# Hours > 70 ppb		# Hours > 120 ppb		# Hours > 70 ppb		# Hours > 120 ppb	
	All Hours	7 am - 7 pm	All Hours	7 am - 7 pm	All Hours	7 am - 7 pm	All Hours	7 am - 7 pm
Open Ambient Plots	5	5	0	0	1	1	0	0
Charcoal Filtered	1	1	0	0	0	0	0	0
0.5 X Ambient	1	1	0	0	2	2	1	1
1.0 X Ambient	3	3	0	0	0	0	0	0
1.5 X Ambient	106	89	0	0	106	141	1	1
2.0 X Ambient	305	249	6	6	467	403	13	13

Table 3

Regression equations for height and diameter

Ozone Treatment	Parameters	1990 Height (cm)	1991 Height (cm)	1990 Diameter (mm)	1991 Diameter (mm)
CF	Intercept	43.6692	135.041	6.3394	17.537
	Linear	9.4902	2.9135	0.0840	0.1197
	Quadratic	0.0232	0.0117		
0.5 X	Intercept	43.1045	147.625	6.4021	17.127
	Linear	9.6006	1.4438	0.0888	0.1084
	Quadratic	0.0234	0.0081		
1.0 X	Intercept	42.4381	138.151	6.4855	17.565
	Linear	6.6672	2.6597	0.0845	0.1178
	Quadratic	0.0167	0.0116		
1.5 X	Intercept	42.4550	125.150	6.0225	15.165
	Linear	4.3300	2.4245	0.0952	0.1077
	Quadratic	0.0114	0.0106		
2.0 X	Intercept	37.7113	106.611	5.7363	14.710
	Linear	5.3418	4.2398	0.0680	0.1129
	Quadratic	0.0132	0.0156		

Note: For 1990 height and diameter, linear independent variable is: Day - 227.67; quadratic independent variable for height is: Day^2 - 51983. For 1991 height and diameter, linear independent variable is: Day - 176.43; quadratic independent variable for height is: Day^2 - 31908.

Table 4

Summary statistics for height and diameter

		Multivariate Analysis P - Values			
		Height		Diameter	
Source	df	1990	1991	1990	1991
Treatment	4	0.0309*	0.0125*	0.0403*	0.0304*
Linear	1	0.0066*	0.0018*	0.0060*	0.0043*
Quadratic	1	0.0967	0.0409*	0.0607	0.3542
Time	2	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
Linear	1	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
Time*Treatment	8	0.0219*	0.2763	0.6660	0.7429
Time*Linear	2	0.0045*	0.4594	0.2814	0.4679
Linear*Linear	1	0.0009*	0.7296	0.3418	0.3674
Time*Quadratic	2	0.1974	0.0650	0.3603	0.7477
Linear*Quadratic	1	0.3079	0.0071*	0.1679	0.5959
Linear*Treatment	4	0.0079*	0.0262*	0.2874	0.4085
Quadratic*Treatment	4	0.0421*	0.3466	0.3024	0.8603

*Values are significantly different at $p \leq 0.05$.

reductions persisted into the summer of 1991 and showed a strong linear decline with ozone (Table 3). Plots of treatment means versus exposure days, and the derived regression lines illustrate these fits (Figs. 3 & 4). Note that the growth curves for height are curvilinear, since the quadratic contrasts for time were significant, while the diameter growth curves showed only linear responses (Table 4). Significant linear*linear and linear*quadratic contrasts were found for height in 1990 and 1991, respectively, indicating that the slopes for the height growth curves differed among treatments. Diameter growth response curves were similar across treatments in both years.

The significant time effect found in the multivariate analysis meant that the response of plants to ozone had to be tested for each day, using univariate analysis of variance. Results of the univariate analysis indicated that height growth was reduced in a linear fashion, starting on August 17, 1990, 47 days after fumigation started. In 1991, height was again linearly reduced, but by the last four dates, the reductions became non-linear and were modeled using quadratic curves.

Diameter growth was significantly reduced starting by July 31, 1990, only 30 days after fumigation began. In 1991, diameter was linearly reduced all season. At the end of the summer of 1990, heights and diameters in 2.0 X ambient were reduced 15% and 14%, respectfully, as compared to 1.0 X ambient. At the end of the 1991 season, reductions in height and diameter in 2.0 X ambient were 16% and 14%, respectively, as compared to 1.0 X ambient.

Figure 3. Height growth of tulip poplar seedlings as a function of ozone exposure. (A) Height growth in 1990. (B) Height growth in 1991. Lines through points are best fit linear regressions. Symbols are as follows: ▫ CF, ▪ 0.5 X, ▵ 1.0 X, ▲ 1.5 X, ◇ 2.0 X.

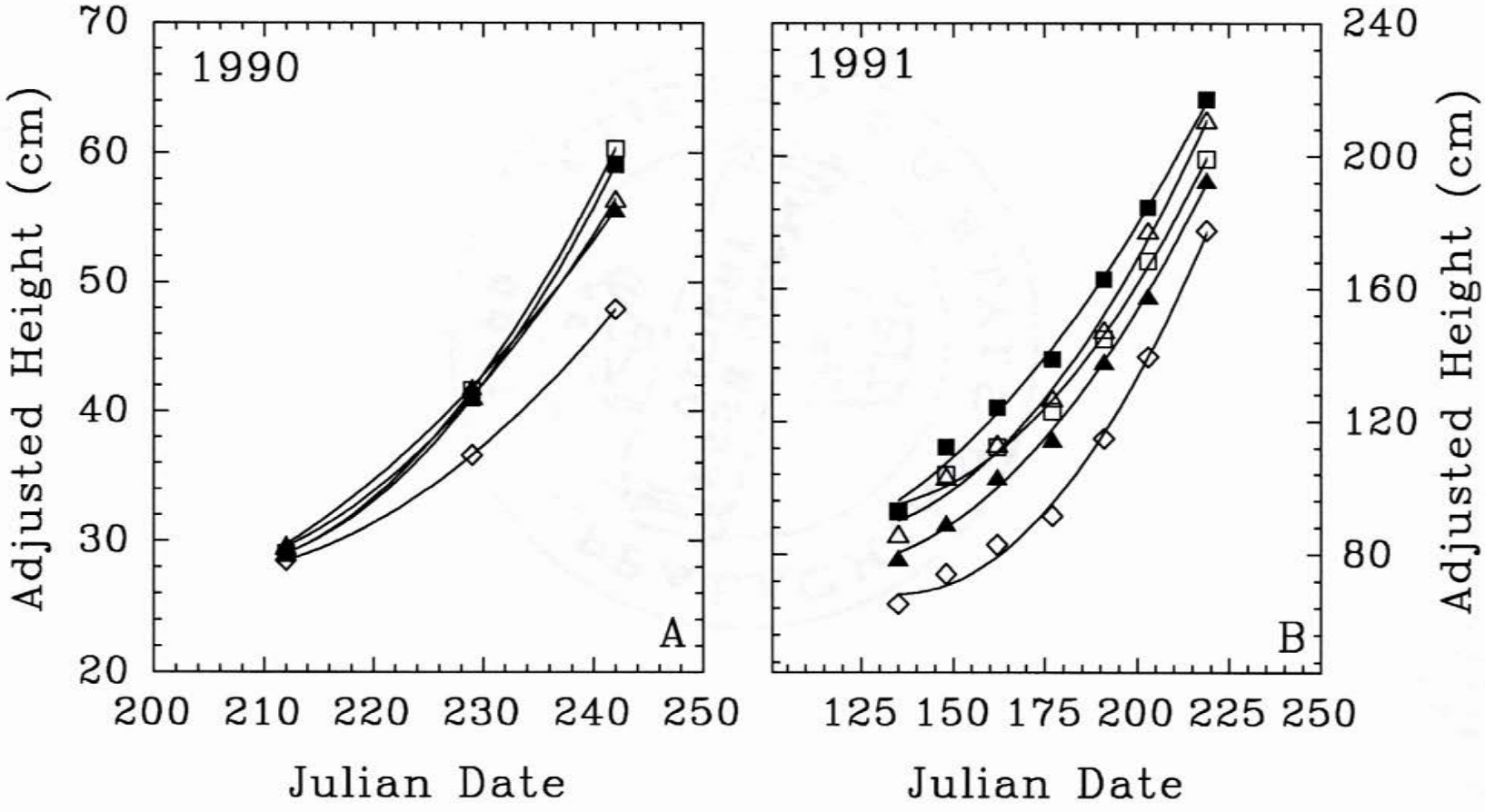

1990
A
1991
B
Adjusted Height (cm)
Adjusted Height (cm)
Julian Date
Julian Date
70
60
50
40
30
20
240
200
160
120
80
200 210 220 230 240 250
125 150 175 200 225 250

Figure 4. Diameter growth of tulip poplar seedlings as a function of ozone exposure. (A) Diameter growth in 1990. (B) Diameter growth in 1991. Lines through points are best fit linear regressions. Symbols are as follows: ▫ CF, ▪ 0.5 X, ▵ 1.0 X, ▲ 1.5 X, ◇ 2.0 X.

Adjusted Diameter (mm)
8
7
6
5
4
1990
A
210 220 230 240 250
Julian Date
Adjusted Diameter (mm)
25
20
15
10
5
1991
B
125 150 175 200 225 250
Julian Date

Biomass Accumulation and Allocation

Analysis of variance for ozone effects on biomass for 1990 is summarized in Table 5. In 1990, initial seedling diameters were significant covariates for total leaf count and weight, tap and total root weight, aboveground and total dry weight, and the data were therefore adjusted prior to analysis. Increased cumulative ozone exposure (from 9.6 ppm-hr in CF to 76.7 ppm-hr in 2.0 X) caused a significant linear decrease in total leaf count and weight (Fig. 5), tap and total root weight (Fig. 6), aboveground dry weight and total dry weight (Fig. 7). The differences between the CF and 2.0 X treatments for total leaf count and weight, tap and total root weight, aboveground and total dry weight were: 14%, 22%, 30%, 21% and 25% respectively. No treatment effects were found for the other biomass parameters (Table 5). Leaf area was also not significantly reduced by high ozone (Table 5).

In 1991, initial seedling heights and diameters were no longer significant covariates for any biomass parameters (Table 6). Increased total cumulative ozone (from 18.67 ppm-hr to 172.48 ppm-hr) resulted in significant linear decreases in branch and total leaf count (Fig. 8), main stem and total leaf weight (Fig. 9), and total leaf area (Fig. 10). The differences between CF and 2.0 X for total and branch leaf count, total leaf area, main stem and total leaf weight were 26%, 28%, 20%, 22% and 22%, respectfully. Although there were no significant treatment effects for other biomass parameters (Table 6), these values tended to be lower in the higher ozone treatments.

Table 5

Analysis of variance summary for biomass - 1990.

Parameter	Mean Square Error Chamber (Treatment)	Mean Square Error (Treatment)	P-values	
			Treatment	Linear
Total leaf count‡	91.84†	284.14	0.06	0.02*
Total leaf weight‡	27.33	53.19	0.04*	0.02*
Total leaf area	12.91 x 10^5	17.76 x 10^5	0.42	0.31
Wood weight	14.70	16.11	0.22	0.20
Aboveground weight‡	166.43	248.21	0.09	0.05*
Tap root length	0.36	0.47	0.73	0.99
Primary root length‡	0.54	0.46	0.13	0.17
Primary root count	139.64	80.59	0.13	0.34
Tap root weight‡	2.63	6.64	0.03*	0.04*
Secondary root weight‡	24.07	34.09	0.12	0.06
Total root weight‡	38.60	59.54	0.07	0.05*
Total dry weight‡	344.84	512.45	0.07	0.05*
Root to shoot ratio	0.03	0.06	0.36	0.29
LAR	1550.10	1217.38	0.32	0.08

†Average sample size = 24. ‡Covariate is diameter. *Values are significant at $p \leq 0.05$.

Figure 5. Adjusted leaf count and weight as a function of ozone exposure - 1990. Points are chamber means. Line a through filled squares is leaf count. Line b line with open squares is leaf weight. Lines through points are best fit linear regressions: leaf count = 47.2984 - 0.0996*O_3, adjusted R^2 = 0.28; leaf weight = 15.83 - 0.0509*O_3, adjusted R^2 = 0.22.

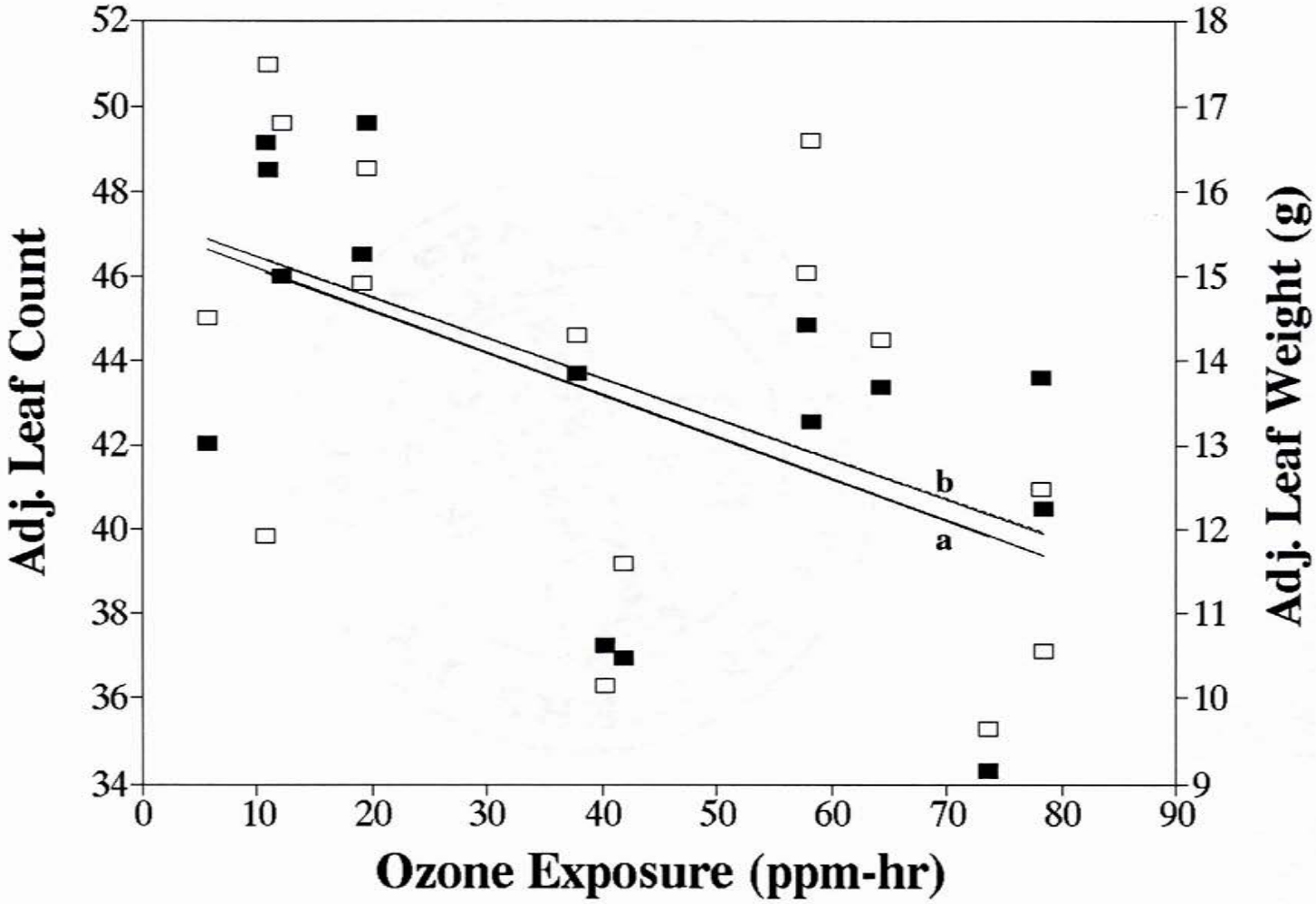

Adj. Leaf Count
Adj. Leaf Weight (g)
Ozone Exposure (ppm-hr)
52
50
48
46
44
42
40
38
36
34
18
17
16
15
14
13
12
11
10
9
0
10
20
30
40
50
60
70
80
90
b
a

Figure 6. Adjusted total and tap root weight as a function of ozone exposure - 1990. Points are chamber means. Line a through filled squares is total root weight. Line b with open squares is tap root weight. Lines through points are best fit linear regressions: total root weight = 14.3376 - 0.0514*O_3, adjusted R^2 = 0.15; tap root weight = 4.673 - 0.0139*O_3, adjusted R^2 = 0.10.

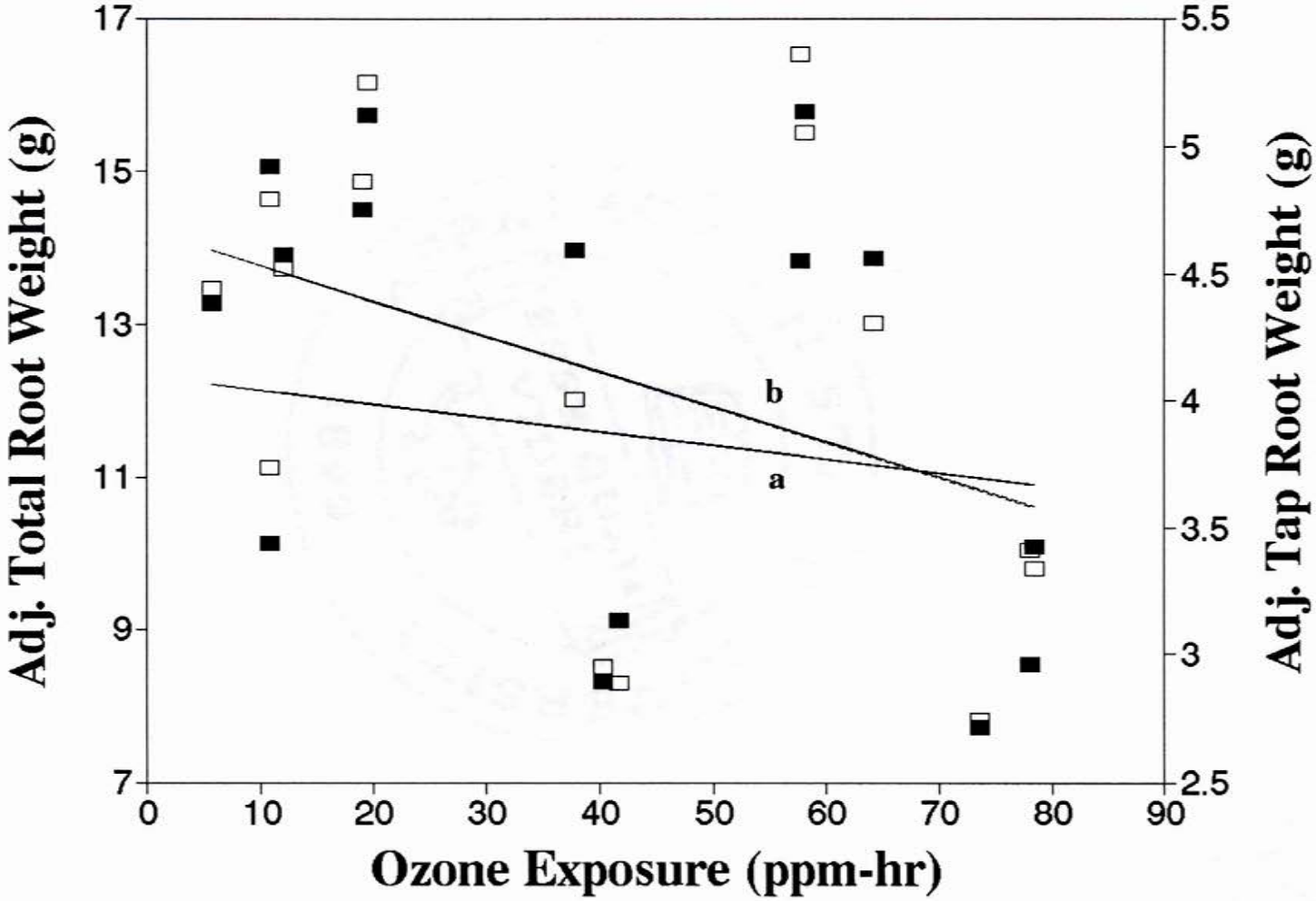

Adj. Total Root Weight (g)
Adj. Tap Root Weight (g)
Ozone Exposure (ppm-hr)
17
15
13
11
9
7
5.5
5
4.5
4
3.5
3
2.5
0
10
20
30
40
50
60
70
80
90
b
a

Figure 7. Adjusted shoot and total weight as a function of ozone exposure - 1990. Points are chamber means. Line a through filled squares is total dry weight. Line b through open squares is shoot weight. Lines through points are best fit linear regressions: total dry weight = 45.1104 - 0.1557*O_3, adjusted R^2 = 0.18; tap root weight = 30.7728 - 0.1044*O_3, adjusted R^2 = 0.17.

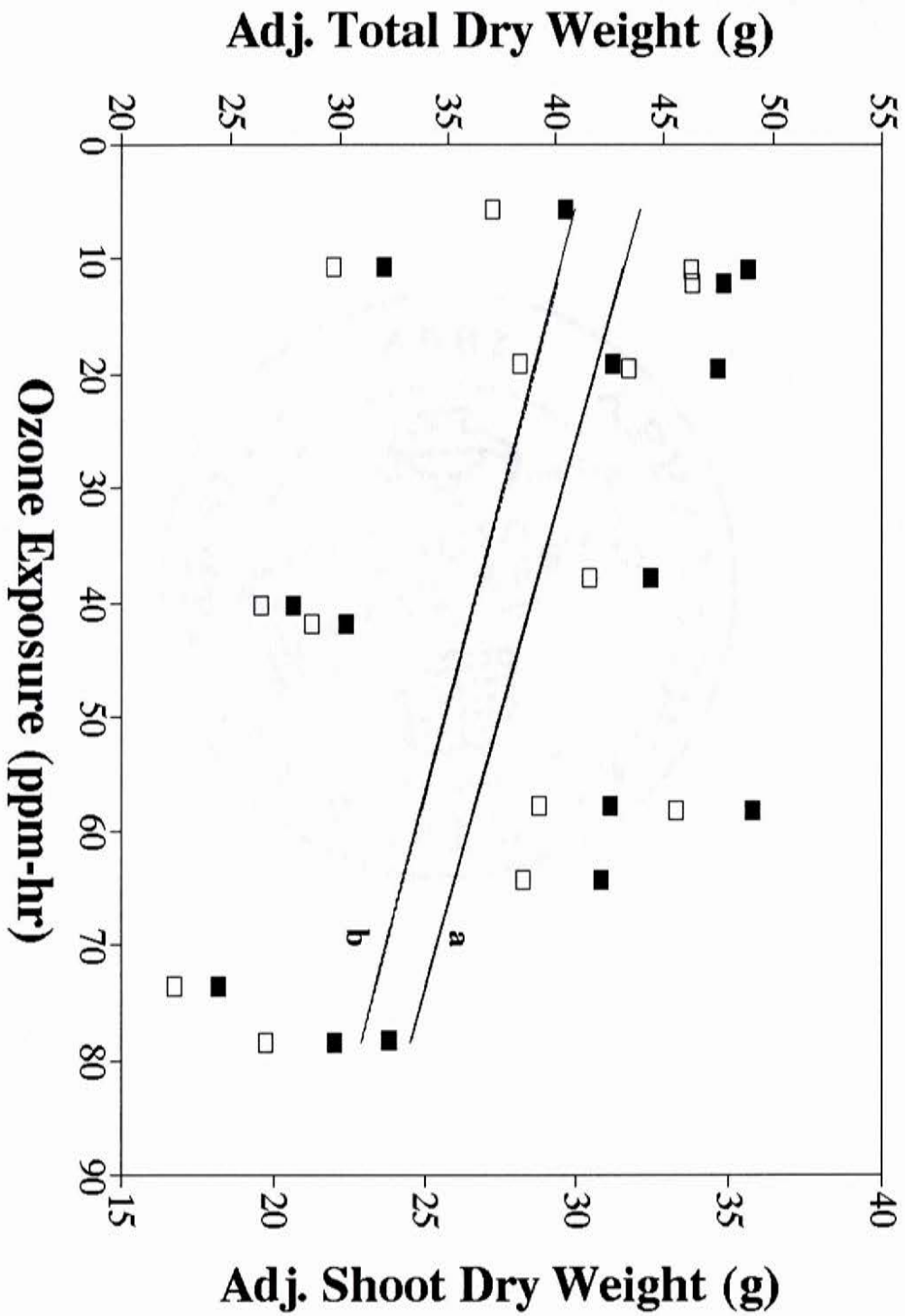

Adj. Total Dry Weight (g)
20
25
30
35
40
45
50
55
Ozone Exposure (ppm-hr)
0
10
20
30
40
50
60
70
80
90
b
a
15
20
25
30
35
40
Adj. Shoot Dry Weight (g)

Table 6.

Analysis of variance summary for biomass - 1991

Parameters[‡]	Mean Square Error Chamber (Treatment)	Mean Square Error (Treatment)	P-values	
			Treatment	Linear
Main stem leaf count	20.40[†]	13.16	0.26	0.09
Branch leaf count	5170.33	3264.77	0.18	0.03*
Total leaf count	5320.75	3338.99	0.17	0.03*
Main stem leaf weight	64.94	74.07	0.14	0.03*
Branch leaf weight	501.92	609.03	0.25	0.08
Total leaf weight	685.05	840.87	0.17	0.03*
Main stem leaf area	55.91×10^5	53.81×10^5	0.76	0.35
Branch leaf area	41.55×10^5	32.30×10^5	0.22	0.07
Total leaf area	46.39×10^6	44.46×10^6	0.23	0.05*
Main stem weight	2180.73	2790.81	0.28	0.12
Branch weight	538.73	345.04	0.84	0.34
Wood weight	4585.10	4642.91	0.43	0.16
Aboveground weight	8545.61	8698.93	0.38	0.11
Tap root weight	364.65	229.69	0.28	0.08
Secondary root weight	1104.05	848.30	0.83	0.44
Total root weight	2155.01	1509.18	0.55	0.20
Total dry weight	17404.77	16204.67	0.41	0.12
Root to shoot ratio	0.02	0.01	0.84	0.59
LAR	4 03.58	502.15	0.69	0.72

[†]Average sample size = 24. [‡]No covariates were needed. *Values are significant at $p \leq 0.05$.

Figure 8. Branch and total leaf count as a function of ozone exposure - 1991. Points are chamber means. Line a through filled squares is total leaf count. Line b through open squares is branch leaf count. Lines through points are best fit linear regressions: total leaf count = 225.7733 - 0.3490*O_3, adjusted R^2 = 0.33; branch leaf count = 203.9783 - 0.3347*O_3, adjusted R^2 = 0.32.

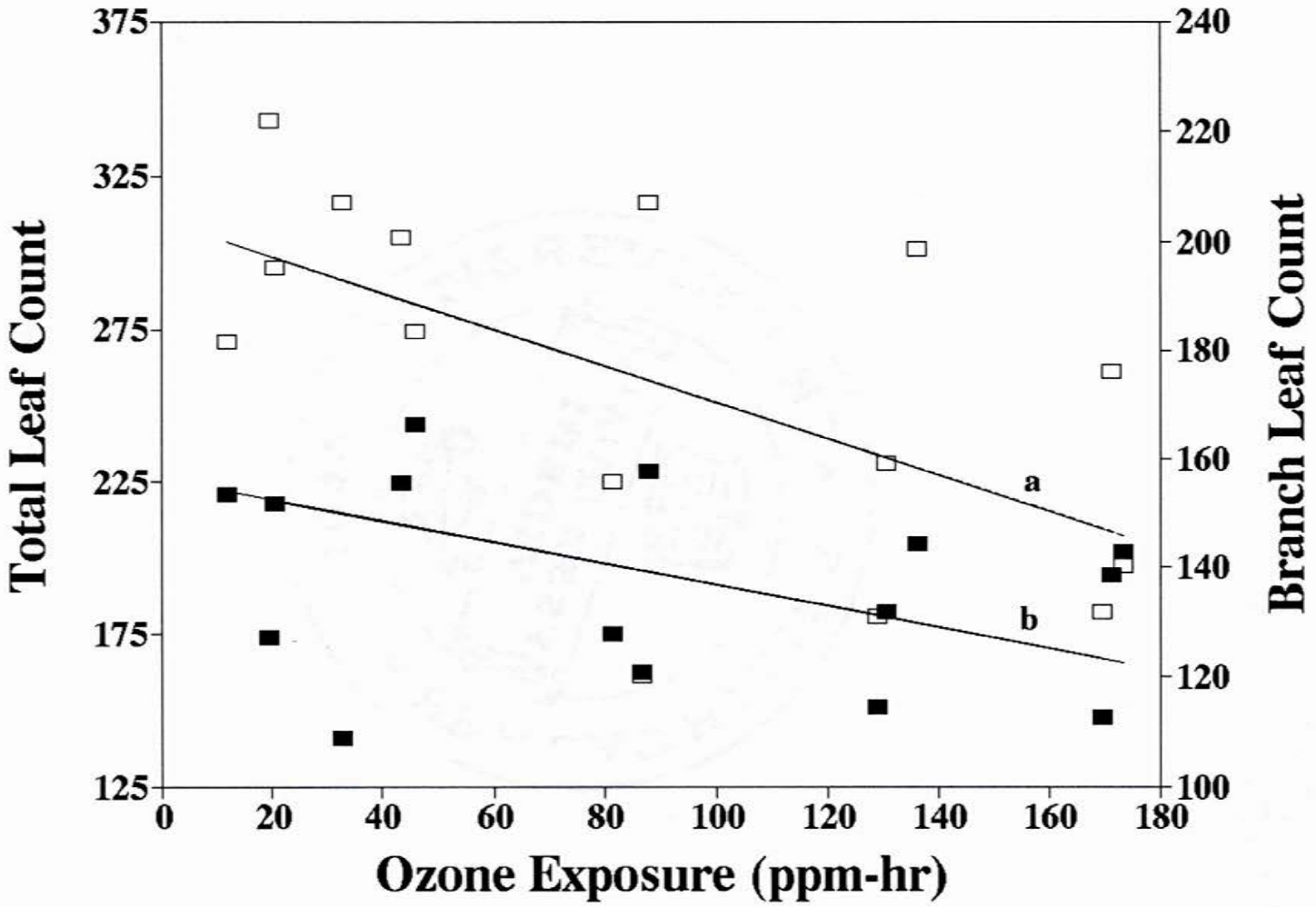

Total Leaf Count
Branch Leaf Count
Ozone Exposure (ppm-hr)
375
325
275
225
175
125
240
220
200
180
160
140
120
100
0
20
40
60
80
100
120
140
160
180
a
b

Figure 9. Main stem and total leaf weight as a function of ozone exposure - 1991. Points are chamber means. Line a through filled squares is total leaf weight. Line b through open squares is main stem leaf weight. Lines through points are best fit linear regressions: total leaf weight = 88.5197 - 0.1163*O_3, adjusted R^2 = 0.29; main stem leaf weight = 27.3242 - 0.0356*O_3, adjusted R^2 = 0.27.

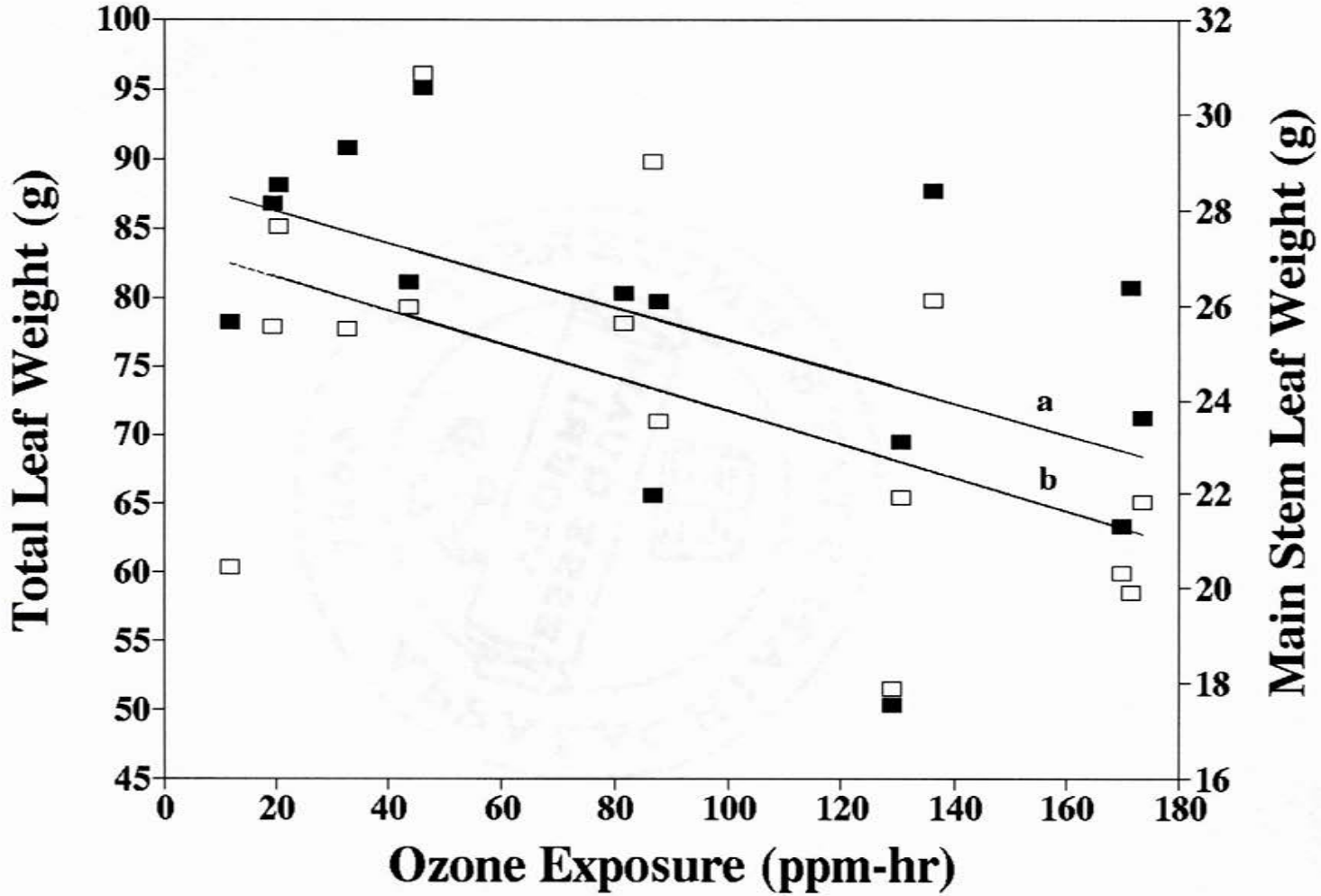
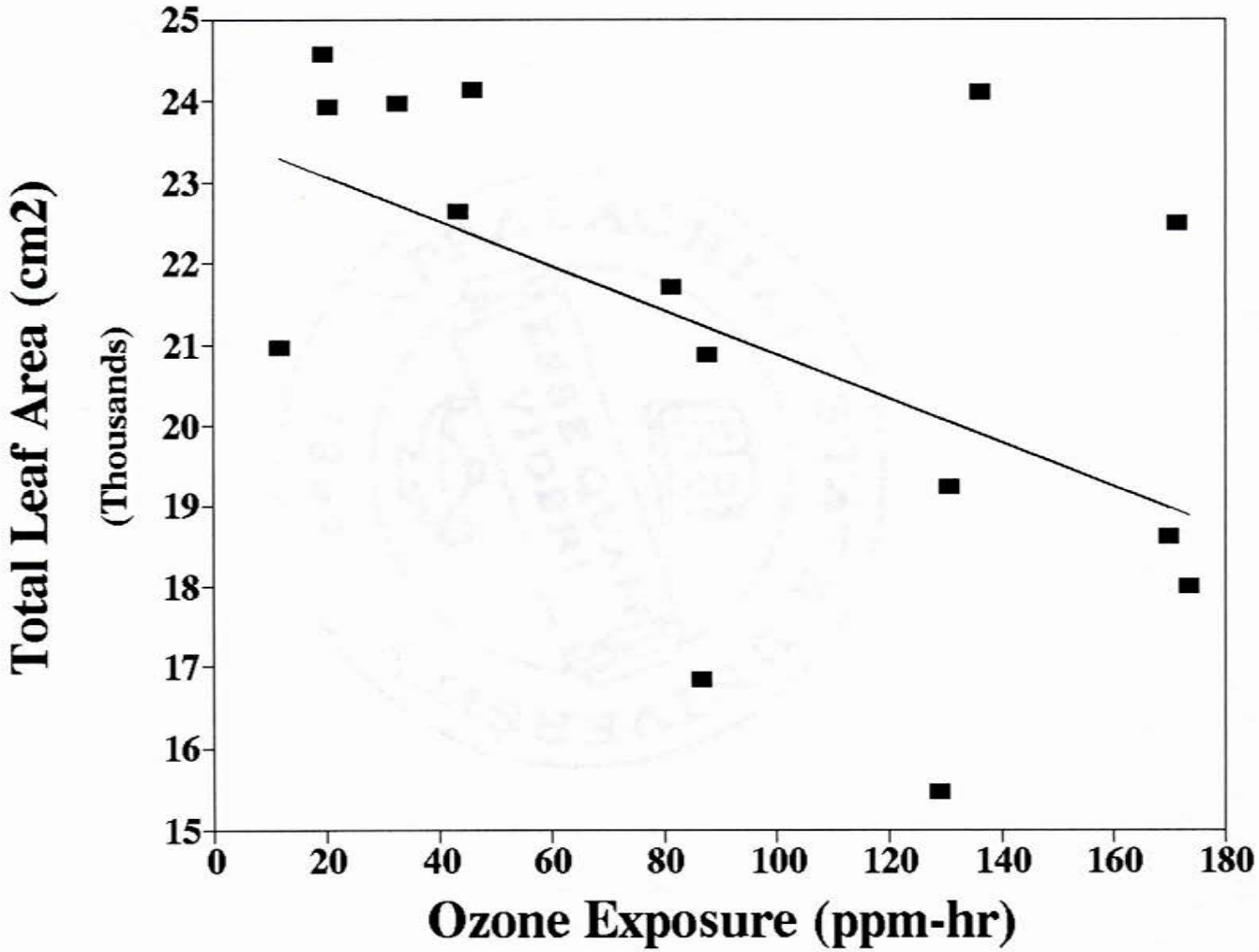

Total Leaf Weight (g)
Main Stem Leaf Weight (g)
Ozone Exposure (ppm-hr)
100
95
90
85
80
75
70
65
60
55
50
45
32
30
28
26
24
22
20
18
16
0
20
40
60
80
100
120
140
160
180
a
b

Figure 10. Total leaf area as a function of ozone exposure - 1991. Points are chamber means. Line through points is best fit linear regression: total leaf weight = 23618 - 27.3599*O_3, adjusted R^2 = 0.25.


Total Leaf Area (cm2)
(Thousands)
25
24
23
22
21
20
19
18
17
16
15
0
20
40
60
80
100
120
140
160
180
Ozone Exposure (ppm-hr)

Chlorophyll Content and Specific Leaf Mass

No significant differences were found in total leaf chlorophyll content, chlorophyll a, chlorophyll b or chlorophyll a:b ratio in 1990 (Table 7). In 1991, at the end of May, plants in CF, 0.5 X and 2.0 X ambient had a lower chlorophyll a:b ratio than plants in 1.5 X and 1.0 X ambient treatments. By July 20, 1991, chl b was reduced in 2.0 X ambient as compared to CF, whereas chl a, total chlorophyll, and the chlorophyll a:b ratio were not affected. On the last date, September 3, 1991, chl a and total chlorophyll contents were significantly reduced due to ozone exposure. Leaves in CF and 1.0 X had significantly higher chlorophyll contents as compared to leaves in 1.5 X and 2.0 X ambient.

Leaf age effects turned out significant for chlorophyll a, chlorophyll b, and total chlorophyll contents on the second date, while on the last date chlorophyll a, chlorophyll b, total chlorophyll contents, and the chlorophyll a:b ratio were significantly affected. For all these parameters, old leaves had lower chlorophyll contents than young leaves. There were no treatment*age interactions at any time during the season (Table 7).

No treatment effects were found for specific leaf mass (SLM) in the summer of 1990 (Table 8). SLM was significantly higher for leaves in the 1.5 X treatment than CF at the end of May, 1991 (Table 8), but there were no differences by July nor in September. Significant leaf age effects were found in the last two sets of measurements in 1991. Older leaves had a higher SLM than younger leaves. No treatment*age interactions were significant (Table 8).

Table 7

Leaf chlorophyll content (µg/g dry weight) as affected by ozone in 1990 and 1991.

	Young Leaves					Old Leaves				
	CF	0.5 X	1.0 X	1.5 X	2.0 X	CF	0.5 X	1.0 X	1.5 X	2.0 X
Sept.-90										
Chl a	11.61 (1.05)	11.65 (0.67)	9.68 (0.87)	9.91 (0.60)	9.73 (0.42)	--	--	--	--	--
Chl b	3.63 (0.35)	3.43 (0.21)	3.06 (0.29)	3.30 (0.29)	3.03 (0.16)	--	--	--	--	--
Total	15.24 (1.37)	15.08 (0.86)	12.75 (1.14)	13.21 (0.87)	12.76 (0.55)	--	--	--	--	--
a:b	3.30 (0.15)	3.42 (0.08)	3.24 (0.11)	3.16 (0.16)	3.29 (0.13)	--	--	--	--	--
May-91										
Chl a	14.90 (0.85)	15.88 (1.00)	1.75 (0.59)	12.16 (0.80)	14.40 (1.07)	--	--	--	--	--
Chl b	4.68 (0.52)	4.42 (0.44)	5.54 (0.22)	5.39 (0.46)	4.93 (0.44)	--	--	--	--	--
Total	19.58 (1.29)	20.30 (1.40)	18.29 (0.74)	17.56 (1.21)	19.47 (1.43)	--	--	--	--	--
a:b*	3.76[a] (0.46)	3.81[a] (0.18)	2.32[b] (0.10)	2.34[b] (0.10)	3.23[a] (0.34)	--	--	--	--	--

Table 7 (cont.)

	Young Leaves					Old Leaves				
	CF	0.5 X	1.0 X	1.5 X	2.0 X	CF	0.5 X	1.0 X	1.5 X	2.0 X
July-91										
Chl a†	25.59 (1.96)	23.56 (1.97)	27.38 (2.30)	27.91 (1.60)	27.16 (1.32)	21.59 (1.60)	18.02 (1.92)	17.86 (1.78)	18.65 (1.06)	15.26 (0.97)
Chl b*†	9.98^{a} (0.71)	7.05^{b} (0.55)	7.59^{b} (0.47)	7.49^{b} (0.59)	8.83^{b} (0.34)	8.83^{a} (0.80)	5.90^{b} (0.89)	3.79^{b} (0.51)	4.09^{b} (0.61)	4.39^{b} (0.55)
Total†	35.57 (2.59)	30.01 (2.18)	34.97 (2.64)	35.4 (2.06)	35.99 (1.44)	30.42 (2.27)	23.91 (2.66)	21.65 (2.15)	22.74 (1.53)	19.66 (1.41)
a:b	2.58 (0.10)	3.28 (0.23)	3.64 (0.24)	3.95 (0.29)	3.11 (0.16)	2.51 (0.11)	3.34 (0.25)	4.50 (0.44)	4.35 (0.49)	4.23 (0.55)
Sept.-91										
Chl a*†	30.75^{ab} (1.50)	28.95^{bc} (1.21)	36.02^{a} (2.16)	28.89^{dc} (1.42)	26.94^{d} (1.82)	29.80^{ab} (1.97)	27.03^{bc} (1.49)	28.29^{a} (1.38)	22.72^{dc} (1.76)	20.79^{d} (1.77)
Chl b†	9.98 (0.60)	9.14 (0.43)	10.92 (0.80)	9.20 (0.65)	8.71 (0.55)	9.81 (0.67)	8.73 (0.55)	8.99 (0.50)	7.42 (0.59)	7.08 (0.52)
Total*†	40.73^{ab} (2.06)	38.09^{bc} (1.62)	46.94^{a} (2.88)	38.09^{dc} (1.89)	35.65^{d} (2.33)	39.62^{ab} (2.59)	35.75^{bc} (2.02)	37.27^{a} (1.83)	30.15^{dc} (2.28)	27.88^{d} (2.25)
a:b†	3.13 (0.10)	3.18 (0.05)	3.40 (0.21)	3.27 (0.17)	3.10 (0.10)	3.07 (0.08)	3.13 (0.07)	3.18 (0.08)	3.10 (0.14)	2.92 (0.12)

Values are mean ± (SE). *Indicates a significant treatment effect ($p \leq 0.05$). †Indicates a significant leaf age effect ($p \leq 0.05$): Young leaves had higher chlorophyll contents or a:b ratio than old leaves. Means followed by different letters are statistically different from one another according to Duncan's Multiple Range Test. Dashes indicate no samples were taken.

Table 8

Specific leaf mass (mg/cm^2) as affected by ozone - 1990 and 1991.

	Young Leaves					Young Leaves				
	CF	0.5 X	1.0 X	1.5 X	2.0 X	CF	0.5 X	1.0 X	1.5 X	2.0 X
Sept.-90										
SLM	65.99 (5.00)	62.68 (3.72)	95.99 (22.76)	74.29 (5.51)	73.04 (3.48)	--	--	--	--	--
May-91										
SLM*	44.22^{b} (3.93)	55.06^{ab} (2.43)	50.00^{ab} (2.61)	57.28^{a} (4.54)	44.44^{b} (4.15)	--	--	--	--	--
July-91										
SLM†	39.75^{b} (3.51)	40.25^{b} (2.45)	39.01^{b} (2.63)	44.69^{b} (3.77)	36.29^{b} (1.81)	41.73^{a} (2.95)	42.96^{a} (2.10)	44.44^{a} (2.45)	52.59^{a} (2.94)	45.68^{a} (2.62)
Sept.-91										
SLM†	42.59^{b} (3.72)	42.72^{b} (2.77)	43.39^{b} (2.50)	41.97^{b} (2.28)	37.04^{b} (1.86)	52.65^{a} (2.40)	49.14^{a} (2.46)	52.38^{a} (2.23)	56.79^{a} (3.90)	47.62^{a} (1.90)

Values are mean ± (SE). *Indicates a significant treatment effect ($p \leq 0.05$). †Indicates a significant leaf age effect ($p \leq 0.05$). Means followed by different letters are statistically different from one another according to Duncan's Multiple Range Test. Dashes indicate no sample were taken.

Gas Exchange

Diurnal Patterns

Net photosynthetic rates versus time for three diurnal curves taken in 1991 are shown in Figs. 11, 12, and 13. Points on the curves represent the average of 6 plants per treatment. In July and August 1991, no treatment effects were found for either **A** or g_s. However, photosynthetic rates of younger leaves were significantly higher than those for older leaves in both treatments. There were no significant age differences among treatments for stomatal conductance. Photosynthesis and g_s were both higher in the morning for both leaf age classes rather than in the afternoon. By September, net photosynthetic rates of the older leaves in the 2.0 X treatment were significantly depressed as compared to the other treatments (Fig. 13).

Light Response Curves

Figures 14 and 15 illustrate the relationship between **A** and irradiance on two separate dates. Each data point represents the mean of five leaves per treatment. Significant treatment effects were found for $\mathbf{A}_{max}$ and C_i at $\mathbf{A}_{max}$ in June 1991 (Table 9). $\mathbf{A}_{max}$ for plants in the 2.0 X ambient treatment was significantly reduced in comparison to plants in the CF and 1.0 X treatments, as determined using Duncan's Multiple Range Test. C_i at $\mathbf{A}_{max}$ was highest in plants from the 2.0 X ambient treatment and lowest in plants from the CF treatment, with intermediate values for plants in the 1.0 X ambient treatment.

Figure 11. Diurnal curve of gas exchange - July, 1991. (A) Net photosynthesis. (B) Stomatal conductance to water vapor. Lines are smooth fits to data. Points are means of six observations ± SE. Symbols are: ▫ CF, old leaves, ▪ CF, new leaves, ▵ 1.0 X. old leaves, ▲ 1.0 X, new leaves, ◇ 2.0 X, old leaves, ◆ 2.0 X, new leaves.

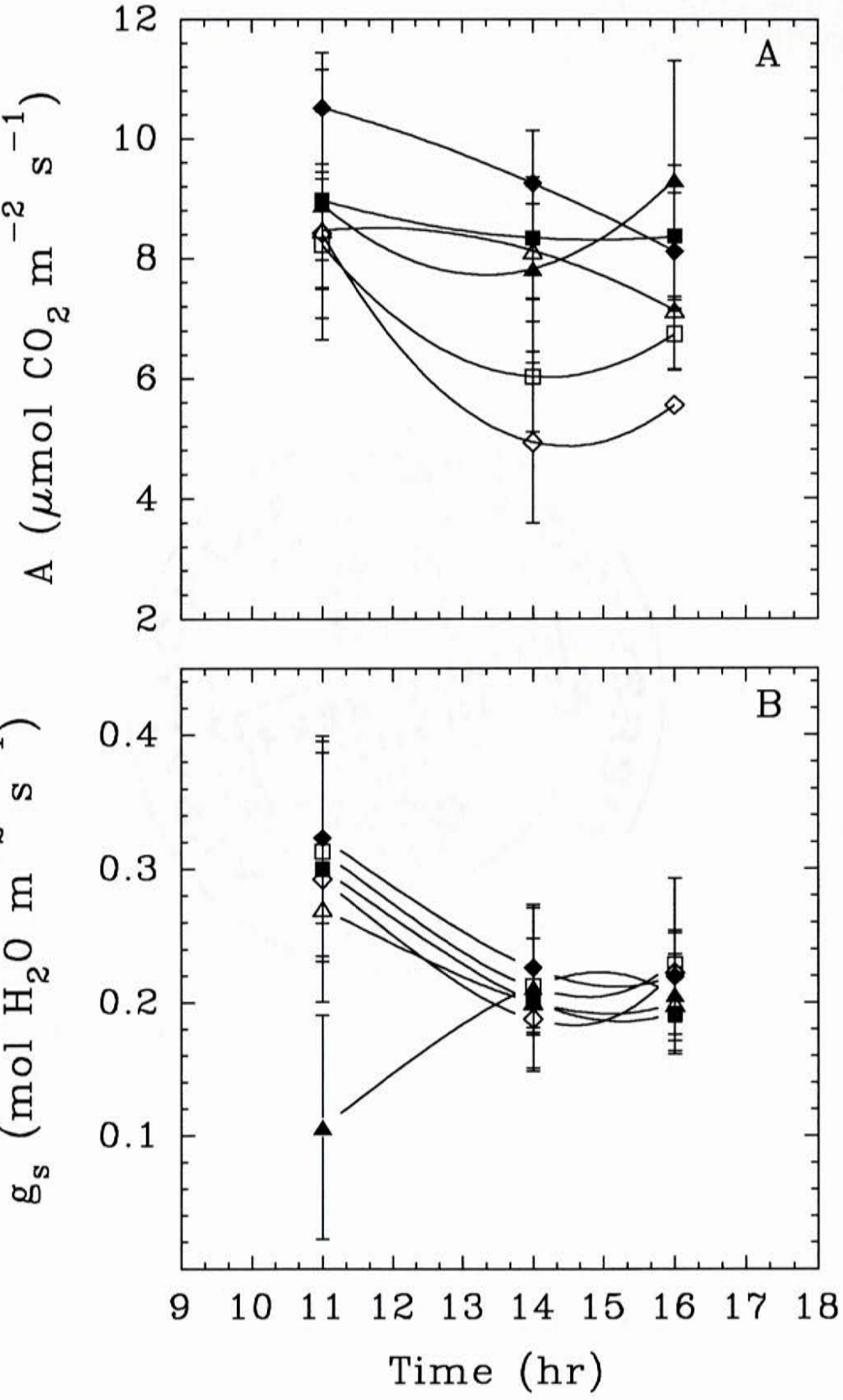

A
B
A (μmol CO_2 m^{-2} s^{-1})
g_s (mol H_2O m^{-2} s^{-1})
12
10
8
6
4
2
0.4
0.3
0.2
0.1
9 10 11 12 13 14 15 16 17 18
Time (hr)

Figure 12. Diurnal curve of gas exchange - August, 1991. (A) Net photosynthesis. (B) Stomatal conductance to water vapor. Lines are smooth fits to data. Points are means of six observations ± SE. Symbols are: ▫ CF, old leaves, ▪ CF new, leaves, ▵ 1.0 X, old leaves, ▲ 1.0 X, new leaves, ◇ 2.0 X, old leaves, ◆ 2.0 X, new leaves.

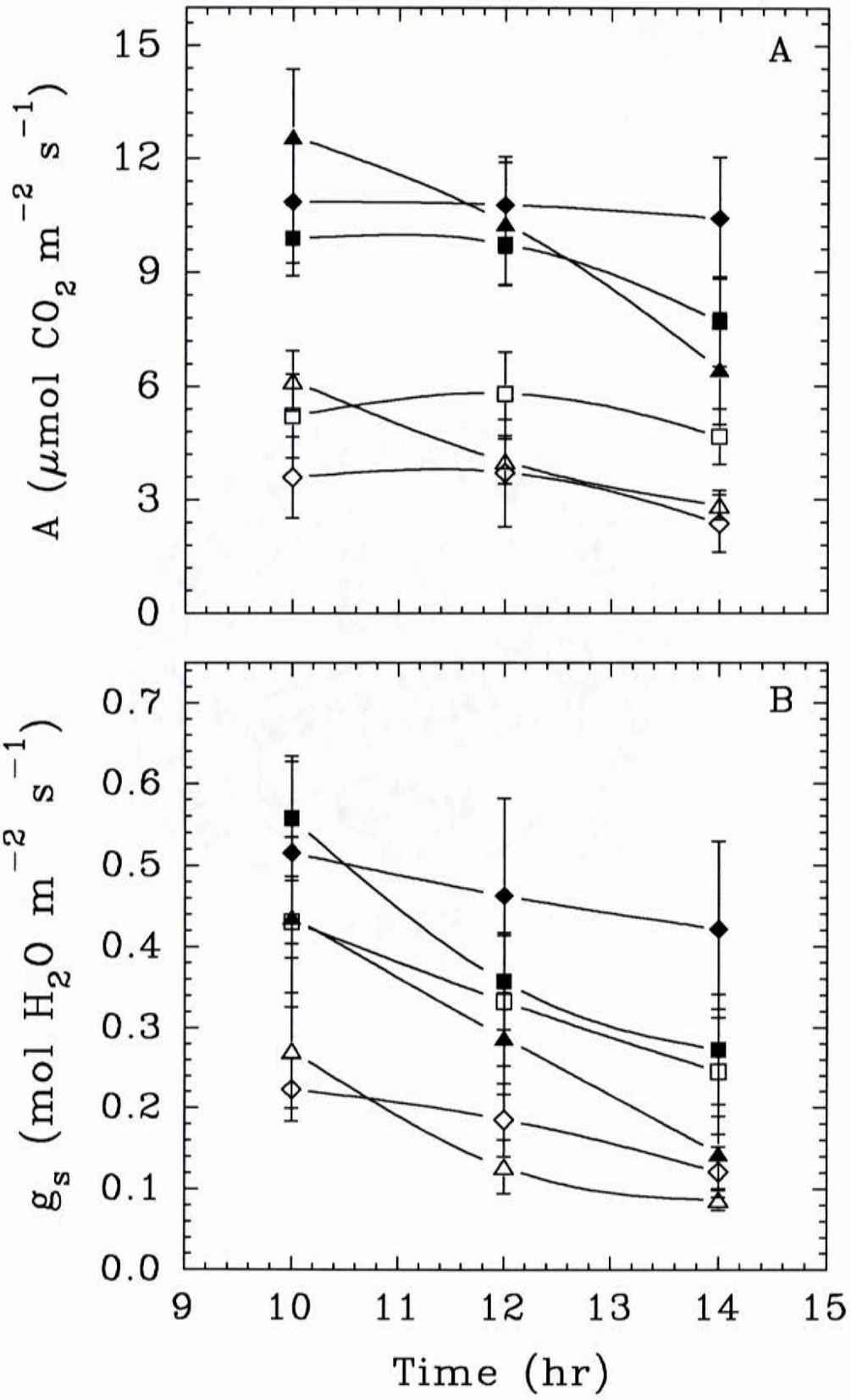

A
B
A (μmol CO_2 m^{-2} s^{-1})
g_s (mol H_2O m^{-2} s^{-1})
Time (hr)
0
3
6
9
12
15
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
9
10
11
12
13
14
15

Figure 13. Diurnal curve of gas exchange - September, 1991. (A) Net photosynthesis. (B) Stomatal conductance to water vapor. Lines are smooth fits to data. Points are means of six observations ± SE. Symbols are: ▫ CF old leaves, ▪ CF, new leaves, ▵ 1.0 X, old leaves, ▲ 1.0 X, new leaves, ◇ 2.0 X, old leaves, ♦ 2.0 X, new leaves.

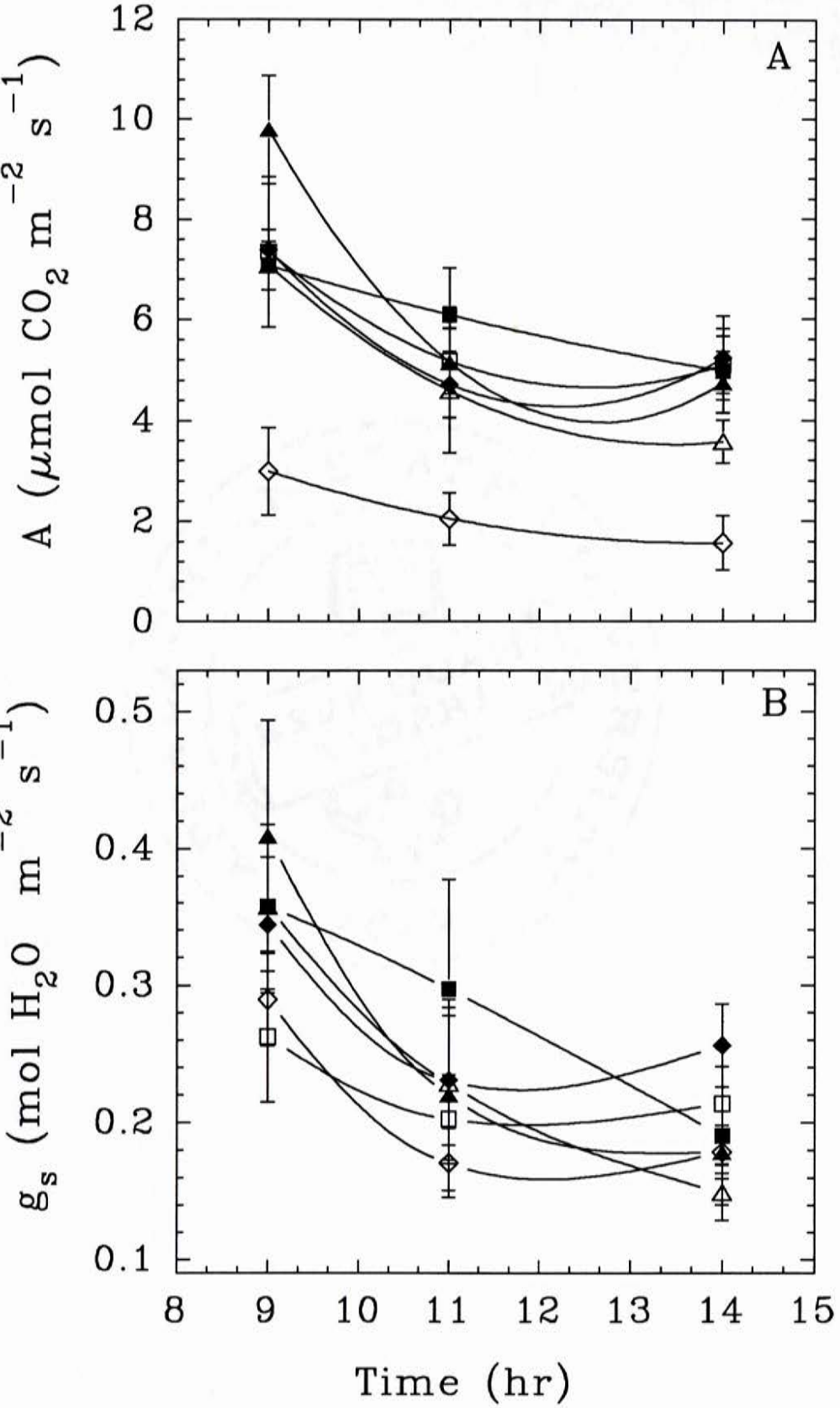

A
B
A (μmol CO_2 m^{-2} s^{-1})
g_s (mol H_2O m^{-2} s^{-1})
Time (hr)
0
2
4
6
8
10
12
0.1
0.2
0.3
0.4
0.5
8
9
10
11
12
13
14
15

Figure 14. Response of gas exchange to light - June, 1991. (A) Net photosynthesis. (B) Stomatal conductance to water vapor. Lines are smooth fits to data. Points are means of six observations ± SE. Symbols are: ▫ CF, ▪ 1.0 X, ▵ 2.0 X.

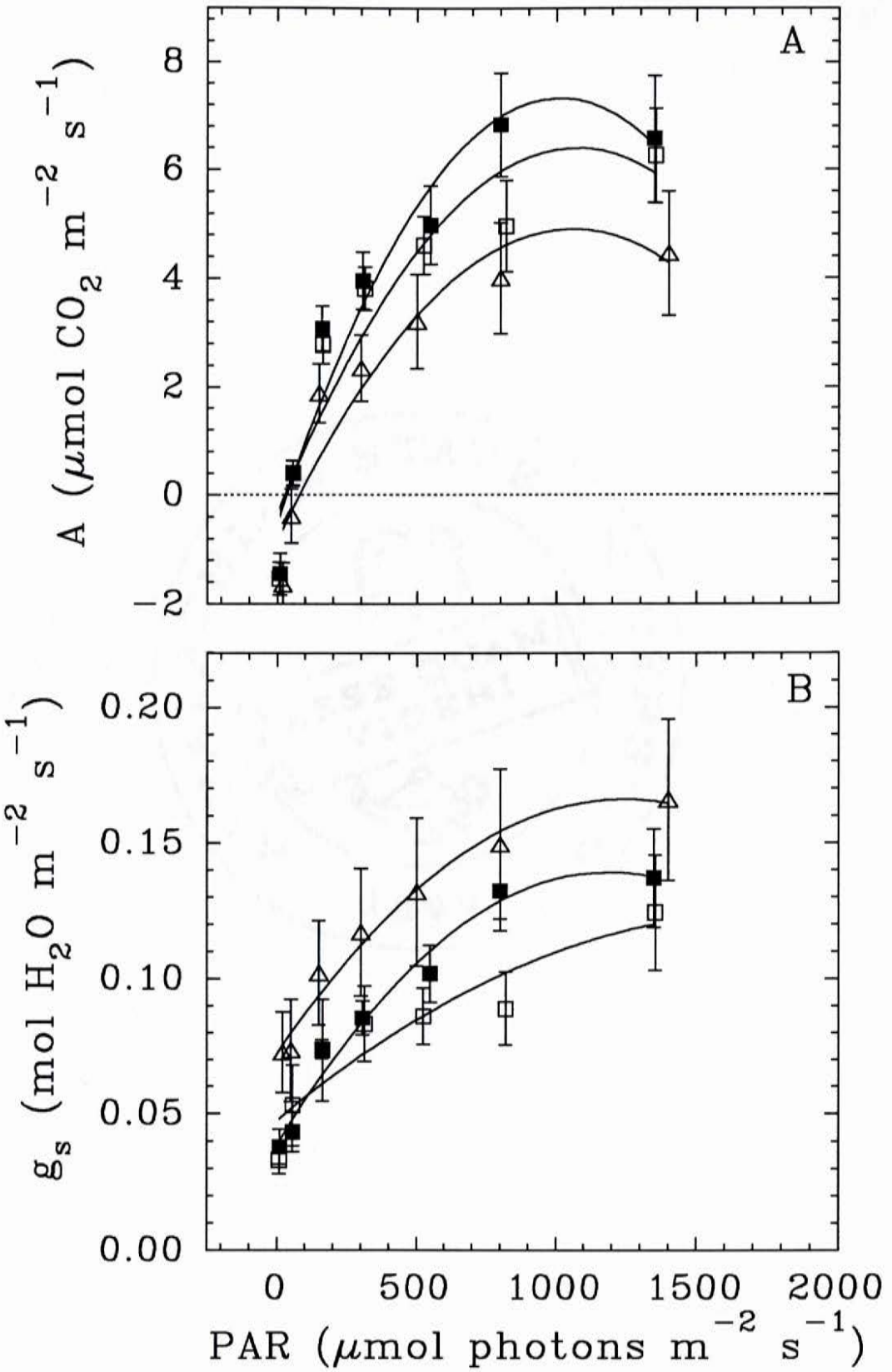

A
B
A (μmol CO_2 m^{-2} s^{-1})
g_s (mol H_2O m^{-2} s^{-1})
PAR (μmol photons m^{-2} s^{-1})
8
6
4
2
0
−2
0.20
0.15
0.10
0.05
0.00
0
500
1000
1500
2000

Figure 15. Response of gas exchange to light - August, 1991. (A) Net photosynthesis. (B) Stomatal conductance to water vapor. Lines are smooth fits to data. Points are means of six observations ± SE. Symbols are: ▫ CF, old leaves, ▪ CF, new leaves, ▵ 2.0 X, old leaves, ▲ 2.0 X, new leaves.

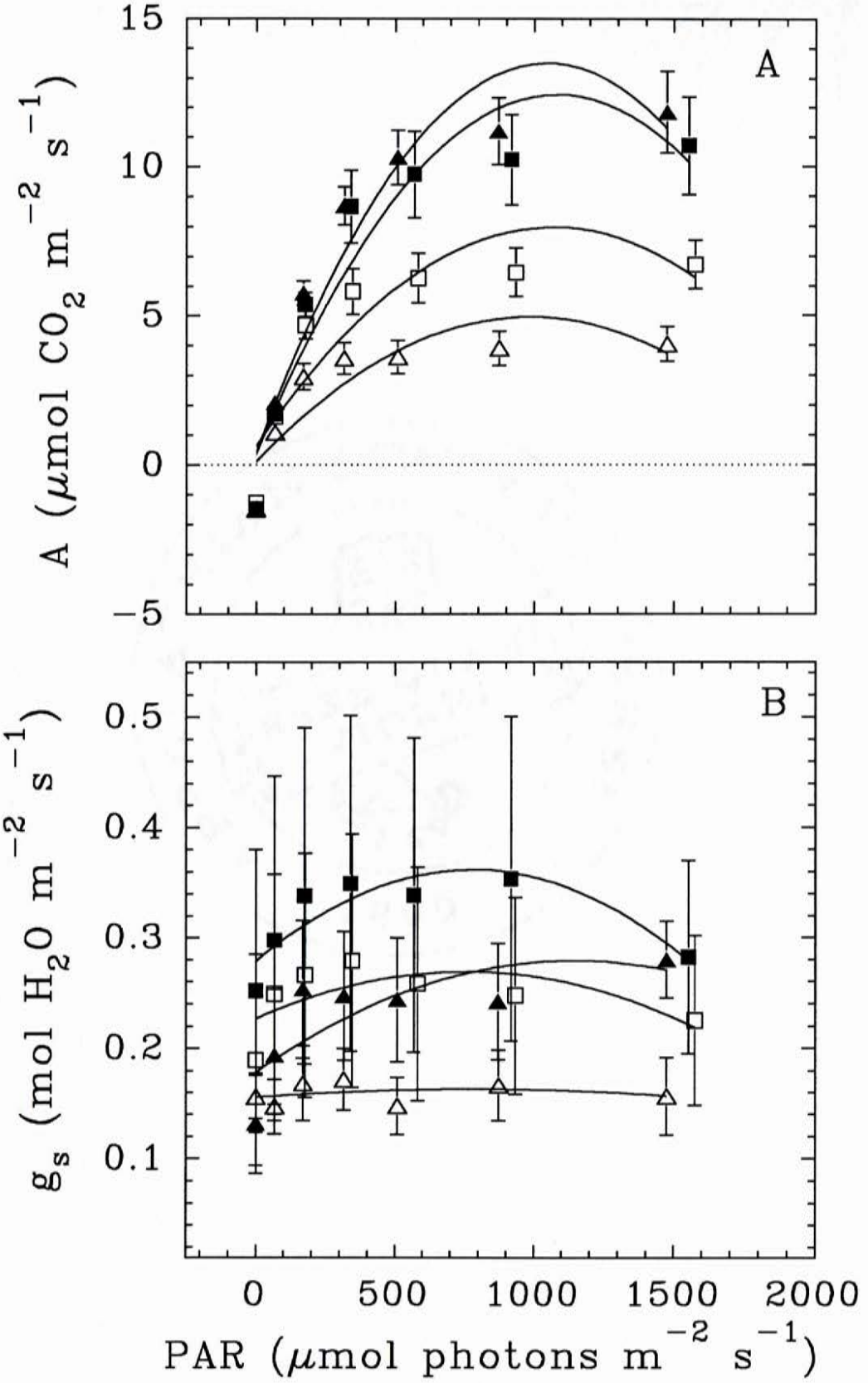

A
B
A (μmol CO_2 m^{-2} s^{-1})
g_s (mol H_2O m^{-2} s^{-1})
PAR (μmol photons m^{-2} s^{-1})
15
10
5
0
−5
0.5
0.4
0.3
0.2
0.1
0
500
1000
1500
2000

Table 9.

Summary statistics for light curve parameters - June, 1991

Variables	Ozone Treatment	Mean ± SE
A_{max} (μmol CO_2 m^{-2} s^{-1})	CF	5.99 ± 0.40[a]
	1.0 X	6.06 ± 0.57[a]
	2.0 X	3.88 ± 0.56[b]
Initial Slope (μmol CO_2/μmol photons)	CF	0.02 ± 0.002[a]
	1.0 X	0.02 ± 0.002[a]
	2.0 X	0.01 ± 0.002[a]
C_i at A_{max} (ppm)	CF	243.19 ± 6.50[a]
	1.0 X	268.11 ± 2.63[a]
	2.0 X	289.54 ± 6.04[a]
g_s at A_{max} (mol H_2O m^{-2} s^{-1})	CF	0.11 ± 0.01[a]
	1.0 X	0.12 ± 0.01[a]
	2.0 X	0.15 ± 0.02[a]
Dark Respiration Rate (μmol CO_2 m^{-2} s^{-1})	CF	-1.53 ± 0.30[a]
	1.0 X	-1.45 ± 0.38[a]
	2.0 X	-1.63 ± 0.39[a]
Light Compensation Point (μmol photons m^{-2} s^{-1})	CF	57.89 ± 6.30[a]
	1.0 X	54.30 ± 11.60[a]
	2.0 X	98.48 ± 28.44[a]

Means followed by different letters are statistically different ($p \leq 0.05$) from one another according to Duncan's Multiple Range Test.

No significant treatment effects were found for dark respiration rate, ø, light compensation point, or g_s at A_{max} (Table 9).

Later in the season, in August, there were no significant treatment effects for A_{max}, C_i at A_{max}, dark respiration rate, ø, light compensation point, or g_s at A_{max} (Table 10). However, plants in the CF treatment had higher ø, lower light compensation points, and dark respiration rates, as compared to the 1.0 X and 2.0 X treatments. Plants from the CF treatment tended to have a higher A_{max} and lower C_i at A_{max}, although the differences were not significant. Significant leaf age effects were found for ø, A_{max}, and C_i at A_{max}, with older leaves having a lower A_{max} and lower ø and higher C_i at A_{max}. No leaf age effects were detected for the light compensation point or dark respiration rate. There was a significant treatment*leaf age interaction effect for A_{max}. New leaves in the 2.0 X ambient treatment had a higher A_{max} than new leaves in CF.

CO_2 Response Curves

The two sets of CO_2 curves, measured in June and August of 1991, are shown in Figs. 16 and 17. Each data point represents an average of 6 leaves. No significant differences were detected for carboxylation efficiency (dA/dC_i), J_{max}, C_i at J_{max}, CO_2 compensation point, g_s at J_{max}, A_o, or A_a in either set of curves (Table 11). Stomatal limitation was marginally significant on the first date ($p = 0.0535$), with plants in CF having a higher stomatal limitation than plants in 2.0 X ambient. There were no differences in the degree of stomatal limitation by the

Table 10

Summary statistics for light curve parameters - August, 1991

Variables	Ozone Treatment	Age	Mean ± SE
A_{max} † (μmol CO_2 m^{-2} s^{-1})	CF	Old	6.48 ± 0.45[b]
		Young	10.23 ± 0.85[a]
	2.0 X	Old	3.84 ± 0.31[b]
		Young	11.12 ± 0.65[a]
Initial Slope† (μmol CO_2/μmol photons)	CF	Old	0.030 ± 0.003[b]
		Young	0.030 ± 0.004[a]
	2.0 X	Old	0.017 ± 0.003[b]
		Young	0.030 ± 0.002[a]
C_i at A_{max}† (ppm)	CF	Old	236.40 ± 15.22[a]
		Young	221.40 ± 10.50[b]
	2.0 X	Old	276.14 ± 8.27[a]
		Young	223.59 ± 7.92[b]
g_s at A_{max} (mol H_2O m^{-2} s^{-1})	CF	Old	0.24 ± 0.05[a]
		Young	0.33 ± 0.07[a]
	2.0 X	Old	0.16 ± 0.02[a]
		Young	0.26 ± 0.03[a]
Dark Respiration Rate (μmol CO_2 m^{-2} s^{-1})	CF	Old	-1.28 ± 0.21[a]
		Young	-1.48 ± 0.20[a]
	2.0 X	Old	-1.49 ± 0.17[a]
		Young	-1.53 ± 0.11[a]
Light Compensation Point (μmol photons m^{-2} s^{-1})	CF	Old	26.22 ± 10.52[a]
		Young	25.73 ± 6.66[a]
	2.0 X	Old	40.01 ± 11.24[a]
		Young	21.58 ± 4.24[a]

†Indicates a significant leaf age effect ($p \leq 0.05$). Means followed by different letters are statistically different ($p \leq 0.05$) from one another according to Duncan's Multiple Range Test.

Table 11

Summary statistics for CO_2 curve parameters - June, 1991

Variables	Ozone Treatment	Mean ± SE
A_a (μmol CO_2 m^{-2} s^{-1})	CF	8.45 ± 1.29
	1.0 X	7.70 ± 0.92
	2.0 X	7.16 ± 0.99
Initial Slope (mol m^{-2} s^{-1})	CF	0.06 ± 0.01
	1.0 X	0.04 ± 0.01
	2.0 X	0.04 ± 0.01
Stomatal Limitation (%)	CF	0.41 ± 0.03
	1.0 X	0.31 ± 0.31
	2.0 X	0.26 ± 0.05
Biochemical Limitation (CF-2.0 X)*100/CF (%)		15.3
J_{max} (μmol CO_2 m^{-2} s^{-1})	CF	20.71 ± 2.17
	1.0 X	19.20 ± 2.10
	2.0 X	10.07 ± 2.13
CO_2 Compensation Points (ppm)	CF	101.30 ± 13.79
	1.0 X	93.57 ± 5.20
	2.0 X	83.81 ± 6.90

Note: No treatment effects were significant for any parameters.

Figure 16. Response of gas exchange to CO_2 - June, 1991. (A) Net photosynthesis. (B) Stomatal conductance to water vapor. Lines are smooth fits to data. Points are means of six observations ± SE. Symbols are: ▫ CF, ▪ 1.0 X, ▵ 2.0 X.

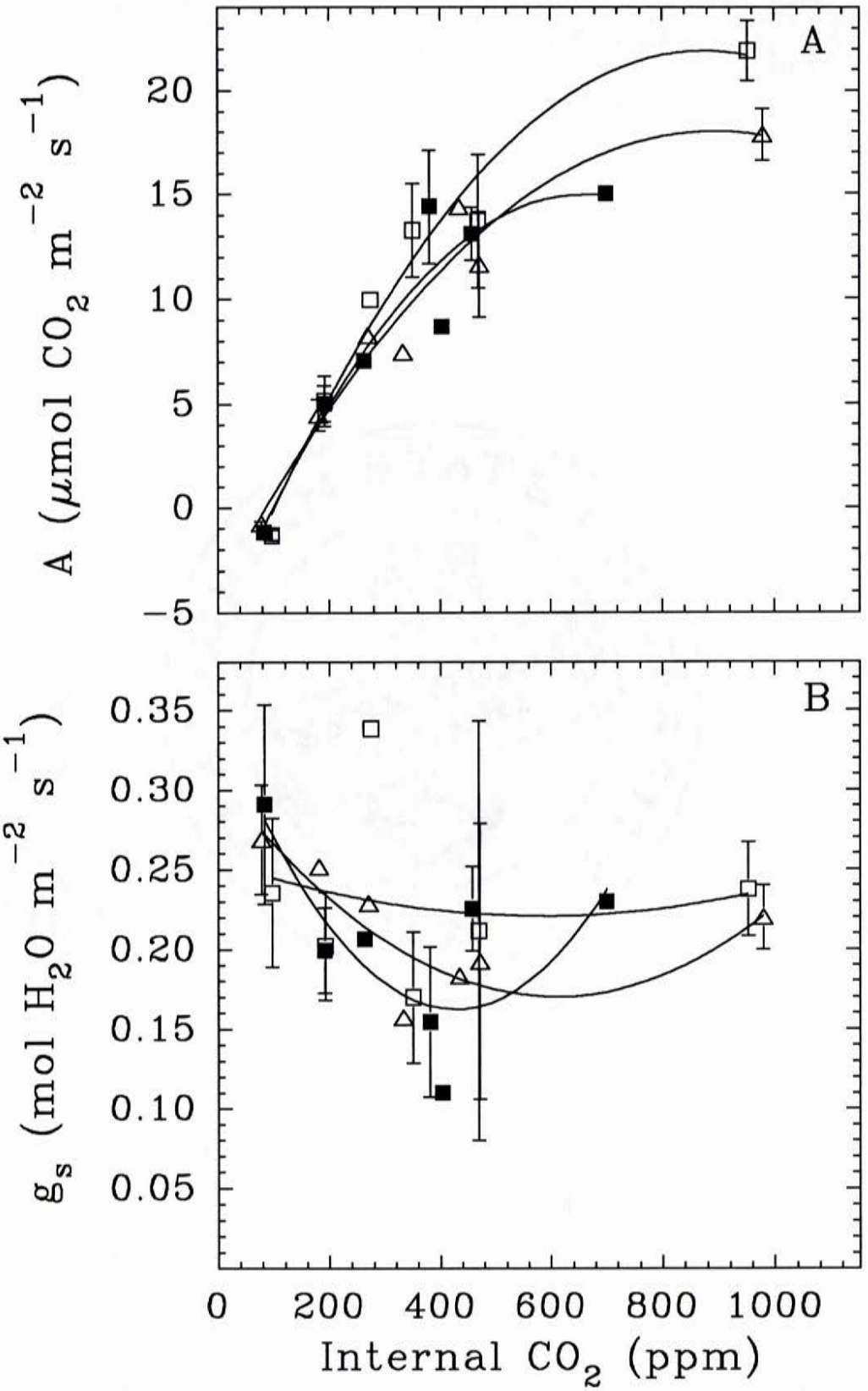
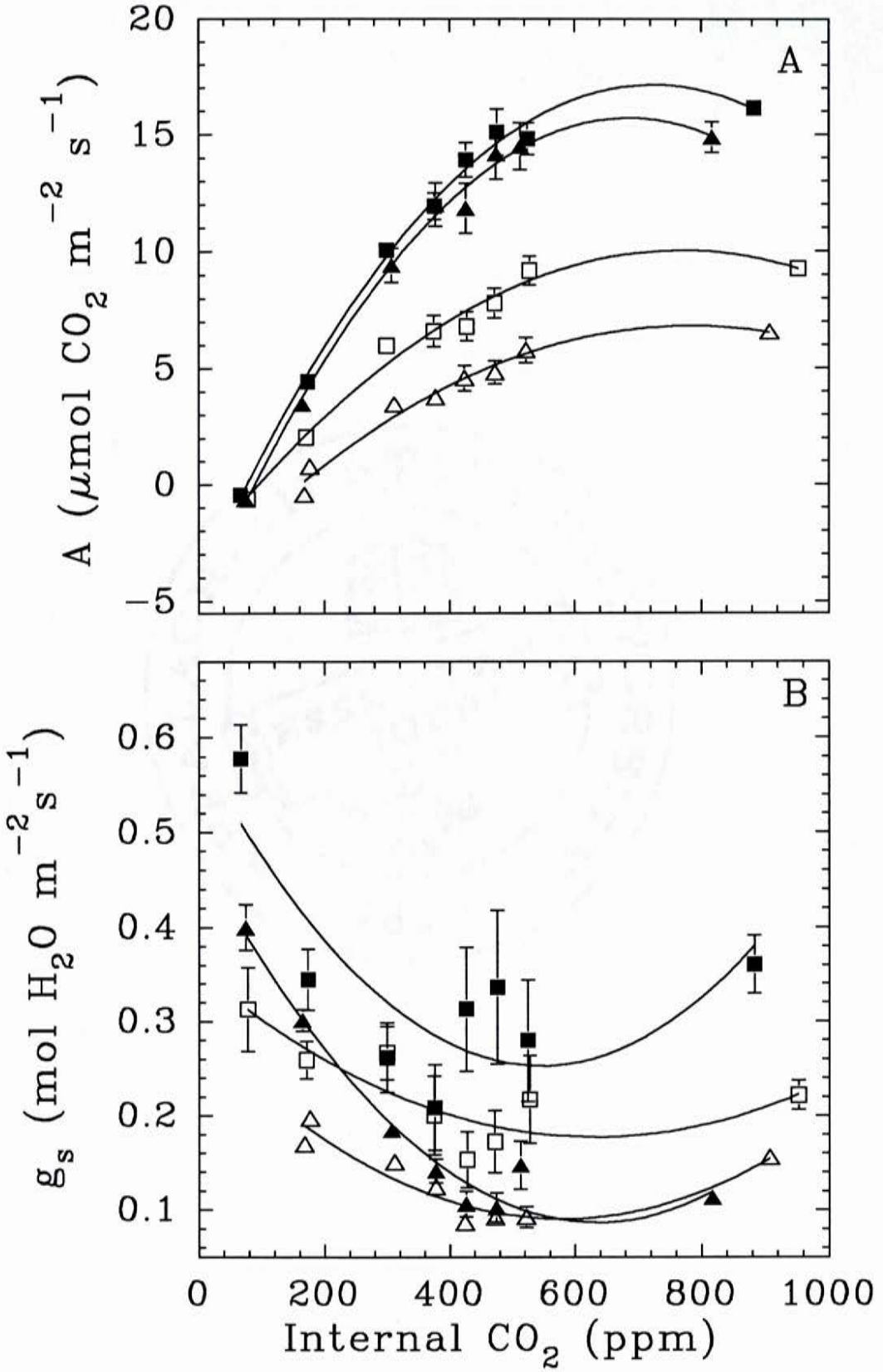

A
B
A (μmol CO_2 m^{-2} s^{-1})
g_s (mol H_2O m^{-2} s^{-1})
20
15
10
5
0
−5
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
200
400
600
800
1000
Internal CO_2 (ppm)

Figure 17. Response of gas exchange to CO_2 - August, 1991. (A) Net photosynthesis. (B) Stomatal conductance to water vapor. Lines are smooth fits to data. Points are means of six observations ± SE. Symbols are: ▫ CF, old leaves, ▪ CF, new leaves, ▵ 2.0 X, old leaves, ▲ 2.0 X, new leaves.


A
B
A (μmol CO2 m-2 s-1)
gs (mol H2O m-2 s-1)
Internal CO2 (ppm)
20
15
10
5
0
−5
0.6
0.5
0.4
0.3
0.2
0.1
0
200
400
600
800
1000

second date. The biochemical limitation between CF and 2.0 X ambient on the first date was about 15%, and was about 50% in older leaves on the later date (Table 12). CF plants had a slightly higher A_a and carboxylation efficiency at the later date. Leaf age effects were also observed in carboxylation efficiency, J_{max}, and CO_2 compensation points on this date. For example, older leaves had higher CO_2 compensation and CO_2 saturation points as compared to new leaves, while new leaves had higher carboxylation efficiencies and J_{max} than older leaves in the last measurements (Table 12).

Table 12

Summary statistics for CO_2 curve parameters - August, 1991

Variables	Ozone Treatment	Age	Mean ± SE
A_a (μmol CO_2 m^{-2} s^{-1})	CF	Old	4.9 ± 1.23[a]
		Young	5.5 ± 1.07[a]
	2.0 X	Old	2.7 ± 0.80[a]
		Young	6.1 ± 0.98[a]
Initial Slope† (mol CO_2 m^{-2} s^{-1})	CF	Old	0.02 ± 0.030[b]
		Young	0.04 ± 0.003[a]
	2.0 X	Old	0.03 ± 0.003[b]
		Young	0.05 ± 0.010[a]
Stomatal Limitation (%)	CF	Old	23 ± 10[a]
		Young	29 ± 6[a]
	2.0 X	Old	17 ± 1[a]
		Young	25 ± 3[a]
Biochemical Limitation (CF - 2.0 X)*100/CF (%)		Old	50
		Young	-0.24
J_{max}† (μmol CO_2 m^{-2} s^{-1})	CF	Old	9.00 ± 1.08[b]
		Young	14.86 ± 1.16[a]
	2.0 X	Old	5.99 ± 1.38[b]
		Young	17.18 ± 2.87[a]
CO_2 Compensation Point† (ppm)	CF	Old	87.8 ± 11.6[b]
		Young	66.6 ± 6.9[a]
	2.0 X	Old	164.6 ± 37.6[b]
		Young	77.9 ± 9.6[a]

†Indicates a significant leaf age effect ($p \leq 0.05$). Means followed by different letters are statistically different ($p \leq 0.05$) from one another according to Duncan's Multiple Range Test.

DISCUSSION

Growth and Biomass Accumulation

Height and Diameter Growth

The impact of ozone on tulip poplar seedling growth has been investigated in the past, but with conflicting results. Most of those studies were conducted using indoor fumigation facilities, such as continuously stirred tanker reactors, which are controlled environmental exposure systems. Results from these systems are only relevant to the conditions under which they were obtained and extrapolation to ambient conditions is questionable. In addition, ozone exposures on tulip poplar historically have not been realistic in either exposure amount or dynamics. Square-wave dosing regimes, with constant daytime ozone concentrations instead of realistic diurnal patterns, have often been employed. For example, in a number of studies, plants were only exposed to certain ozone concentrations for several hours a day, and for three to five days a week (Mahoney et al., 1984; Chappelka et al., 1985; Jensen and Patton, 1990). In addition, some researchers have used extraordinarily high ozone concentrations (Jensen, 1973; Kress and Skelly, 1982). Another factor affecting interpretation of these studies is that plants grown in indoor environments differ morphologically and physiologically from those grown outdoors (Lewis and Brennan, 1977). Indoor plants generally have lower leaf conductances than plants growing in the field, and therefore may

take up less ozone, making them appear less sensitive to ozone than they are in the field (Lewis and Brennan, 1977). These unrealistic ozone fumigation profiles and plant growth conditions, plus the short lengths over which the studies have been conducted, might be reasons for previous failures in detecting negative impacts of ozone on the growth of tulip poplar seedlings.

In the present study, ozone had a negative impact on both height and diameter growth. As cumulative ozone concentrations in the fumigation chambers increased from less than 7.9 ppm-hr to 60.5 ppm-hr in 1990, and the two seasons total increased from less than 19.94 ppm-hr to 172.48 ppm-hr through the end of the 1991 season, significant decreases in height and diameter were observed.

The significant time by ozone interaction indicated that the response of height and diameter to ozone differed throughout the season. In particular, ozone did not reduce height until day 47 of exposure in 1990, at which time the cumulative ozone exposure in the 1.5 X treatment was 38.2 ppm-hr. The time effects may have occurred because it took a minimum amount of ozone to allow the negative impact to become detectable (Hogsett et al., 1985; Jensen and Patton, 1991).

Tulip poplar is one of the most abundant trees in GRSM, and it ranges from an altitude of 259 m to approximately 1373 m in the Park (Stupka, 1964; White, 1982). Ozone exposures for the 1.5 X and 2.0 X treatments in this study are comparable in amount to ambient ozone concentrations found at high elevations

(1264 m) in the Park (Neufeld et al., 1992). Thus, the results of the present study suggest that under current ambient ozone conditions, height and diameter growth of tulip poplar seedlings in the Park are likely to be reduced during the first year of growth.

Biomass

Treatment effects were found for some of the biomass parameters, but these differed from one season to the next. Other studies have found few or no effects of ozone on growth (Jensen, 1973; Kress & Skelly, 1982; Mahoney et al., 1984; Chappelka et al., 1985; Chappelka et al., 1988; Tjoelker and Luxmoore, 1991). In the present study, even though certain parameters were not significantly affected by the ozone, there were consistent trends for many of those values to be reduced as ozone increased. The elimination in 1991 of the total dry weight effect found in 1990 may have resulted from the effect of root binding (a result of being grown in the PVC pots) on these large trees (average heights for trees in CF and 2.0 X in mid-summer 1991 were around 130 cm and 75 cm respectively). Root-binding has been shown to close stomata, and to affect shoot growth (Thomas and Strain, 1991). This may have reduced ozone uptake by the smaller trees in the 2.0 X ambient treatment, making them less sensitive to ozone, allowing them to grow more than expected, or, conversely, may have reduced growth of the larger trees in the lower ozone treatments. Either way, the trend would be to make the treatment effects smaller. Further studies should

look at responses of trees rooted in the ground to avoid this potentially confounding factor.

It is known that the leaves on a tree are very sensitive to environmental changes (Dickson and Isebrands, 1991) and the present study seems to provide some evidence of this. Significant reductions in total leaf number in both seasons were caused by ozone-induced premature leaf abscission. In the second year, the number of branch leaves was reduced more than the number of main stem leaves (total leaf number was not divided into main stem leaves or branch leaves in the first season). This might be because there were many branches, containing the majority of the older leaves, and which provided a larger sample size. Thus, treatment effects were easier to detect for this biomass fraction. At the end of August 1991, a parallel leaf longevity study (not included in this thesis) showed that 16 out of 26 trees in the above ambient treatments had completely lost their oldest main stem leaves (the first to third leaf formed after fumigation started) while similarly-aged leaves on trees grown in the other treatments were still viable. Tjoelker and Luxmoore (1991) also found that tulip poplar was highly sensitive to ozone-induced leaf abscission. Elevated ozone exposure increased leaf abscission to an average of 37% of total leaf production, relative to 12% at the ambient level. They also found high leaf turnover rates (total leaf production/final harvest standing leaf biomass) in the high ozone treatment. Their work indicated that average leaf lifespans were reduced by half at above ambient ozone levels. Jensen and Patton (1990) also found that seedlings in the

highest ozone treatment (average ozone concentration = 0.2 ppm) had the fewest leaves, and that they tended to lose their leaves faster. Ozone effects on leaf drop are widespread among tree species (Mann et al. 1980; Noble and Jensen, 1980; Coyne and Bingham, 1982; McLaughlin et al. 1982; Reich and Lassoie, 1985; Allen et al. 1992). Constantinidou and Kozlowski (1979) found that the reduction in leaf number for *Ulmus americana* seedlings was not due to reduced leaf production, but rather, a higher rate of leaf loss, as found in the present study.

In addition to a reduction in leaf number, ozone reduced total plant leaf area on tulip poplar seedlings. The response of leaf area to ozone was different over time in the present study. In the first season, large variations in leaf area within treatments (CV=134.3%) prevented the finding of any statistically significant reductions due to ozone, even though values were lower in the higher ozone treatments. Significant reductions in the second year were due to much smaller sample variation (CV=31%). It is not clear why the variation was so much higher in 1990. If the visible foliar damage found on the older leaves in the higher ozone treatments is taken into consideration (results not included in this thesis), the functional leaf area that could be used for photosynthesis would be reduced even further, and the treatment differences would be even more statistically significant.

The significant impairment in total leaf weight in both years could have been due to two factors: reduced leaf number and ozone-induced reductions in leaf

structural and nonstructural carbohydrate levels (Constantinidou and Kozlowski, 1979). As SLM was not reduced in the present study, lowered leaf weight was caused mainly by the loss of leaf material itself. Reduction in aboveground dry weights were due primarily to the loss in leaf weight, rather than wood weight, since wood weight was not significantly reduced.

It is known that leaves and roots are functionally integrated and that root growth is responsive to changes in carbon allocation within shoots. In actively growing plants, roots are considered as weak sinks and tend to receive assimilates primarily from lower leaves after developing leaf demands are met (Dickson and Isebrands, 1991). Assimilates stored in roots may even be re-located to shoots under stress (Huber, 1983), causing reductions in the root to shoot ratio. Plants under ozone stress preferentially allocate carbon to shoots at the expense of roots (Cooley and Manning, 1987; Pye, 1988). The significant linear reductions in total root weight in 1990, and the marginally significant reductions in 1991 ($p=0.08$) might be the result of the loss of older leaves (Cooley and Manning, 1987). The reason why tap root growth was reduced more than that of fine roots is that tap root growth tends to follow older leaf growth in trees (Dickson and Isebrands, 1991). Another consideration is that growth and turnover of fine roots is believed to be largely independent of shoot growth (Johnson-Flanagan and Owens, 1985; Eis, 1986) and more dependent on root environment (Marshall and Waring, 1985; Kuhns et al., 1985; Eissenstat and Caldwell, 1988).

The lack of any significant differences in the root to shoot ratio shows that root and shoot growth were similarly impaired in the first year of exposure, and that there were few reductions in the second year. This observation contrasts somewhat with previous reports for tree seedlings (Hogsett et al., 1985; Chappelka and Chevone, 1986), but is consistent with others (Neufeld et al., 1992).

Reduced leaf number, leaf weight and particularly leaf area, together with the reduced quantity of photosynthetically active tissue in the higher ozone treatments, may have been important factors contributing to the reduced seedling height and diameter growth in this study. Other studies seem to support this explanation. For example, Allen et al. (1992) proposed that ozone's negative effect on foliage longevity, and consequently reduced leaf area duration in loblolly pine, appeared to be the dominant factors governing its effects on growth. Mann et al. (1980) suggested that inhibition of growth in their study of white pine (*Pinus strobus* L.) was associated with premature needle loss and decreased retention of photosynthetic tissue, rather than inhibition of photosynthesis of existing foliage.

Chlorophyll Contents

Chlorophyll contents were reduced by ozone only late in the 1991 season (September). Since ozone is unlikely to penetrate past the cell plasmalemma before exerting an effect (Heath, 1980), it is likely that secondary reaction

products and the consequences of ozone oxidations caused reductions in chlorophyll content in leaves exposed to ozone (Reich, 1983; Sasek and Richardson, 1989). It is known that lipids and proteins are targets of degradation during leaf senescence and that ozone induces early leaf senescence (Pell and Dann, 1991). One of the targets could be the chlorophyll-protein complex, where chlorophyll oxidation would lead to pigment destruction (Pell and Dann, 1991). Ozone-induced leaf senescence likely leads to reduced chlorophyll contents as a result of enzymatic degradation.

Gas Exchange

Diurnal Patterns

The diurnal course of net photosynthesis did not show any ozone inhibition until very late in the season in 1991. This reduction, which was found only in mid-September, was due to reduced rates in older leaves in the 2.0 X treatment. Since no significant stomatal conductance differences were found due to ozone exposure under these field conditions, reduced rates of photosynthesis were probably biochemical in origin, the result of ozone-accelerated aging in older leaves. Reich (1983) found that the effects of ozone were greatest in older leaves in hybrid poplar and proposed that ozone-accelerated-aging was at least partially responsible for those lower rates. The lack of a significant ozone effect among new leaves in tulip poplar may be the result of exposure to less ozone, since leaf responses to ozone are dependent on the cumulative exposure received (Reich,

1987; Tjoelker and Luxmoore, 1991). Another explanation is that decreased sensitivity to ozone occurred because of acclimation. Walmsley et al. (1980) found that when radish plants were continuously fumigated, successive new leaves became less ozone sensitive, suggesting that leaves in certain species of plants may acclimate to the prevailing ozone conditions. It is not possible to distinguish between these two hypotheses in the present study.

Previous studies on tulip poplar seedlings have not shown any significant differences in photosynthesis or stomatal conductance as a result of fumigation with ozone (Jensen and Patton, 1990; Roberts, 1990; Tjoelker and Luxmoore, 1991). This was probably because only young leaves were used. These are frequently the most recently expanded leaves, and may not be very sensitive to ozone. In the present study two different aged leaves were examined. Only older leaves showed lower rates of photosynthesis. Thus future investigations should take into account leaf age when assessing ozone effects on leaf photosynthesis.

Since this study did not show any ozone effects on photosynthesis during most of the season, these results and those of Reich et al. (1986) suggest that repeated, instantaneous measurements of photosynthesis on one or two leaves per plant do not adequately reflect total plant photosynthetic rates after exposure to ozone. Tulip poplar is a continuously flushing species, with all-age populations of leaves. Differently aged leaves may have varying responses to ozone. This would make it difficult to explain whole-plant responses based on a limited

sampling of just a few leaves per plant, sampled on a limited number of days each season (Zelitch, 1971, 1982). For example, Reich (1983) found that in hybrid poplar, ozone treated leaves reached their maximum rates of photosynthesis just prior to full expansion in the high ozone treatment while leaves in the control treatment reached their peak rate of photosynthesis at full expansion. Most of the measurements of photosynthesis in the present study were conducted on green or partly chlorotic leaves, while many other leaves in the higher ozone treatments were visibly senescent. Therefore the rates of photosynthesis measured may not reflect the values for all older leaves, since senescing leaves would likely have lower rates of photosynthesis (Reich et al., 1986).

Diagnostic Gas Exchange

Diagnostic measurements were used to test the impact of ozone on various physiological aspects of the photosynthetic process. Additionally, since these measurements were conducted inside the lab instead of the fumigation chambers, they were used to test for residual damage to the photosynthetic apparatus caused by ozone. Responses of different aged leaves were measured in the second set of diagnostic curves to see if young and old leaves responded differently to ozone.

(1) Ozone Effect on Stomatal Conductance

Stomatal conductance was not significantly affected by ozone in most of the diagnostic studies, a result consistent with that found in the diurnal curves. However, in the CO_2 response curves, there did appear to be a greater stomatal limitation on photosynthesis in CF than in 2.0 X ambient, especially in the first CO_2 curves (P=0.054). Therefore, ozone apparently did not cause stomatal closure in 2.0 X ambient; in fact, it may have increased stomatal conductance for seedlings in that treatment. Similar observations were obtained by Taylor and Dobson (1989) for beech (*Fagus sylvatica*). Second flush leaves grown in CF air had a lower g_s than leaves on trees grown in unfiltered air. No explanation was offered by the authors for why g_s was higher in polluted as opposed to clean air. In the present study, it appears that ozone did not cause closure of plant stomata or that it any potential effect was short-lived.

(2) Effect of Ozone on Photochemical Process

The present study showed a trend for ozone to impair photochemical reactions carried out by PSI and/or PSII, although the treatment differences were not always statistically significant. There are two parameters in the diagnostic gas exchange measurements that can indicate an effect of ozone on light-dependent electron transport: the apparent quantum efficiency (ø) and J_{max}, the maximum photosynthetic capacity, an indicator of the RUBP regeneration rate. The ø of photosynthesis at 2.0 X ambient treatment was reduced in comparison to CF on

the first date, indicating impaired electron transport on this date. By the second date, those leaves used in the first curves (now referred to as the older leaves) had lower values in the 2.0 X ambient treatment as compared to new leaves (most recently fully expanded leaves). However, even though these older leaves received an additional exposure of 67.3 ppm-hr, the reduction in ø was only 5% greater at this later time. Thus these reductions in ø appeared to be fairly constant during the season and not a function of increasing ozone exposure. Since the decline in ø was maintained in the absence of fumigation, it suggests an irreversible or slowly recoverable effect by ozone. That ozone can affect the ø is supported by other researchers. Sasek and Richardson (1989) found that for loblolly pine ø was reduced by exposure to high ozone. They found a 38% reduction at 2.0 X ambient as compared to CF. Ozone fumigation caused similar reductions in the ø in poplar trees (Reich, 1983). von Caemmermer and Farquhar (1981) indicated that J_{max} is mostly limited by the amount of ATP and NADPH, which are products from the light reactions. The decreases in J_{max} in older tulip poplar leaves exposed to ozone suggests that less ATP and NADPH were available, thereby reducing the activity of the Tricarboxylic Acid Cycle (TCA cycle, or Calvin cycle).

Both reduced ø and J_{max} in older tulip poplar leaves suggests that elevated ozone may have caused changes in the efficiency of the light reactions of photosynthesis. Davison et al. (1988) suggest that the production of free radicals from ozone exposure may impair electron transport in the light reactions. A

reduced ø could be due to a decrease in photolysis (Schreiber et al. 1978; and Shimazaki, 1988), impaired plastoquinone reoxidation, and reduced electron flow from the water-splitting component of photosystem II (Barnes et al. 1988), or a reduction in chlorophyll content. The exact reasons for the decline of ø and J_{max} in this study need further investigation. Chlorophyll content measurements taken at the same time as when J_{max} was significantly reduced did not show any declines across treatments, and therefore, it is unlikely that the reduced photochemical efficiency at this sampling time was a result of the loss of chlorophyll.

(3) Effect of Ozone on Biochemical Process

A_{max} in the light curves, and initial slopes in the CO_2 curves are indicators of the level of activity of the dark reactions in photosynthesis. In the first set of light curves, A_{max} was significantly impaired in the higher ozone treatment, but did not show an ozone treatment effect in the second set of light curves. However, since most leaves measured in the first curve were used as the older aged leaves in the second curves, there was a reduction in A_{max} in 2.0 X as compared to CF. The lower A_{max} for older leaves suggests that biochemical processes of photosynthesis in older leaves have been impaired by ozone. Reduced A_{max} might be caused by the following possible reasons: stomatal closure (Temple, 1986), direct damage to the dark reactions, accelerated leaf aging, or stimulated dark respiration rates (Reich, 1983). Since significant reductions in A_{max} in the 2.0 X treatment occurred without any reductions in g_s, stomatal

closure was unlikely responsible for the lower A_{max}. This suggests that reductions in the dark reactions, or increases in the dark respiration rate are responsible for the lowered A_{max}. The damaging effects of ozone on the dark reactions could be related to its negative impact on RUBISCO, the carboxylating enzyme in the TCA cycle. Ozone has been reported to reduce RUBISCO activity and quantity in several species (Dann and Pell, 1989). The initial slopes of the CO_2 curves, which are crude indicators of the efficiency of carboxylation (i.e. amount of active RUBISCO), decreased 33% in 2.0 X ambient compared to CF in June ($p=0.07$), but there were no differences in August ($p=0.8$). The decrease in initial slopes suggests that exposure to ozone might have reduced carboxylation activity in June, but the activity or amount of RUBISCO recovered somewhat by August. I have no explanation for why RUBISCO activity would increase later in the season.

Biochemical limited photosynthesis did show a reduction of 15% at A_a in both treatments if age differences were omitted, and caused a 50% reduction in older leaves if one compares CF and 2.0 X ambient in August. Since older leaves measured in August were used in the previous measurements, this means that they had an additional 35% reduction after two more months fumigation (additional ozone: 67.3 ppm-hr). This again indicates that the dark reactions in older leaves were impaired.

A_a for new leaves on plants in 2.0 X was 19% higher than both new and older leaves in CF. There are several possible reasons for this apparent

stimulation of photosynthesis by ozone. First, older leaves in the high ozone treatment were undergoing senescence and nutrients, especially N (released from degradation of RUBISCO) might have been retranslocated to young leaves and made available for young leaf photosynthesis (Dann and Pell, 1989). Second, new leaves might have acclimated to ozone stress (Atkinson et al. 1988; Walmsley et al. 1980). Finally, hormonal alterations, due to the loss of older leaves, might also have affected photosynthetic rates (Meinzer et al., 1991).

(4) Effects of Ozone on Dark Respiration

Studies show that dark respiration rates in plants are often stimulated by ozone exposure (Barnes, 1972; Skärby et al., 1987). In the present study, dark respiration rates were slightly higher (but not significantly) for older leaves in the high ozone treatment as compared to CF. Stimulated dark respiration rates can be caused by repair of ozone injury, which involves energy consuming processes (Amthor, 1988). Ozone may also result in a decrease in respiratory efficiency, such that to perform the same respiratory functions requires an increase in respiration rates. Ozone exposure is known to enhance ethylene production (Mehlhorn and Wellburn, 1987; Heath, 1988) and enhanced biosynthesis of stress ethylene may cause a rise in respiratory rates (Amthor, 1988). Since in this study I did not find a significant stimulation of the dark respiration rate, I hypothesize that the reductions found in photosynthesis in this study were not caused by increased dark respiration rates, but rather, by direct biochemical effects of ozone

or its byproducts on the photosynthetic mechanisms, accelerated leaf aging, or both.

Overall, in the gas exchange studies, reduced photosynthesis was found mainly in the older leaves on the later dates. Stomatal conductance was more insensitive than photosynthesis to ozone exposure. Both the dark and light reactions of photosynthesis were impaired by ozone exposure, and older leaves showed more impairment. Since stomatal conductance was not affected by ozone in this study, physiological impairment of photosynthesis and leaf abscission appear to be the main reasons for altered carbon assimilation in tulip poplar seedlings. However, to what extent the lowered rates of photosynthesis contributed to the growth reductions remains difficult to answer. As mentioned earlier, this is because of the poor correlations that often exist between short term measurements of the rate of photosynthesis and growth (Zelitch, 1971, 1982; Kozlowski et al., 1991). The poor correlation is believed partly due to the fact that dry matter production depends on leaf area and leaf area duration (as discussed in the introduction) while photosynthesis is usually measured on only a few leaves or branches a few times during the growing season (Kozlowski et al., 1991). However, Taylor and Dobson (1989) indicated that small changes in gas exchange may nevertheless be very important for woody species if they are consistent or are repeated for many years, because they eventually will lead to significant alterations in biomass accumulation and allocation.

In summary, during two years of ozone fumigation, tulip poplar was found to be a very sensitive species in terms of visible injury and leaf abscission. However, if one considers height and diameter growth, biomass accumulation and allocation, and gas exchange patterns, tulip poplar should be considered only moderately sensitive to ozone.

Conclusions

This is the first study on tulip poplar to investigate the mechanisms by which photosynthesis is affected by ozone. It is also the first known report to demonstrate that ozone may cause significant reductions in height, diameter, biomass and rates of photosynthesis in tulip poplar seedlings under long-term ozone fumigation in open-top chambers. Nevertheless, extrapolation of these seedling responses to mature trees, especially in field situations, remains difficult, particularly when one considers the differences in environmental conditions and growth behavior between seedlings and mature trees. Environmental conditions for seedlings and mature trees, such as net radiation, wind speed, and air and leaf temperatures, decrease from above to below the canopy, while humidity and CO_2 concentrations tend to increase. Such environmental changes may affect a plant's sensitivity to ozone. In addition, ozone concentrations may be depleted as air passes through the tree canopy (Pye, 1988; Neufeld et al., 1992). Also, trees growing in the field are more likely to experience nutrient and water stresses, thus altering their susceptibility to ozone (Harkov and Brennan, 1980; Chappelka

and Chevone, 1992). Seedlings and mature trees may also have different growth behaviors, such as different patterns of photosynthate transport and allocation, and overall leaf-stem-root relations, making it difficult to extrapolate responses to mature trees. Nevertheless, experiments on seedlings do provide convincing evidence that ozone can exert significant affects on growth (Pye, 1988). In the future, in order to evaluate the ecological impacts of ozone on forests, researchers should scale the effects of ozone up to mature trees, and eventually, to the ecosystem level.

LITERATURE CITED

Allen, H.L., T.K. Stow, A.H. Chappelka, L.W. Kress, and R.O. Teskey. 1992. Ozone impacts on foliage dynamics of loblolly pine. *In* R. Floger [ed.], The response of southern commercial forests to air pollution, Air and Waste Management Association, Pittsburgh, PA (In press).

Altshuller, A.P. 1986. The role of nitrogen oxides in non-urban ozone formation in the planetary boundary layer over North America, Western Europe and adjacent areas of ocean. *Atmosphere Environment* 20: 245-268.

Amthor, J.S. 1984. The role of maintenance respiration in plant growth. *Plant, Cell and Environment* 7: 561-569.

Amthor, J.S. 1986. Evolution and applicability of a whole plant respiration model. *Journal of Theoretical Biology* 122: 473-490.

Amthor, J.S. 1988. Growth and maintenance respiration in leaves of bean (*Phaseolus vulgaris* L.) exposed to ozone in open-top chambers in the field. *New Phytologist* 110: 319-325.

Amthor, J.S., and J.R. Cumming. 1988. Low levels of ozone increase bean leaf maintenance respiration. *Canadian Journal of Botany* 66: 724-726.

Atkinson, C.J., S.V. Robe and W.E. Winner. 1988. The relationship between changes in photosynthesis and growth for radish plants fumigated with SO_2 and O_3. *New Phytologist* 110: 173-184.

Barnes, L.L. 1972. Effect of chronic exposure to ozone on photosynthesis and respiration of pines. *Environmental Pollution* 3: 133-138.

Barnes, J.D., K. Reiling, A.W. Davison, and C.J. Renner. 1988. Interactions between ozone and winter stress. *Environmental Pollution* 53: 235-254.

Benoit, L.F., J.M. Skelly, L.D. Moore, and L.S. Dochinger. 1982. Radial growth reductions of *Pinus strobus* L. correlated with foliar ozone sensitivity as an indicator of ozone induced losses in eastern forests. *Canadian Journal of Forest Research* 12: 673-678.

Chameides, W.L., and J.P. Lodge. 1992. Troposphere ozone: formation and fate. *In* L.S. Allen [ed.], Surface Level Ozone Exposures and Their Effects on Vegetation. pp. 5-30. Lewis Publishers, Inc., Chelsea, MI.

Chappelka, A.H., and B.I. Chevone. 1986. White ash seedling growth response to ozone and stimulated acid rain. *Canadian Journal of Forest Research* 16: 786-790.

Chappelka, A.H., B.I. Chevone, and T.E. Burk. 1985. Growth response of yellow-poplar (*Liriodendron tulipifera* L.) seedlings to ozone, sulfur dioxide, and simulated acidic precipitation, alone and in combination. *Environmental and Experimental Botany* 25: 233-244.

Chappelka, A.H., B.I. Chevone, and J.R. Seiler. 1988. Growth and physiological response of yellow-poplar seedlings exposed to ozone and stimulated acidic rain. *Environmental Pollution* 49: 1-18.

Chappelka, A.H., and B.I. Chevone. 1992. Tree responses to ozone. *In* L.S. Allen [ed.], Surface Level Ozone Exposures and Their Effects on Vegetation. pp. 271-325. Lewis Publishers, Inc., Chelsea, MI.

Chevone, B.I., J.M. Skelly, and Y.S. Yang. 1983. The influences of ambient pollution on the growth of seedling forest trees and native vegetation. *Aquilo Series of Botany* 19: 198-207.

Constantinidou, H.A., and T.T. Kozlowski. 1979. Effects of sulfur dioxide and ozone on *Ulmus americana* seedlings. II. Carbohydrates, proteins and lipids. *Canadian Journal of Botany* 57: 176-184.

Cooley, D.R., and W.J. Manning. 1987. The impact of ozone on assimilate partitioning in plants: A review. *Environmental Pollution* 47: 95-113.

Coulson, C., and R.L. Heath. 1974. Inhibition of photosynthetic capacity of isolated chloroplasts by ozone. *Plant Physiology* 53: 32-38.

Coyne, P.I., and G.E. Bingham. 1981. Comparative ozone dose response of gas exchange in a ponderosa pine stand exposed to long-term fumigations. *Journal of the Air Pollution Control Association* 28: 1119-1123.

Coyne, P.I., and G.E. Bingham. 1982. Variation in photosynthesis and stomatal conductance in an ozone-stressed Ponderosa Pine stand: Light response. *Forest Science* 28: 257-273.

Dann, M.S., and E.J. Pell. 1989. Decline of activity and quantity of ribulose bisphosphate carboxylase/oxygenase and net photosynthesis in ozone-treated potato foliage. *Plant Physiology* 91: 427-432.

Davison, A.W., J.D. Barnes, and C.J. Renner. 1988. Interactions between air pollution and cold stress. *In* S. Schulte-Hostede, N.M. Darrall, L.W. Blank and A.R. Wellburn [eds.], Air Pollution and Plant Metabolism. pp. 307-328. Elsevier, London, UK.

Dickson, R.E. and J.G. Isebrands. 1991. Leaves as regulators of stress response. *In* H.A. Mooney, W.E. Winner, and E.J. Pell [eds.], Responses of Plants to Multiple Stresses. pp. 3-33. Academic Press, Inc., San Diego, CA.

Duchelle, S.F., J.M. Skelly, and B.I. Chevone. 1982. Oxidant effects on forest tree seeding growth in the Appalachian Mountains. *Water, Air, and Soil Pollution* 18: 363-373.

Edwards, N.T., G.E. Taylor, M.B. Adams, G.L. Simmons, and J.M. Kelly. 1990. Ozone, acid rain and soil magnesium effects on growth and foliar pigments of *Pinus taeda* L. *Tree Physiology* 6: 95-104.

Eis, S. 1986. Differential growth of individual components of trees and their interrelationships. *Canadian Journal of Forest Research* 16: 352-359.

Eissenstat, D.M., and M.M. Caldwell. 1988. Seasonal timing of root growth in favorable microsites. *Ecology* 69: 870-873.

Evans, L.S., and I.P. Ting. 1974. Ozone sensitivity of leaves: Relationship to leaf water content, gas transfer resistance, and anatomical characteristics. *American Journal of Botany* 61: 592-587.

Farquhar, G.D., S. von Caemmerer, and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* 149: 78-90.

Farquhar, G.D., and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* 33: 317-345.

Farrar, J.F., and F.W. Rayns. 1987. Respiration of leaves of barley infected with powdery mildew: Increased engagement of the alternative oxidase. *New Phytologist* 107: 119-125.

Harkov, R. and E. Brennan. 1980. The influence of soil fertility and water stress on the ozone response of hybrid poplar trees. *Phytopathology* 70: 991-994.

Heagle, A.S., R.B. Philbeck, and W.W. Heck. 1973. An open-top chamber to assess the impact of air pollution on plants. *Journal of Environmental Quality* 2: 365-368.

Heath, R.T. 1980. Initial events in injury to plants by air pollutants. *Annual Review of Plant Physiology* 31: 395-431.

Heath, R.T. 1988. Biochemical mechanisms of pollution stress. *In* W.W. Heck, O.C. Taylor and D.T. Tingey [eds.], Assessment of Crop Loss from Air Pollutants. pp. 259-286. Elsevier Applied Science, London, England.

Hill, A.C., and N. Littlefield. 1969. Ozone-effect on apparent photosynthesis, rate of transpiration, and stomatal closure in plants. *Environmental Science & Technology* 3: 52-56.

Hogsett, W.E., M. Plocher, V. Wildman, D.T. Tingey, and J.P. Bennett. 1985. Growth response of two varieties of slash pine seedlings to chronic ozone exposures. *Canadian Journal of Botany* 63: 2369-2376.

Huber, S.C. 1983. Relation between photosynthetic starch formation and dry-weight partitioning between the shoot and root. *Canadian Journal of Botany* 61: 2709-2716.

Jackson, J.E. 1963. Relationship of relative leaf growth rate to net assimilation rate and its relevance to the physiological analysis of plant yield. *Nature* 200: 909.

Jensen, K.F. 1973. Response of nine forest tree species to chronic ozone fumigation. *Plant Disease Reporter* 57: 914-917.

Jensen, K.F. 1983. Growth relationships in silver maple fumigated with O_3 and SO_2. *Canadian Journal of Forest Research* 13: 298-302.

Jensen, K.F. 1985. Response of yellow-poplar seedlings to intermittent fumigation. *Environmental Pollution* 38: 183-191.

Jensen, K.F., and R.L. Patton. 1990. Response of yellow-poplar (*Liriodendron tulipifera* L.) seedlings to simulated acid rain and ozone 1. Growth modifications. *Environmental and Experimental Botany* 30: 59-66.

Johnson-Flanagan, A.M., and J.N. Owens. 1985. Development of white spruce (*Picea glauca*) seedling roots. *Canadian Journal of Botany* 63: 456-462.

Kozlowski, T.T., P.J. Kramer, and S.G. Pallardy. 1991. The Physiological Ecology of Woody Plants. Academic Press, Inc., San Diego, CA.

Krause, G.H.M., U. Arndt, C.J. Brandt, J. Bücker, G. Krenk, and E. Matzner. 1986. Forest decline in Europe: Development and possible causes. *Water, Air and Soil Pollution* 31: 647-668.

Krause, G.H.M. 1988a. Forest decline - A European ecological challenge. The International Congress on Nature Management and Sustainable Development, 6.9.12. 1988, University of Groningen, P.O. Box 1141, NL-9701 BC Groningen, The Netherlands.

Krause, G.H.M. 1988b. Impact of air pollution on above-ground plant parts on forest trees. *In*: P. Mathy [ed.], Air Pollution and Ecosystems, pp. 168-216. Proceedings of the International Symposium, Grenoble, France 18.22.5, 1987. D. Reidel Publishing Company, Dordrecht.

Kress, L.W., and J.M. Skelly. 1982. Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. *Plant Disease* 66: 1149-1152.

Kress, L.W., H.L. Allen, J.E. Mudano, and W.W. Heck. 1988. Response of loblolly pine to acidic precipitation and ozone. *In* Proceedings of the 81st annual meeting of the air pollution control association, pp19-24 June, 1988, Dallas, TX, Air pollution Control Association, Pittsburgh, PA.

Krupa, S.V., and W.J. Manning. 1988. Atmospheric ozone: Formation and effects on vegetation. *Environmental Pollution* 50: 101-137.

Kuhns, M.R., H.E. Garrett, R.O. Teskey, and T.M. Hinckley. 1985. Root growth of black walnut trees related to soil temperature, soil water potential, and leaf water potential. *Forest Science* 31: 617-629.

Li-Cor Application Note #103. 1991. A protocol for measuring assimilation rate versus internal CO_2 concentration using the Li-6200.

Lewis, E., and E. Brennan. 1977. A disparity in the ozone response of bean plants grown in a greenhouse, growth chamber or open-top chamber. *Journal of Air Pollution Control Association* 27: 889-891.

Long, S.P. and J-E Hällgren. 1985. Measurement of CO_2 assimilation by plants in the field and the laboratory. *In* J. Coombs, D.O. Hall, S.P. Long, and J.M.O. Scurlock [ed.], Techniques in Bioproductivity and Photosynthesis. pp. 62-95. Pergamon Press, Oxford, England.

Mahoney, M.J., J.M. Skelly, B.I. Chevone, and L.D. Moore. 1984. Response of yellow poplar (*Liriodendron tulipifera* L.) seedling's shoot growth to low concentrations of O_3, SO_2, and NO_2. *Canadian Journal of Forest Research* 14: 150-153.

Mann, L.K., S.B. Mclaughlin, and D.S. Shriner. 1980. Seasonal physiological responses of white pine under chronic air pollutant stress. *Environmental and Experimental Botany* 20: 99-105.

Mansfield, T.A. 1973. The role of stomata in determining the responses of plants to air pollutants. *Science* 2: 11-20.

Marshall, J.D., and R.H. Waring. 1985. Predicting fine root production and turnover by monitoring root starch and soil temperature. *Canadian Journal of Forest Research* 15: 791-800.

Mehlhorn, H., and A.R. Wellburn. 1987. Stress ethylene formation determines plant sensitivity to ozone. *Nature* 327: 417-418.

Meinzer, F.C., D.A. Grantz, and B. Smit. 1991. Root signals mediate coordination of stomatal and hydraulic conductance in growing sugarcane. *Australian of Journal of Plant Physiology* 18: 329-338.

McLaughlin, S.B., R.K. McConathy, D. Duvick, and L.K. Mann. 1982. Effects of chronic air pollution stress on photosynthesis, carbon allocation, and growth of white pine trees. *Forest Science* 28: 60-70.

McLaughlin, S.B. 1985. Effects of air pollution on forests: A critical review. *Journal of Air Pollution Control Association* 35: 512-534.

Miller, P.R. 1977. Photochemical oxidant air pollutant effects on a mixed conifer forest ecosystem. Annual Progress Report, 1975-1976. EPA-600/3-77-104. NITS No. PB 274 532/AS. U.S. Environmental Protection Agency.

Miller, P.R. 1983. Ozone effects in the San Bernardino National Forest. *In* D.D. Davis, A.A. Miller, and L. Dochinger [eds.], Air Pollution and the Productivity of the Forest, pp. 161. Izaak Walton League and Pennsylvania State University.

Moran, R., and D. Porath. 1980. Chlorophyll determination in intact tissues using N,N-Dimethylformamide. *Plant Physiology* 65: 478-479.

Moran, R. 1982. Formulae for determination of chlorophyllous pigments extracted with N,N-Dimethylformamide. *Plant Physiology* 69: 1376-1381.

Nakamura, H., and H. Saka. 1978. Photochemical oxidants injury in rice plants. III. Effect of ozone on physiological activities in rice plants. *Japan Journal of Crop Science* 47: 704-714.

Neufeld, H.S. 1989. Long-term ozone exposure response studies for tree seedlings from the southern Appalachian mountains. A proposal to the U.S. Environmental Protection Agency.

Neufeld, H.S., J.R. Renfro, W.D. Hacker, and D. Silsbee. 1992. Ozone in Great Smoky Mountains National Park: Dynamics and effects on plants. *In* R. Floger [ed.], The Response of Southern Commercial Forests to Air Pollution, Air & Waste Management Association, Pittsburgh, PA (In press).

Noble, R.D., and K.F. Jensen. 1980. Effects of sulfur dioxide and ozone on growth of hybrid poplar leaves. *American Journal of Botany* 67: 1005-1009.

Olszyk, D.M., and T.W. Tibbitts. 1981a. Stomatal response and leaf injury of *Pisum sativum* L. with SO_2 and O_3 exposure. I. Influence of pollutant level and leaf maturity. *Plant Physiology* 67: 539-544.

Olszyk, D.M., and T.W. Tibbitts. 1981b. Stomatal response and leaf injury of *Pisum sativum* L. with SO_2 and O_3 exposure II. Influence of moisture stress and time exposure. *Plant Physiology* 67: 545-549.

Parks, C.R., and J.F. Wendel. 1990. Molecular divergence between Asian and North American species of *Liriodendron* (Magnoliaceae) with implications for interpretation of fossil floras. *American Journal of Botany* 77: 1243-1256.

Pell, E.J., and N.S. Pearson. 1983. Ozone-induced reduction in the quantity of ribulose-1,5-bisphosphate carboxylase in alfalfa foliage. *Plant Physiology* 73: 185-187.

Pell, E.J., and M.S. Dann. 1991. Multiple stress-induced foliar senescence and implications for whole-plant longevity. *In* H.A. Mooney, W.E. Winner, and E.J. Pell [eds.]., pp. 189-205. Response of Plants to Multiple Stresses. Academic Press, Inc., San Diego, CA.

Potter J.R., and J.W. Jones. 1977. Leaf area partitioning as an important factor in growth. *Plant Physiology* 59: 10-14.

Pye, J.M. 1988. Impact of ozone on the growth and yield of trees: a review. *Journal of Environmental Quality* 17: 347-360.

Reich, P.B. 1983. Effects of low concentrations of O_3 on net photosynthesis, dark respiration, and chlorophyll contents in aging hybrid poplar leaves. *Plant Physiology* 73: 291-296.

Reich, P.B., and R.G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. *Science* 230: 566-570.

Reich, P.B., J.P. Lassoie, and R.G. Amundson. 1984. Reduction in growth of hybrid poplar following field exposure to low levels of O_3 and/or SO_4. *Canadian Journal of Botany* 62: 2835-2841.

Reich, P.B., and J.P. Lassoie. 1985. Influence of low concentrations of ozone on growth, biomass partitioning and leaf senescence in young hybrid poplar plants. *Environmental Pollution* 39: 39-51.

Reich, P.B., A.W. Schoettle, R.M. Raba, and R.G. Amundson. 1986. Response of soybean to low concentration of ozone. I. Reductions in leaf and whole plant net photosynthesis and leaf chlorophyll content. *Journal of Environmental Quality* 15: 31-36.

Reich, P.B. 1987. Quantifying plant response to ozone: a unifying theory. *Tree Physiology* 3: 63-91.

Renfro, J.R. 1989. Evaluating the effects of ozone on the plants of Great Smoky Mountains National Park. *Park Science* 9: 22-24.

Retzlaff, W.A., L.E. Williams, and T.M. DeJong. 1991. The effect of different atmospheric ozone partial pressures on photosynthesis and growth of nine fruit and nut tree species. *Tree Physiology* 8: 93-105.

Richardson, C.J., T.W. Sasek, and R.T. Di Giukio. 1990. Use of physiological and biochemical markers for assessing air pollution stress in trees. *In* W. Wang, J.W. Gorsuch, and W.R. Lower, [eds.], Plants for Toxicity Assessment. ASTM STP 1091. pp. 143-155. American Society for Testing and Materials, Philadelphia, PA.

Ridge, C.R., T.M. Hinckley, R.F. Stettler, and E.V. Volkenburgh. 1986. Leaf growth characteristics of fast-growing poplar hybrids *Populus trichocarpa* x *P. deltoides*. *Tree Physiology* 1: 209-216.

Roberts, B.R. 1990. Physiological response of yellow-poplar seedlings to simulated acid rain, ozone fumigation, and drought. *Forest Ecology and Management* 31: 215-224.

Sakaki, T., N. Kondo, and K. Sugahara. 1983. Breakdown of photosynthetic pigments and lipids in spinach leaves with ozone fumigation: Role of active oxygen. *Plant Physiology* 59: 28-34.

Sasek, T.W., and C.J. Richardson. 1989. Effects of chronic doses of ozone on loblolly pines: photosynthetic characteristics in the third growing season. *Forest Science* 35: 745-755.

Schreiber, U., W. Vidaver, V.C. Runeckles, and D. Rosen. 1978. Chlorophyll fluorescence assay for ozone injury in intact plants. *Plant Physiology* 61: 80-84.

Seinfeld, M.A. 1989. Urban air pollution: State of science. *Science* 243: 745-752.

Shafer, S.R., A.S. Heagle, and D.M. Camberato. 1987. Effects of chronic doses of ozone on field-grown loblolly pine: Seedling responses in the first year. *Journal Air Pollution Control Association* 37: 1179-1184.

Shimazaki, K. 1988. Thylakoid membrane reactions to air pollutants. *In* S. Schulte-Hostede, N.M. Darrall, L.W. Blank, and A.R. Wellburn [eds.], Air Pollution and Plant Metabolism. pp. 116-133. Elsevier, London, England.

Skärby, L.E. Troeng, and C. Bostrom. 1987. Ozone uptake and effects on transpiration, net photosynthesis, and dark respiration in Scots pine. *Forest Science* 33: 801-808.

Skelly, J.M., B.I. Chevone, and Y.S. Yang. 1982. Effects of ambient concentrations of air pollutants on vegetation indigenous to the Blue Ridge Mountains of Virginia. *In* International Symposium in Hydrometeorology, American Water Resources Association, June, pp. 69-73.

Skelly, J.M., D.D. Davis, W. Merrill, E.A. Cameron, H.D. Brown, D.B. Drummond, and L.S. Dochinger, [eds.]. 1987. Diagnosing injury to eastern forest trees. The Pennsylvania State University Pennsylvania.

Stupka, A. 1964. Trees, shrubs, and wood vines of Great Smoky Mountains National Park. The University of Tennessee Press, Knoxville, TN.

Sutton, R., and I.P. Ting. 1977. Evidence for the repair of ozone-induced membrane injury. *American Journal of Botany* 64: 404-411.

Taylor, G., and M.C. Dobson. 1989. Photosynthetic characteristics, stomatal responses and water relations of *Fagus sylvatica*: impact of air quality at a site in southern Britain. *New Phytologist* 113: 265-273.

Temple, P.J. 1986. Stomatal conductance and transpirational responses of field-grown cotton to ozone. *Plant, Cell and Environment* 9: 315-321.

Thomas, R.B., and B.R. Strain. 1991. Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. *Plant Physiology* 96: 627-634.

Tingey, D.T., and G.E. Taylor, Jr. 1982. Variation in plant response to ozone: A conceptual model of physiological events. *In* M. H. Unsworth, and D.P. Ormrod [eds.], Effects of Gaseous Air Pollution in Agriculture and Horticulture, pp. 113-138. Butterworth Scientific, London, U.K.

Tjoelker, M.G., and R.J. Luxmoore. 1991. Soil nitrogen and chronic ozone stress influence physiology, growth and nutrient status of *Pinus taeda* L. and *Liriodendron tulipifera* L. seedlings. *New Phytologist* 119: 69-81.

Volz, A., and D. Kley. 1988. Evaluation of the Montsouris Series of ozone measurements made in the nineteenth century. *Nature* 332: 240-242.

Walmsley, L., M.R. Ashmore, and J.N.B. Bell. 1980. Adaptation of radish *Raphanus sativus* L. in response to continuous exposure to ozone. *Environmental Pollution* 23: 165-177.

Wang, D., D.F. Karnosky, and F.H. Bormann. 1986. Effects of ambient ozone on the productivity of *Populus tremuloides* Michx. grown under field conditions. *Canadian Journal of Forest Research* 16: 47-55.

West, D.C., S.B. McLaughlin, and H.H. Shugart. 1980. Simulated forest response to chronic air pollution stresses. *Journal of Environmental Quality* 9: 43-49.

White, P.S. 1982. The flora of Great Smoky Mountains National Park: An annotated checklist of the vascular plants and a review of previous floristic work. Research/Resource Management Report SER-55. National Park Service.

Winner, W.E., and C.J. Atkinson. 1986. Absorption of air pollution by plants, and consequences for growth. *Tree* 1: 15-18.

Woodman, J.N. 1987. Pollution-induced injury in North American forests: facts and suspicions. *Tree Physiology* 3: 1-15.

von Caemmerer, S., and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153: 376-387.

Yang, Y.S., J.M. Skelly, B.L. Chevone, and J.B. Birch. 1983a. Effects of short-term ozone exposure on net photosynthesis, dark respiration and transpiration of three eastern white pine clones. *Environment International* 9: 265-269.

Yang, Y.S., J.M. Skelly, B.L. Chevone, and J.B. Birch. 1983b. Effects of long-term ozone exposure on photosynthesis and dark respiration of eastern white pine. *Environmental Science and Technology* 17: 371-373.

Zelitch, I. 1971. Photosynthesis, Photorespiration, and Plant Productivity. Academic Press, New York, NY.

Zelitch, I. 1982. The close relationship between net photosynthesis and crop yield. *Bioscience* 32: 796-802.

VITA

Songqiao Huang was born in Lanzhou, China on August 27, 1964 to Dazhi Huang and Zhiyun Liu. She attended elementary schools in that city and graduated from Beijing Petroleum College's Middle School in Beijing in July 1982. She then entered Beijing Teachers' College the following September and received her Bachelor of Science degree in Biology in July, 1986. She was hired as a faculty member in the Department of Biology, the Branch Campus of Beijing Teachers' College, immediately after her graduation.

In 1989, she entered the Master's program in the Dept. of Biology, Appalachian State University. She anticipates receiving her M.S. degree in Biology in August, 1992 from Appalachian State University in Boone, North Carolina, U.S.A. She is a member of the Association of Southeastern Biologists and Phi Kappa Phi.

Ms. Huang's address is: c/o Mr. Dazhi Huang, P. O. Box 914, Beijing China.