

# Stable Isotopes and Plant Carbon–Water Relations

Edited by

**James R. Ehleringer**

Stable Isotope Ratio Facility  
for Environmental Research  
Department of Biology  
University of Utah  
Salt Lake City, Utah

**Anthony E. Hall**

Department of Botany and Plant Science  
University of California  
Riverside, California

**Graham D. Farquhar**

Plant Environmental Biology Group  
Research School of Biological Sciences  
Australian National University  
Canberra, Australia

## 16

### Carbon Isotope Composition and Gas Exchange of Loblolly and Shortleaf Pine as Affected by Ozone and Water Stress

Christine G. Elsik, Richard B. Flagler, and Thomas W. Boutton



**Academic Press, Inc.**

A Division of Harcourt Brace & Company

San Diego Boston New York  
London Sydney Tokyo Toronto

#### I. Introduction

Ozone ( $O_3$ ), a photochemical oxidant, has been recognized as the most phytotoxic of the widespread air pollutants (Reich, 1987) and has been implicated as a factor in the recent forest growth decline in the southeastern United States (Sheffield and Cost, 1987). Injury to plant tissue results from oxidation of biological compounds by  $O_3$  and the free radicals it forms, impacting several biochemical and physiological processes, and leading to alterations in growth and biomass allocation (Guderian *et al.*, 1985). Air pollution is among the environmental factors affecting leaf internal carbon dioxide concentration ( $c_i$ ) through effects on rates of net photosynthesis ( $A$ ) and stomatal conductance ( $g$ ), justifying stable carbon isotope analysis as a tool in the study of plant response to air pollution. Stable carbon isotope analysis has been used previously in the study of pollutant effects on trees and crop plants (Freyer, 1979; Greitner and Winner, 1988; Martin *et al.*, 1988; Becker *et al.*, 1989; Boutton and Flagler, 1990; Martin and Sutherland, 1990; Saurer *et al.*, 1991; Taylor, 1991).

Growth declines resulting from elevated  $O_3$  levels have been correlated with decreased photosynthesis in crop and tree species (Reich and Amundson, 1985). Whether the  $O_3$ -induced decrease in  $A$  results from stomatal or nonstomatal limitations remains controversial, but information on the effect of  $O_3$  on  $c_i$  can clarify the mechanism (Runeckles and Chevone, 1992). Ozone may decrease  $A$  through increased mesophyll resistance, resulting in increased  $c_i$  (Reich, 1987). Alternatively,  $O_3$  may affect guard cells directly, decreasing  $g$ , causing decreased  $c_i$ , and resulting in diminished  $A$  (Moldau

*et al.*, 1990). There is no direct evidence supporting an immediate effect of  $O_3$  on guard cell function in tree species (Chappelka and Chevone, 1992); however, stable carbon isotope analysis has indicated stomatal limitation to  $A$  in tree and crop species (Greitner and Winner, 1988; Martin *et al.*, 1988; Boutton and Flagler, 1990; Saurer *et al.*, 1991; Taylor, 1991).

Since  $O_3$  enters the leaf through the stomata, environmental variables which affect  $g$ , such as water stress, alter plant response to  $O_3$ . Stomatal regulation can be a protective mechanism against both drought and air pollution; stomatal closure minimizes water loss (Teskey and Hinckley, 1986) and reduces  $O_3$  injury through decreased  $O_3$  uptake (Harkov and Brennan, 1980; Olszyk and Tibbitts, 1981). Alternatively,  $O_3$  exposure may alter plant response to water stress by modifying  $g$  (Reich and Lassoie, 1984). The effects of  $O_3$  on  $g$  in tree species are inconsistent. Ozone may increase stomatal sensitivity to vapor pressure deficit (Chappelka *et al.*, 1988), preventing possible drought injury, or may reduce stomatal responsiveness and increase transpiration (Keller and Hasler, 1984; Reich and Lassoie, 1984), increasing the likelihood of desiccation during periods of drought.

Ozone and water deficit can alter transpiration efficiency ( $W$ ), the ratio of biomass produced to total water transpired. As a result of the different diffusive conductances for carbon dioxide and water vapor, reduced  $g$  decreases transpiration to a greater extent than carbon dioxide uptake (Nobel, 1991). Moderate water deficit generally increases  $W$  by inducing partial stomatal closure. Ozone also alters  $W$  through effects on  $g$  and the biochemical reactions of photosynthesis. Ozone-induced stomatal closure, without a reduction in  $A$ , would result in increased  $W$ , while decreased stomatal sensitivity to water deficit or decreased  $A$  would cause a reduction in  $W$ . Since  $W$  is related to integrated  $c_i$ , carbon isotope analysis may be used to assess  $W$  (Farquhar *et al.*, 1989b).

The purpose of this study was to investigate the effects of  $O_3$  and water deficit and their interaction on  $\delta^{13}C$  and  $c_i$  of loblolly and shortleaf pines. Seedlings were exposed to different levels of  $O_3$  and soil moisture in open-top chambers during their first growing season. Stable carbon isotope composition was determined in order to assess integrated gas exchange characteristics. In addition, conventional gas exchange methods were used to measure  $A$ ,  $g$ , and  $c_i$ .

## II. Experimental Methods

### A. Study Area

The research site was located in the USDA Forest Service Stephen F. Austin Experimental Forest (31° 30' N latitude, 94° 46' W longitude), roughly 12 km southwest of Nacogdoches, Texas. The mean annual maximum and minimum temperatures are 24.2°C and 11.2°C, respectively, and the mean



annual precipitation is 115.6 cm. The area immediately surrounding the site consists of mature loblolly and shortleaf pine forest.

### B. Plant Material

Seeds from one half-sib shortleaf pine (*Pinus echinata* Mill.) family (S2PE-3) and one half-sib loblolly (*P. taeda* L.) pine family (GR1-8) were stratified for 90 days and then sown, in January 1990, in 7-liter pots containing a fritted clay medium that had been leached with reverse osmosis water. Until treatments began, the seedlings were maintained in a greenhouse and were fertilized weekly beginning 12 weeks after the sowing date with 15 : 30 : 15 (N : P : K) and a micronutrient mix supplemented with chelated iron.

### C. Ozone Exposure Chambers

The seedlings were exposed to O<sub>3</sub> in 10 cylindrical open-top field chambers 3 m in diameter and 2.5 m in height (Heagle *et al.*, 1973). Chambers were equipped with a fixed cap to exclude ambient rainfall. Air was forced through a plenum surrounding the lower portion of each chamber at approximately 60 m<sup>3</sup> min<sup>-1</sup>, during the hours 0600 to 2400 CST daily.

Ozone was generated from O<sub>2</sub> by a corona discharge type generator and was metered to chambers through needle valves. Air from inside each chamber was sampled through Teflon tubing. The O<sub>3</sub> concentration was monitored continually with a uv-photometric O<sub>3</sub>-specific analyzer on a time-shared basis in each chamber. The O<sub>3</sub> monitors were calibrated with a uv-photometry transfer standard.

### D. Experimental Design and Treatment Regimes

The experimental design was a split-split plot conducted within a completely randomized design. The whole plots were five levels of O<sub>3</sub>; the subplots were two water regimes; the sub-subplots were two species. Each treatment combination was replicated twice, requiring 10 chambers. Each treatment combination included 18 seedlings per replication, for a total of 720 seedlings.

Seedlings were placed in the chambers and O<sub>3</sub> treatments were initiated on 25 June 1990, approximately 24 weeks after sowing, when substantial secondary needle tissue had developed in both species. The five O<sub>3</sub> treatments ranged from a subambient level to 2.5 times ambient O<sub>3</sub> concentration. Charcoal filters were used to remove O<sub>3</sub> for the subambient treatment (CF); the ambient treatment consisted of nonfiltered air (NF). The three O<sub>3</sub> addition treatments were 1.7, 2.0, and 2.5 times the ambient O<sub>3</sub> concentration (1.7×, 2.0×, and 2.5×, respectively), and fluctuated as a proportion of ambient O<sub>3</sub>, during the hours 0800 to 2000 CST.

All seedlings were watered daily to field capacity with reverse osmosis water to maintain the  $\psi_{\text{soil}}$  at -0.08 MPa until 10 August 1990. After this date, the two water regimes, well-watered (WW) and water-stressed (WS), were imposed. The WW and WS treatments received water whenever the

soil volumetric water content was less than 34% ( $\psi_{\text{soil}} = -0.08$  MPa) and 28% ( $\psi_{\text{soil}} = -0.3$  MPa), respectively. The intent was to allow WW seedlings to experience virtually no water stress, while the WS seedlings undergo mild water deficit. Soil moisture was characterized with a Trase System soil moisture device (Soil Moisture Equipment Corp., Santa Barbara, CA) based on time domain reflectometry. A moisture retention curve for the medium was produced, using the pressure plate technique, so that volumetric water content could be related to soil water potential. Trase measurements were taken daily for two randomly selected seedlings per plot to determine whether that plot needed to be watered, at which time reverse osmosis water was added to field capacity. All treatments were watered with fertilizer solution once every 2 weeks on a day that water addition was necessary.

#### E. Carbon Isotope Composition

Carbon isotope composition was determined for foliage and stems of five seedlings per plot. Carbon isotope composition, expressed as  $\delta^{13}\text{C}$ , was determined using the technique of Boutton (1991). Five milligrams of dried tissue, ground to pass a 40-mesh screen, was combusted to  $\text{CO}_2$  in sealed quartz tubes at  $850^\circ\text{C}$ . The  $\text{CO}_2$  was purified cryogenically and analyzed on a VG-903 dual-inlet, triple collector, gas isotope ratio mass spectrometer (VG Isogas, Middleswich, UK). Precision was approximately 0.1‰ for all  $\delta^{13}\text{C}$  measurements.

#### F. Gas Exchange Characteristics

Gas exchange characteristics ( $A$ ,  $g$ , and  $c_i$ ) were measured biweekly with a portable photosynthesis system (LI-6200, LI-COR, Inc., Lincoln, NE) equipped with a 0.25-liter leaf chamber. Measurements were made on detached fascicles from the oldest flush of each seedling. Previous studies have shown that gas exchange characteristics of shortleaf pine are not significantly affected by fascicle detachment for up to 90 s (Lock and Flagler, 1992), and that photosynthetic rates of loblolly pine are not affected by detachment of fascicles from branches which had been removed from the tree for up to 30 min (Ginn *et al.*, 1991). During each sample period, measurements were made on one fascicle per seedling and two seedlings per treatment combination, on all treatment combinations. The replications were measured on 2 consecutive days, between 1000 and 1400 h CST. During measurements, the leaf chamber was kept in a light box, so that photosynthetically active radiation reaching the chamber would be kept constant at approximately  $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$  using two 300-Watt cool beam lamps (General Electric, Cleveland, OH). The light box was equipped with an air blower for cooling and supplying fresh ambient air to the leaf chamber.

#### G. Data Analysis and Statistics

Response variables were analyzed by analysis of variance (ANOVA) for differences due to  $\text{O}_3$ , water regime, and interactions for species sepa-



rately. Ozone effects were broken down to linear and curvilinear orthogonal contrasts. Regression analysis was performed on the moisture treatments separately, using the seasonal sum of hourly  $O_3$  averages between the hours 0800 and 2000 CST (12 h sum zero) as the regressor.

### III. Results

#### A. Ozone Exposures and Meteorology

During the exposure period (25 June–31 October) the mean ambient 12 h  $d^{-1}$   $O_3$  concentration for the hours 0800 to 2000 CST was 0.047 ppm. The highest ambient 1-h peak  $O_3$  concentration was 0.109 ppm. The federal secondary ambient air quality standard for  $O_3$  of 0.120 ppm was exceeded by the three  $O_3$  addition treatments. Ozone exposure statistics for the five treatments are given in Table I. Seasonal and diurnal mean  $O_3$  concentration trends have been illustrated in a previous paper (Elsik *et al.*, 1992). The average temperature and relative humidity during the treatment period (25 June–31 October) was 19.2°C and 81.7%, respectively. The average daily maximum and average daily minimum temperatures were 27.1 and 13.1°C, respectively. The maximum and minimum 1-h temperatures were 33.1, and -0.6°C, respectively. The average daily maximum and average daily minimum relative humidities were 97.2 and 52.9%, respectively. The maximum and minimum 1-h relative humidities were 99.5 and 28.8%, respectively.

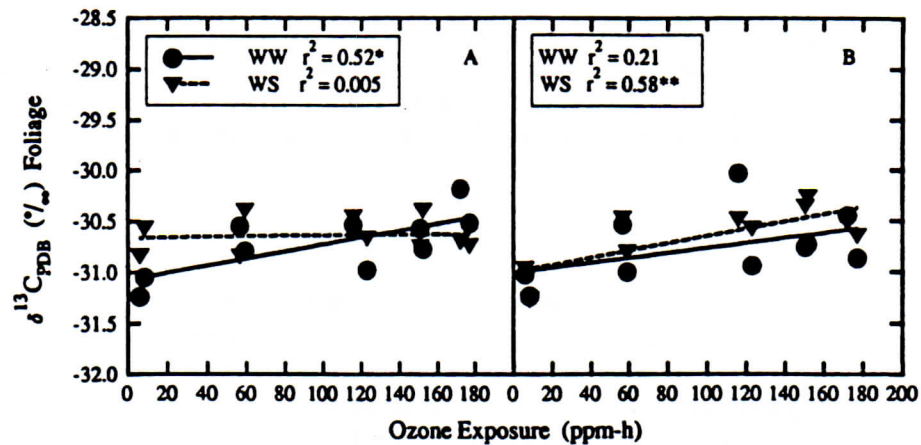
**Table I** Ozone Exposure Statistics for Ambient Air and Loblolly and Shortleaf Pine Exposed to Five Levels of  $O_3$  in Open-Top Chambers in East Texas\*

Ozone level <sup>b</sup>	12 h day <sup>-1</sup> $O_3$ concn (ppm)		1 h day <sup>-1</sup> peak $O_3$ concn (ppm)		Sum zero <sup>c</sup> (ppm-h)
	Seasonal mean	Highest	Seasonal mean	Highest	
CF	0.005	0.018	0.013	0.066	9.6
NF	0.037	0.076	0.058	0.108	58.1
1.7×	0.078	0.161	0.123	0.252	118.3
2.0×	0.099	0.218	0.157	0.348	148.7
2.5×	0.114	0.238	0.178	0.357	170.5
AA	0.047	0.079	0.069	0.109	44.1

\*Each value is the mean from two chambers except for AA values, which are the mean from one ambient air monitor, for the daily period 0800 to 2000 h CST from 25 June to 31 October 1990.

<sup>b</sup>Ozone levels CF, NF, 1.7×, 2.0×, 2.5×, and AA are charcoal filtered, non-filtered, 1.7 times ambient, 2.0 times ambient, 2.5 times ambient, and ambient air, respectively.

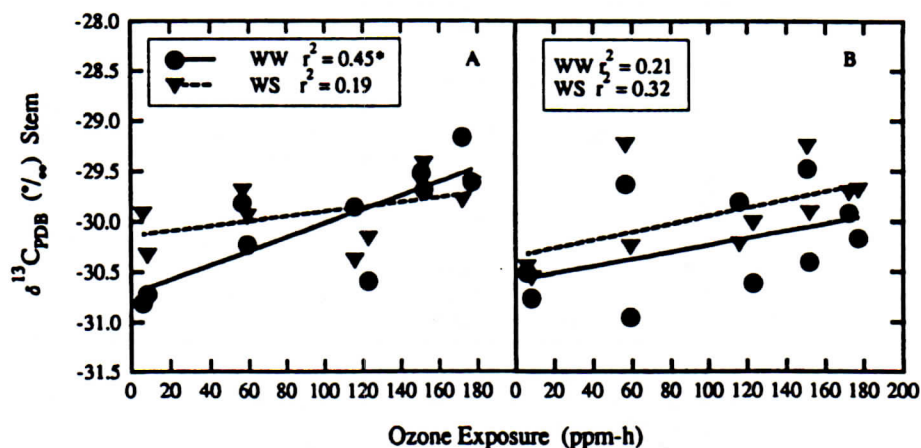
<sup>c</sup>Seasonal sum of hourly  $O_3$  averages between the hours 0800 and 2000 CST from 25 June to 31 October 1990.



**Figure 1.** Foliar  $\delta^{13}\text{C}$  of loblolly (A) and shortleaf (B) pine versus  $\text{O}_3$  exposure ( $\text{O}_3$  sum zero) for well-watered (WW) and water-stressed (WS) seedlings. Significance at the 0.05 and 0.01 levels are signified by \* and \*\*, respectively ( $n = 10$ ).

### B. Carbon Isotope Composition

Foliar  $\delta^{13}\text{C}$  increased with  $\text{O}_3$  exposure in both species ( $P < 0.1$ ) (Table II). Increased  $\delta^{13}\text{C}$  indicates decreased long-term internal  $\text{CO}_2$  concentration, and, thus, increased  $W$  with elevated  $\text{O}_3$ . Stem  $\delta^{13}\text{C}$  increased significantly ( $P < 0.05$ ) with  $\text{O}_3$  exposure in loblolly pine only (Table II). A tendency for  $\delta^{13}\text{C}$  to increase with  $\text{O}_3$  exposure was observed in shortleaf pine stems, but this trend was not significant. A consistent increase in  $\delta^{13}\text{C}$  attributable to moisture deficit was observed only in stem tissue of shortleaf pine ( $P < 0.1$ ) (Table II). Linear regressions for separate moisture regimes are shown in Figs. 1 and 2 for foliage and stem, respectively. Regression analysis indi-



**Figure 2.** Stem  $\delta^{13}\text{C}$  of loblolly (A) and shortleaf (B) pine versus  $\text{O}_3$  exposure ( $\text{O}_3$  sum zero) for well-watered (WW) and water-stressed (WS) seedlings. Significance at the 0.05 level is signified by \* ( $n = 10$ ).



**Table II** Carbon Isotope Composition of Foliage and Stem Tissue of Loblolly and Shortleaf Pine Seedlings Exposed to O<sub>3</sub> and Water Deficit<sup>a</sup>

Ozone level <sup>b</sup>	Water regime <sup>c</sup>	$\delta^{13}\text{C}(\text{‰})$			
		Loblolly pine		Shortleaf pine	
		Foliage	Stem	Foliage	Stem
CF	WW	-31.65 (0.09)	-30.77 (0.21)	-31.14 (0.11)	-30.65 (0.12)
NF	WW	-30.68 (0.12)	-30.03 (0.21)	-30.77 (0.23)	-30.30 (0.63)
1.7x	WW	-30.77 (0.22)	-30.23 (0.75)	-30.48 (0.45)	-30.22 (0.41)
2x	WW	-30.68 (0.10)	-29.60 (0.09)	-30.75 (0.01)	-29.95 (0.47)
2.5x	WW	-30.36 (0.17)	-29.38 (0.22)	-30.66 (0.21)	-30.05 (0.13)
CF	WS	-30.70 (0.14)	-30.14 (0.25)	-31.12 (0.15)	-30.52 (0.06)
NF	WS	-30.63 (0.22)	-29.83 (0.12)	-30.65 (0.16)	-29.75 (0.54)
1.7x	WS	-30.57 (0.10)	-30.28 (0.11)	-30.54 (0.04)	-30.13 (0.10)
2.0x	WS	-30.58 (0.18)	-29.52 (0.09)	-30.32 (0.05)	-29.59 (0.33)
2.5x	WS	-30.73 (0.02)	-29.71 (0.09)	-30.59 (0.06)	-29.71 (0.03)
ANOVA <sup>d</sup>					
O <sub>3</sub>					
Linear		0.24	0.04	0.19	0.53
Quadratic		0.06	0.01	0.07	0.17
Residual		0.45	0.81	0.10	0.55
Water regime		0.57	0.08	0.95	0.75
Interaction		0.43	0.25	0.24	0.05
		0.26	0.42	0.54	0.68

<sup>a</sup>Values are means and standard errors (in parentheses) of 10 samples.

<sup>b</sup>Ozone levels CF, NF, 1.7x, 2.0x, and 2.5x are charcoal filtered, nonfiltered, 1.7 times ambient, 2.0 times ambient, and 2.5 times ambient, respectively.

<sup>c</sup>Water regimes WW and WS are well-watered and water-stressed, respectively.

<sup>d</sup>Tabular values are probability levels associated with ANOVA. Degrees of freedom associated with the sources of variation are O<sub>3</sub>, 4 df; O<sub>3</sub> contrasts, 1 df; water regime, 1 df; interaction, 4 df.

**Table III** Net Photosynthesis (*A*), Stomatal Conductance (*g*), and Internal CO<sub>2</sub> Concentration (*c<sub>i</sub>*) of Loblolly Pine Seedlings Exposed to O<sub>3</sub> and Water Deficit<sup>a</sup>

Ozone level <sup>b</sup>	Water regime <sup>c</sup>	13 October 1990				27 October 1990			
		<i>A</i> ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	<i>g</i> ( $\text{mol m}^{-2} \text{s}^{-1}$ )	<i>c<sub>i</sub></i> (ppm)	<i>A</i> ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	<i>g</i> ( $\text{mol m}^{-2} \text{s}^{-1}$ )	<i>c<sub>i</sub></i> (ppm)		
CF	WW	4.33 (0.17)	0.129 (0.003)	271 (4)	4.34 (0.23)	0.116 (0.002)	265 (2)		
NF	WW	4.08 (0.12)	0.112 (0.021)	268 (16)	3.76 (0.16)	0.090 (0.009)	278 (18)		
1.7x	WW	4.02 (0.20)	0.111 (0.008)	267 (2)	3.42 (0.16)	0.090 (0.015)	271 (21)		
2x	WW	3.10 (0.93)	0.078 (0.027)	285 (18)	2.24 (0.35)	0.062 (0.004)	288 (15)		
2.5x	WW	2.53 (0.33)	0.060 (0.002)	272 (11)	2.50 (0.87)	0.088 (0.029)	301 (6)		
CF	WS	4.51 (0.13)	0.092 (0.000)	245 (0)	4.25 (0.76)	0.095 (0.020)	257 (5)		
NF	WS	5.70 (0.97)	0.109 (0.022)	242 (6)	4.77 (0.54)	0.104 (0.025)	257 (11)		
1.7x	WS	4.47 (0.12)	0.089 (0.004)	243 (7)	3.87 (0.64)	0.075 (0.017)	267 (29)		
2x	WS	3.69 (0.70)	0.073 (0.014)	256 (11)	2.55 (0.20)	0.047 (0.014)	255 (35)		
2.5x	WS	3.25 (0.20)	0.077 (0.015)	267 (9)	2.65 (0.74)	0.067 (0.008)	271 (10)		
		ANOVA <sup>d</sup>							
O <sub>3</sub>		0.11	0.22	0.65	0.10	0.27	0.87		
Linear		0.02	0.04	0.25	0.02	0.09	0.36		
Quadratic		0.25	0.59	0.64	0.91	0.42	0.80		
Residual		0.62	0.78	0.72	0.43	0.46	0.95		
Water regime		0.02	0.08	0.00	0.14	0.17	0.02		
Interaction		0.39	0.06	0.24	0.59	0.59	0.47		

<sup>a</sup>Values are means and standard errors (in parentheses) of four measurements taken during the last two sample periods.

<sup>b</sup>Ozone levels CF, NF, 1.7x, 2.0x, and 2.5x are charcoal filtered, nonfiltered, 1.7 times ambient, 2.0 times ambient, and 2.5 times ambient, respectively.

<sup>c</sup>Water regimes WW and WS are well-watered and water-stressed, respectively.

<sup>d</sup>Tabular values are probability levels associated with ANOVA. Degrees of freedom associated with the sources of variation are O<sub>3</sub>, 4 df; O<sub>3</sub> contrasts, 1 df; water regime, 1 df; interaction, 4 df.



**Table IV** Net Photosynthesis (A), Stomatal Conductance (g), and Internal CO<sub>2</sub> Concentration (c<sub>i</sub>) of Shortleaf Pine Seedlings Exposed to O<sub>3</sub> and Water Deficit<sup>a</sup>

Ozone level <sup>b</sup>	Water regime <sup>c</sup>	13 October 1990				27 October 1990			
		A ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	g ( $\text{mol m}^{-2} \text{s}^{-1}$ )	c <sub>i</sub> (ppm)	A ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	g ( $\text{mol m}^{-2} \text{s}^{-1}$ )	c <sub>i</sub> (ppm)		
CF	WW	5.51 (0.14)	0.169 (0.008)	275 (0)	4.54 (0.05)	0.128 (0.016)	273 (12)		
NF	WW	5.42 (0.43)	0.154 (0.006)	274 (0)	4.42 (0.54)	0.126 (0.030)	284 (16)		
1.7x	WW	3.48 (0.58)	0.094 (0.015)	271 (0)	4.71 (0.93)	0.107 (0.024)	264 (10)		
2x	WW	3.62 (1.97)	0.107 (0.041)	293 (5)	3.59 (0.44)	0.098 (0.005)	288 (9)		
2.5x	WW	4.17 (0.08)	0.107 (0.013)	274 (8)	2.42 (0.23)	0.073 (0.016)	300 (0)		
CF	WS	4.54 (0.51)	0.108 (0.033)	253 (14)	4.76 (0.04)	0.100 (0.004)	252 (5)		
NF	WS	6.07 (0.80)	0.122 (0.011)	246 (0)	5.37 (0.43)	0.115 (0.017)	255 (5)		
1.7x	WS	5.83 (1.05)	0.128 (0.017)	248 (7)	4.63 (1.94)	0.099 (0.052)	253 (9)		
2x	WS	4.43 (1.06)	0.094 (0.012)	264 (2)	4.94 (0.24)	0.105 (0.013)	264 (2)		
2.5x	WS	5.09 (0.88)	0.127 (0.018)	269 (7)	4.44 (0.23)	0.084 (0.013)	254 (1)		
ANOVA <sup>d</sup>									
O <sub>3</sub>									
Linear		0.64	0.48	0.79	0.61	0.57	0.34		
Quadratic		0.35	0.19	0.56	0.21	0.17	0.16		
Residual		0.94	0.46	0.90	0.40	0.56	0.52		
Water regime		0.49	0.61	0.09	0.99	0.88	0.65		
Interaction		0.15	0.37	0.05	0.06	0.69	0.02		
		0.36	0.16	0.29	0.48	0.88	0.47		

<sup>a</sup>Values are means and standard errors (in parentheses) of four measurements taken during the last two sample periods.

<sup>b</sup>Ozone levels CF, NF, 1.7x, 2.0x, and 2.5x are charcoal filtered, nonfiltered, 1.7 times ambient, 2.0 times ambient, and 2.5 times ambient, respectively.

<sup>c</sup>Water regimes WW and WS are well-watered and water-stressed, respectively.

<sup>d</sup>Tabular values are probability levels associated with ANOVA. Degrees of freedom associated with the sources of variation are O<sub>3</sub>, 4 df; O<sub>3</sub> contrasts, 1 df; water regime, 1 df; interaction, 4 df.

cated that  $\delta^{13}\text{C}$  of foliage and stem tissue in WW loblolly pine seedlings were linearly related to  $\text{O}_3$  exposure ( $P < 0.05$ ), but  $\delta^{13}\text{C}$  of WS loblolly pine seedlings was not significantly related to  $\text{O}_3$ . This suggests an  $\text{O}_3 \times$  water stress interaction. The opposite relationship was observed in shortleaf pine foliage, in which  $\delta^{13}\text{C}$  of WS seedlings was linearly related to  $\text{O}_3$  exposure ( $P < 0.01$ ), but  $\delta^{13}\text{C}$  of WW seedlings was not significantly related to  $\text{O}_3$ .

### C. Gas Exchange Characteristics

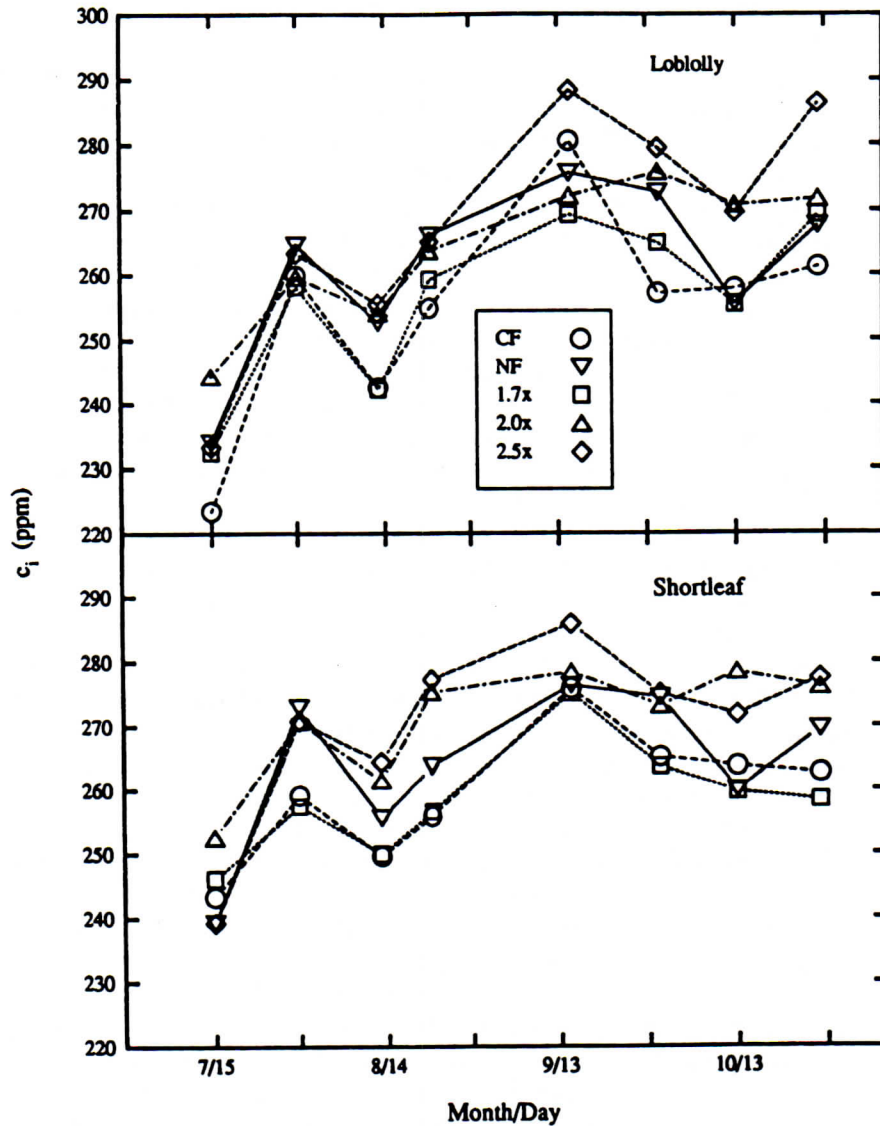
Results for  $A$  and  $g$ , which have been reported previously (Elsik *et al.*, 1992), are reviewed, to provide a basis for comparison with carbon isotope and instantaneous  $c_i$  data. Divergence in  $A$  and  $g$  were initially observed during the 13 September measurement period in both species; however, significant differences were not observed until the 13 October measurement period. The seasonal trends in  $A$  and  $g$  for each  $\text{O}_3$  level have been illustrated elsewhere (Elsik *et al.*, 1992). Assimilation rate and  $g$  decreased linearly due to  $\text{O}_3$  in loblolly pine during the last two measurement periods (Table III). Downward trends in  $A$  and  $g$  with  $\text{O}_3$  exposure were observed in shortleaf pine, but were not significant (Table IV). Water-stressed seedlings of both species tended to possess higher  $A$  than WW seedlings, but this was significant in loblolly pine only during the 13 October measurement period ( $P < 0.05$ ) and in shortleaf pine only during the 27 October measurement period ( $P < 0.1$ ). Water stress tended to decrease  $g$  in both species, but this was significant in loblolly pine only during the 13 October measurement period ( $P < 0.1$ ) and was not significant in shortleaf pine. There were no consistent  $\text{O}_3 \times$  water stress interactions on  $A$  or  $g$  in either species.

There were no consistent  $\text{O}_3 \times$  water stress interactions on  $c_i$  (Tables III and IV), so data were averaged over moisture regimes to reveal the seasonal pattern in  $\text{O}_3$  main effects on  $c_i$  (Fig. 3). Internal  $\text{CO}_2$  concentration tended to increase due to  $\text{O}_3$  exposure during most measurement periods, but this was significant ( $P < 0.05$ ) only in shortleaf pine during the 13 October measurement period (Table IV). The pattern was not consistent throughout the season, often with a decrease in  $c_i$  in the  $1.7\times$  treatment compared to CF. Water deficit caused decreased  $c_i$  during all measurement periods beginning 22 August in loblolly pine ( $P < 0.001$  to  $P < 0.05$ ) and shortleaf pine ( $P < 0.01$  to  $P < 0.05$ ).

## IV. Discussion

The linear increase in  $\delta^{13}\text{C}$  with  $\text{O}_3$  exposure in both species is consistent with previous studies using stable carbon isotope composition to assess  $\text{O}_3$  response by loblolly pine (Taylor, 1991), shortleaf pine (Boutton and Flagler, 1990), and other  $\text{C}_3$  plant species (Greitner and Winner, 1988; Martin *et al.*, 1988; Saurer *et al.*, 1991). These data indicate a decreased  $c_i$ ,





**Figure 3.** Seasonal pattern of instantaneous internal  $\text{CO}_2$  concentration ( $c_i$ ) of loblolly and shortleaf pine seedlings as affected by five levels of  $\text{O}_3$  ( $n = 8$ ).

which could result from either increased  $A$  or, more likely, decreased  $g$ . Instantaneous gas exchange measurements have indicated a linear decrease in  $A$  due to  $\text{O}_3$  in loblolly pine. Consequently, it can be concluded that decreased  $c_i$  due to  $\text{O}_3$  is a result of decreased  $g$ . These results are in agreement with measurements of  $g$ , which tended to decrease in response to  $\text{O}_3$  exposure in both species, although significant in loblolly pine only. The  $\delta^{13}\text{C}$  values indicate increased  $W$  with higher  $\text{O}_3$  concentrations, in

agreement with a previous study on shortleaf pine (Boutton and Flagler, 1990).

The increase in  $\delta^{13}\text{C}$  in stem tissue of shortleaf pine due to water deficit indicated a water-stress-induced increase in  $W$ , in agreement with instantaneous water-use efficiency ( $A/E$ ) (Elsik *et al.*, 1992). This is in accordance with previous studies on the effect of water deficit on  $\delta^{13}\text{C}$  in  $\text{C}_3$  plants (Hubick *et al.*, 1986; Martin and Thorstenson, 1988). However, the water stress did not have a consistent effect on  $\delta^{13}\text{C}$  in loblolly pine, and water deficit did not affect  $A/E$  in this species (Elsik *et al.*, 1992).

Increased instantaneous  $c_i$  values attributable to  $\text{O}_3$  suggested that the  $\text{O}_3$ -induced decrease in  $A$  was a result of biochemical processes, such as light harvesting or dark reactions of photosynthesis, rather than  $g$ , in accordance with a previous study on loblolly pine (Sasek and Richardson, 1989). This does not agree with the integrated  $c_i$  values as measured by  $\delta^{13}\text{C}$ . Instantaneous water-use efficiency was not significantly related to  $\text{O}_3$  (Elsik *et al.*, 1992), also contrary to  $\delta^{13}\text{C}$ , which indicated increased  $W$  with elevated  $\text{O}_3$ . This variation may be related to the calculation of  $c_i$  in instantaneous gas exchange measurements. The calculation assumes that stomata are uniformly open or there is sufficient conductance between substomatal cavities, but a significant overestimation of  $c_i$  may occur if stomatal apertures vary or if lateral diffusion is limited (Laisk, 1983; Downton *et al.*, 1988; Terashima *et al.*, 1988). The homogeneity of stomatal response to  $\text{O}_3$  has not been reported in loblolly or shortleaf pine. This may explain findings in a previous study, in which instantaneous gas exchange measurements indicated nonstomatal limitations to photosynthesis attributable to  $\text{O}_3$  in loblolly pine (Sasek and Richardson, 1989).

In addition to possible errors in the calculation of  $c_i$  values in instantaneous gas exchange measurements, error may arise due to the need for constant environmental conditions throughout each measurement period of gas exchange. Light, humidity, and temperature were maintained relatively constant in the cuvette of the gas analyzer during each measurement, but the seedlings experienced different levels of soil moisture, humidity, and temperature throughout the day. Although the daily time frame within which measurements were made was kept to a minimum, there was still a notable change in atmospheric temperature and relative humidity during this period. Gas exchange characteristics exhibit a diurnal pattern, which may be affected by treatments. Therefore, seedlings may not be at the peak level of carbon assimilation when measurements were made. Both  $c_i$  and  $A/E$  are especially sensitive to environmental conditions, because they are dependent on  $A$  and  $g$ . Instantaneous gas exchange measurements may also have been obscured by the method in which treatments were applied, because seedlings within the same water regime but different chambers were at varying levels of moisture stress when gas exchange measurements were made.

In addition to variability in immediate environmental conditions, physiological responses to  $\text{O}_3$  exposure may confound the relationship between



$\delta^{13}\text{C}$  and  $c_i$ . Changes in respiration due to  $\text{O}_3$  have been reported in pine species (Barnes, 1972; McLaughlin *et al.*, 1982; Yang *et al.*, 1983) and would obscure the response of  $\delta^{13}\text{C}$  to  $\text{O}_3$  if fractionation is associated with respiration. It is assumed that isotope discrimination associated with respiration is negligible, but this has not been confirmed (O'Leary, 1988). PEP carboxylation is another process affecting carbon isotope composition. A fourfold increase in PEP carboxylase activity due to  $\text{O}_3$  was observed in Scots pine (*Pinus sylvestris*) (Leuthy-Krause *et al.*, 1990). Since PEP carboxylation discriminates against  $^{12}\text{C}$  (Farquhar *et al.*, 1989a), an increase in PEP carboxylase activity would cause increased  $\delta^{13}\text{C}$ . Fractionation during secondary metabolism could also obscure  $\delta^{13}\text{C}$  response to  $\text{O}_3$ . Sharkey *et al.* (1991) reported fractionation associated with the synthesis of isoprene in red oak (*Quercus rubra*), in which the magnitude of discrimination was dependent on plant response to environmental conditions. Finally, variability in resistance to  $\text{CO}_2$  diffusion within the leaf may modify  $\delta^{13}\text{C}$  (Vitousek *et al.*, 1990). While  $\delta^{13}\text{C}$  reflects  $c_i$  at the sites of carboxylation, gas exchange parameters yield  $c_i$  of the substomatal cavities (Evans *et al.*, 1986). In this study,  $\text{O}_3$  significantly increased specific leaf area ( $\text{m}^2$  leaf area/g foliage biomass), indicating decreased needle thickness or less densely packed cells (Elsik *et al.*, 1992). In either case, the result may be decreased internal resistance to  $\text{CO}_2$  diffusion to the sites of carboxylation. Thus, the effect of  $\text{O}_3$  on specific leaf area would result in increased  $c_i$  at the sites of carboxylation, consequently decreasing  $\delta^{13}\text{C}$ . This would conceal the effect of stomatal response to  $\text{O}_3$  on  $\delta^{13}\text{C}$ , in which decreased  $g$  causes decreased  $c_i$  in the substomatal cavities, increasing  $\delta^{13}\text{C}$ .

There were no  $\text{O}_3 \times$  water-stress interactions on instantaneous  $c_i$  or other gas exchange measurements. The relationship between watering and fertilizing schedules for each chamber with the timing of  $\text{O}_3$  peaks may have obscured this potential interaction. Since  $\text{O}_3$  was added in proportion to ambient, and watering and fertilizing cycles within a water stress treatment were different in each chamber, it is possible that seedlings within the same watering regime, but different chambers, were at different water-stress levels when  $\text{O}_3$  peaks occurred. The integrated nature of stable carbon isotope composition allowed an interaction to be detected, because, unlike instantaneous gas exchange measurements,  $\delta^{13}\text{C}$  is not immediately dependent on environmental variables at the time of measurement, which may or may not represent typical conditions.

The interaction suggested by regression analysis, in which the effects of  $\text{O}_3$  on  $\delta^{13}\text{C}$  of foliage and stem tissue in WW seedlings of loblolly pine were greater than those of WS seedlings, indicated that water stress provided protection from  $\text{O}_3$  through stomatal closure. This is supported by the tendency of WS loblolly pine seedlings to possess lower  $g$  than WW loblolly pine seedlings. Similar interactions, in which the WS treatment lessened the  $\text{O}_3$  effect, were observed in foliage biomass of loblolly pine (Elsik *et al.*, 1992). The opposite interaction was observed in foliar  $\delta^{13}\text{C}$  of shortleaf pine, with a greater  $\text{O}_3$  effect in WS seedlings than in WW seedlings. This

interaction in shortleaf pine suggested that  $O_3$  may have increased stomatal response to water stress. Instantaneous measurements of  $g$  in shortleaf pine, indicating that water stress tended to increase  $g$  at the higher  $O_3$  levels, do not support this hypothesis. Alternatively, this apparent interaction may be a result of the high degree of variability in  $O_3$  response of foliar  $\delta^{13}C$  of WW seedlings as opposed to WS seedlings.

The results reported here and the previously reported growth measurements (Elsik *et al.*, 1992) indicate that the loblolly pine family was more sensitive to  $O_3$  than the shortleaf pine family. Differences in  $O_3$  sensitivity have been attributed to inherent differences in  $g$ , because  $O_3$  uptake is limited by stomatal aperture (Reich, 1987). This is not the case in the present study. The loblolly pine family used here (GR1-8) is drought-hardy (van Buijtenen, 1966) and is expected to exhibit lower  $g$  than a drought-sensitive family. Seiler and Johnson (1988) reported that this family had lower rates of transpiration than two drought-susceptible families. In this study, instantaneous gas exchange measurements indicated that loblolly pine did indeed possess significantly lower  $g$  ( $P < 0.05$ ) than shortleaf pine throughout the experiment. Alternatively, the difference in  $O_3$  sensitivity may be related to mesophyll properties (Taylor *et al.*, 1982). According to Knauf and Bilan (1977), a drought-hardy loblolly pine family possessed more closely packed mesophyll than loblolly pine families from mesic seed sources. This would provide more surface area on which  $O_3$  molecules can dissolve. The difference may also be related to the ability of the plant to compensate for  $O_3$  damage. Loblolly pine exhibited significantly greater height growth ( $P < 0.001$ ) throughout the season, while shortleaf pine exhibited significantly greater  $A$  ( $P < 0.05$ ) (Elsik *et al.*, 1992). The additional carbon assimilated in shortleaf pine may have been allocated to compensatory processes. Differences in carbon allocation to roots and foliage may also have contributed to the difference in response and has been addressed elsewhere (Elsik *et al.*, 1992).

The difference between the two tissues in their response of  $\delta^{13}C$  to the treatments may be related to the timing of seedling susceptibility to treatment and carbon allocation patterns. Ozone injury does not occur until a plant is unable to compensate for the cellular damage caused by  $O_3$  (Tingey and Taylor, 1982). The stem tissue may contain a higher percentage of the carbon that had been assimilated previous to  $O_3$  injury. Stem tissue may, therefore, provide a better estimate of integrated seedling response. The lack of significant stem  $\delta^{13}C$  response to  $O_3$  in shortleaf pine, despite the significant increase in foliar  $\delta^{13}C$  due to  $O_3$ , is most likely related to the timing of  $O_3$  injury. Loblolly pine, which showed a significant  $O_3$  response in both tissues, and was more responsive to  $O_3$  in growth and gas exchange characteristics (Elsik *et al.*, 1992), was unable to compensate for  $O_3$  damage at a time preceding the onset of  $O_3$  injury in shortleaf pine.

The increase in  $A$  due to water stress may cause one to question the effectiveness of the WS treatment. However, the aim of the WS treatment was merely to provide a mild water deficit compared to the WW treatment.



Although the WS treatment did not consistently result in significantly lower  $g$ , it did tend to decrease  $g$ . The increased  $A$  in WS seedlings may be explained by a fertilizer effect, in which nitrate was leached from the fritted clay medium more quickly in the WW treatment than in the WS treatment. This is supported by higher foliar N content and foliar chlorophyll concentration in WS seedlings than WW seedlings (Elsik, 1992).

## V. Summary

Chronic environmental stress, such as  $O_3$  exposure, may have subtle effects on physiological processes in trees, while greatly impacting growth over time. Instantaneous measurements of plant gas exchange response may not be sufficient to detect subtle changes in physiology. Therefore, an integrated measure is necessary to determine the long-term effect of physiological response to  $O_3$ . In this study, container-grown seedlings were exposed to both chronic  $O_3$  and mild water stress in open-top field chambers throughout one growing season. Stable carbon isotope composition of foliage and stem tissue was determined in order to assess integrated gas exchange response, which may not have been detected by instantaneous measurements.

After 4 months of treatments,  $O_3$  significantly increased  $\delta^{13}C$  values of foliage and stem tissue in loblolly pine and foliage tissue in shortleaf pine, indicating decreased  $c_i$ , evidence that  $O_3$  had a greater effect on  $g$  than on light harvesting or photosynthetic enzyme processes in loblolly and shortleaf pine. The results also indicate that  $O_3$  exposure increased  $W$  in both species. Stable carbon isotope composition did not reflect instantaneous  $c_i$  measurements. Interactions between  $O_3$  and water stress were not observed in gas exchange measurements, but the integrated nature of stable carbon isotope analysis permitted the detection of an interaction effect on  $\delta^{13}C$ . The interaction in loblolly pine suggested that water deficit provided protection from  $O_3$  through partial stomatal closure, in agreement with instantaneous measurements of  $g$ . The interaction in shortleaf pine suggested that  $O_3$  increased stomatal response to water deficit, but this is not supported by instantaneous measurements of  $g$ . Stable carbon isotope analysis proved to be an important tool in resolving long-term  $O_3$  effects, while instantaneous gas exchange measurements were not sufficient to detect effects of chronic  $O_3$  exposure on  $c_i$  and  $W$ . The application of this technique to air pollution studies can be enhanced through the investigation of  $O_3$  effects on processes other than RuBP carboxylation that may affect  $\delta^{13}C$ .

## Acknowledgments

This research was supported by a grant from the Texas Agricultural Experiment Station. We thank John Lock, Brad Touns, Jeff Anderson, Jimmie Exley, Andrew Midwood, Daniel Watts, and Xing Wang for their expert technical assistance.



## References

- Barnes, R. L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. *Environ. Pollut.* 3: 133–138.
- Becker, K., M. Saurer, A. Egger, and J. Fuhrer. 1989. Sensitivity of white clover to ambient ozone in Switzerland. *New Phytol.* 112: 235–243.
- Boutton, T. W. 1991. Stable carbon isotope ratios of natural materials. I. Sample preparation and mass spectrometric analysis, pp. 155–171. In D. C. Coleman and B. Fry (eds.), *Carbon Isotope Techniques*. Academic Press, New York.
- Boutton, T. W., and R. B. Flagler. 1990. Growth and water-use efficiency of shortleaf pine as affected by ozone and acid rain. Proceedings of the 83rd Annual Meeting and Exhibition of the Air & Waste Management Association, 90-187.7. Air & Waste Management Association, Pittsburgh, PA.
- Chappelka, A. H., and B. I. Chevone. 1992. Tree responses to ozone, pp. 271–324. In A. S. Lefohn (ed.), *Surface Level Ozone Exposures and Their Effects on Vegetation*. Lewis, Chelsea, MI.
- Chappelka, A. H., B. I. Chevone, and J. R. Seiler. 1988. Growth and physiological responses of yellow-poplar seedlings exposed to ozone and simulated acid rain. *Environ. Pollut.* 49: 1–18.
- Downton, W. J. S., B. R. Loveys, and W. J. R. Grant. 1988. Non-uniform stomatal closure induced by water stress causes putative non-stomatal inhibition of photosynthesis. *New Phytol.* 110: 503–509.
- Elsik, C. G. 1992. Growth, Physiology, and  $\delta^{13}\text{C}$  of Loblolly and Shortleaf Pine as Affected by Ozone and soil Water Deficit. Thesis. Department of Forest Science, Texas A&M University, College Station, TX.
- Elsik, C. G., R. B. Flagler, and T. W. Boutton. 1992. Effects of ozone and water deficit on growth and physiology of *Pinus taeda* and *Pinus echinata*, pp. 225–245. In R. B. Flagler (ed.), *Response of Southern Commercial Forests to Air Pollution*. Transactions of the Air & Waste Management Association, TR-21. Air & Waste Management Association, Pittsburgh, PA.
- Evans, J. R., T. D. Sharkey, J. A. Berry, and G. D. Farquhar. 1986. Carbon isotope discrimination measured concurrently with gas exchange to investigate  $\text{CO}_2$  diffusion in leaves of higher plants. *Aust. J. Plant Physiol.* 13: 281–292.
- Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989a. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40: 503–537.
- Farquhar, G. D., K. T. Hubick, A. G. Condon, and R. A. Richards. 1989b. Carbon isotope fractionation and plant water-use efficiency, pp. 21–40. In P. W. Rundel, J. R. Ehleringer, and K. A. Nagy (eds.), *Stable Isotopes in Ecological Research*. Springer-Verlag, New York.
- Freyer, H. D. 1979. On the record in tree rings. Part II. Registration of microenvironmental  $\text{CO}_2$  and anomalous pollution effect. *Tellus* 31: 308–312.
- Ginn, S. E., J. R. Seiler, B. H. Cazell, and R. E. Kreh. 1991. Physiological and growth responses of eight-year-old loblolly pine stands to thinning. *For. Sci.* 37: 1030–1040.
- Greitner, C. S., and W. E. Winner. 1988. Increases in  $\delta^{13}\text{C}$  values of radish and soybean plants caused by ozone. *New Phytol.* 108: 489–494.
- Guderian, R., D. T. Tingey, and R. Rabe. 1985. Effects of photochemical oxidants on plants, pp. 129–333. In R. Guderian (ed.), *Air Pollution by Photochemical Oxidants—Formation, Transport, Control, and Effects on Plants*. Springer-Verlag, New York.
- 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., D. E. Body, and W. W. Heck. 1973. An open-top field chamber to assess the impact of air pollution on plants. *J. Environ. Qual.* 2: 365–368.
- Hubick, K. T., G. D. Farquhar, and R. Shorter. 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Aust. J. Plant Physiol.* 13: 803–816.

- Keller, T., and R. Hasler. 1984. The influence of a fall fumigation with ozone on the stomatal behavior of spruce and fir. *Oecologia* 64: 284–286.
- Knauf, T. A., and M. V. Bilan. 1977. Cotyledon and primary needle variation in loblolly pine from mesic and xeric seed sources. *For. Sci.* 23: 33–36.
- Laisk, A. 1983. Calculation of leaf photosynthetic parameters considering the statistical distribution of stomatal apertures. *J. Exp. Bot.* 34: 1627–1635.
- Leuthy-Krause, B., I. Pfenninger, and W. Landolt. 1990. Effects of ozone on organic acids in needles of Norway spruce and Scots pine. *Trees* 4: 198–204.
- Lock, J. E., and R. B. Flagler. 1992. Comparison of gas exchange rates for attached and detached needle fascicles of shortleaf pine seedlings, submitted for publication.
- Martin, B., and E. K. Sutherland. 1990. Air pollution in the past recorded in width and stable carbon isotope composition of annual growth rings of Douglas-fir. *Plant Cell Environ.* 13: 839–844.
- Martin, B., and Y. R. Thorstenson. 1988. Stable carbon isotope composition ( $\delta^{13}\text{C}$ ), water use efficiency, and biomass productivity of *Lycopersicon esculentum*, *Lycopersicon pennellii* and the  $F_1$  hybrid. *Plant Physiol.* 88: 213–217.
- Martin, B., A. Bytnerowicz, and Y. R. Thorstenson. 1988. Effects of air pollutants on the composition of stable carbon isotopes,  $\delta^{13}\text{C}$ , of leaves and wood, and on leaf injury. *Plant Physiol.* 88: 218–223.
- 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. *For. Sci.* 28: 60–70.
- Moldau, H., J. Sober, and A. Sober. 1990. Differential sensitivity of stomata and mesophyll to sudden exposure of bean shoots to ozone. *Photosynthetica* 24: 446–458.
- Nobel, P. 1991. *Physicochemical and Environmental Plant Physiology*. Academic Press, New York.
- O'Leary, M. H. 1988. Carbon isotopes in photosynthesis. *Bioscience* 38: 328–336.
- Olszyk, D. M., and T. W. Tibbitts. 1981. Stomatal response and leaf injury of *Pistia sativum* L. with  $\text{SO}_2$  and  $\text{O}_3$  exposures. II. Influence of moisture stress and time of exposure. *Plant Physiol.* 67: 545–549.
- Reich, P. B. 1987. Quantifying plant response to ozone: A unifying theory. *Tree Physiol.* 3: 63–91.
- 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., and J. P. Lassoie. 1984. Effects of low level  $\text{O}_3$  exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. *Plant Cell Environ.* 7: 661–668.
- Runeckles, V. C., and B. I. Chevone. 1992. Crop response to ozone, pp. 189–269. In A. S. Lefohn (ed.), *Surface Level Ozone Exposures and Their Effects on Vegetation*. Lewis, Chelsea, MI.
- Sasek, T. W., and C. J. Richardson. 1989. Effects of chronic doses of ozone on loblolly pine: Photosynthetic characteristics in the third growing season. *For. Sci.* 35: 745–755.
- Saurer, M., J. Fuhrer, and U. Siegenthaler. 1991. Influence of ozone on the stable carbon isotope composition,  $\delta^{13}\text{C}$ , of leaves and grain of spring wheat (*Triticum aestivum* L.). *Plant Physiol.* 97: 313–316.
- Seiler, J. R., and J. D. Johnson. 1988. Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. *For. Sci.* 34: 487–495.
- Sharkey, T. D., F. Loreto, C. F. Delwiche, and I. W. Treichel. 1991. Fractionation of carbon isotopes during biogenesis of atmospheric isoprene. *Plant Physiol.* 97: 463–466.
- Sheffield, M., and N. D. Cost. 1987. Behind the decline. *J. For.* 85: 29–33.
- Taylor, G. E., D. T. Tingey, and H. C. Ratsch. 1982. Ozone flux in *Glycine max* (L.) Merr.: Sites of regulation and relationship to leaf injury. *Oecologia* 53: 179–186.
- Taylor, G., Jr. 1991. Interaction of ozone with naturally occurring stresses, pp. 103–113. In *Influence of Ozone, Acidic Precipitation and Soil Magnesium Status on the Physiology and Growth of Pinus taeda L. (Loblolly Pine) under Field Conditions*. Biological Sciences Center, Desert Research Institute, University of Nevada System, Reno, NV.

- Terashima, I., S. C. Wong, C. B. Osmond, and G. D. Farquhar. 1988. Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Plant Cell Physiol.* 29: 385–394.
- Teskey, R. O., and T. M. Hinckley. 1986. Moisture: Effects of water stress on trees, pp. 9–33. In T. C. Hennessey, P. M. Dougherty, S. V. Kossuth, and J. D. Johnson (eds.), *Stress Physiology and Forest Productivity*. Nijhoff, The Netherlands.
- Tingey, D. T., and G. E. Taylor. 1982. Variation in plant response to ozone: a conceptual model of physiological events, pp. 113–138. In M. H. Unsworth and D. P. Ormrod, (eds.), *Effects of Gaseous Air Pollution in Agriculture and Horticulture*. Butterworth Scientific, London.
- van Buijtenen, J. P. 1966. Testing Loblolly Pines for Drought Resistance. Texas Forest Service Research Report 13. Texas Forest Service, College Station, TX.
- Vitousek, P. M., C. B. Field, and P. A. Matson. 1990. Variation in foliar  $\delta^{13}\text{C}$  in Hawaiian *Metrosideros polymorpha*: A case of internal resistance? *Oecologia* 84: 362–370.
- Yang, Y. S., J. M. Skelley, B. I. Chevone, and J. B. Birch. 1983. Effects of long-term ozone exposure on photosynthesis and dark respiration of eastern white pine. *Environ. Sci. Technol.* 17: 371–373.