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Comparative Sensitivity, Mechanisms, and Whole Plant Physiological Implications of Responses of Loblolly Pine Genotypes to Ozone and Acid Deposition

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Environmental Sciences Division
Publication No. 3105



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ENVIRONMENTAL SCIENCES DIVISION

COMPARATIVE SENSITIVITY, MECHANISMS, AND WHOLE PLANT PHYSIOLOGICAL
IMPLICATIONS OF RESPONSES OF LOBLOLLY PINE GENOTYPES
TO OZONE AND ACID DEPOSITION

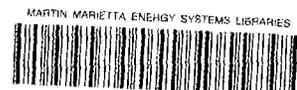
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SUMMARY

McLAUGHLIN, S. B., M. B. ADAMS, N. T. EDWARDS, P. J. HANSON,
P. A. LAYTON, E. G. O'NEILL, and W. K. ROY. 1988.
Comparative sensitivity, mechanisms, and whole
plant physiological implications of responses of
loblolly pine genotypes to ozone and acid deposition.
ORNL/TM-10777. Oak Ridge National Laboratory,
Oak Ridge, Tennessee. 306 pp.

A quantitative and mechanistic basis for evaluating the potential effects of atmospheric pollutants on physiology and growth of seedlings of loblolly pine, an important timber species in southern commercial forests, was evaluated in laboratory and controlled field studies. Fifty-three half-sib families of loblolly pine were examined. Primary objectives were to (1) quantify differences in growth responses of these 53 half-sib families to the individual and interactive effects of simulated acid rain and ozone in the field, (2) characterize the physiological basis of observed responses in field and laboratory studies, (3) compare and contrast results obtained with similar experimental protocols in field and laboratory approaches, and (4) develop experimental protocols for quantifying physiological and growth responses of large trees in the field.

Field exposures of 9950 containerized 12-week-old seedlings were conducted in a 36-plot field research facility composed of 33 open-top chambers and 3 open plots. Six ozone levels [ambient open, ambient chambered, charcoal filtered, ambient + 40 ppb, ambient + 80 ppb, and ambient + 160 ppb, for 6 hr/d and 4 d/w] and three levels of simulated acid rain (pH 3.3, 4.5, and 5.2) were applied. In laboratory studies ozone was added at three levels (0, 160, and 320 ppb) for 6 hr/d, 4 d/week in Continuously Stirred Tank Reactor (CSTR) chambers. A background of pH 4.3 rain (1.1 cm twice each week) was provided, and plants were placed in a charcoal-filtered greenhouse on the other 3 d.

Results of these studies indicated that there were large differences in inherent growth rates among families and significant differences in responses of individual families to ozone and, to a

lesser degree, to acid rain. Seedlings grown under field conditions were generally more sensitive to ozone than those exposed in laboratory chambers, although relative differences in sensitivity among families were similar with both exposure conditions. In the field exposure to ambient air, in which ozone is the principal known phytotoxic component in the Southeast, reduced average height, diameter, and volume (d^2h) growth (-26%, -5%, and -14%, respectively) compared to that observed in charcoal-filtered air in which the exposure dose was reduced by 50%. Increasing ozone levels above those in ambient air resulted in growth responses that were occasionally stimulatory at the lowest ozone level. While these responses became increasingly inhibitory at the highest ozone levels, they did not significantly exceed growth reductions found in ambient air.

Acid rain caused a general stimulation of height growth for most families at ambient levels of rainfall acidity (pH 4.5). By contrast height growth was typically reduced at a mean pH of 3.3. Significant interactions between rainfall acidity and ozone were detected in height growth response. In general the effects of acid rain were greatest in charcoal-filtered air, but decreased as the level of ozone increased.

Physiological measurements on selected families indicated that effects on photosynthesis, carbon allocation, and mycorrhizal colonization were, like the effects on growth, more pronounced under field conditions. Principal responses detected at increasing ozone levels were reduction in photosynthesis, transport of carbon from shoots to roots, and root starch. These changes were in turn associated with reduced mycorrhizal infection of short roots, and reduced of root:shoot ratios. Photosynthesis was stimulated by acid rain at pH 3.3, but this response was not associated with an increase in plant dry-matter accumulation at the end of the 12-week experiment. Physiological changes induced by ozone were generally positively correlated with observed changes in biomass increment.

Collectively the measurements of carbon assimilation and partitioning suggest that growth responses to ozone will be strongly influenced by physiological changes leading to both reduced

availability of carbon (reduced assimilation) and altered partitioning of that carbon, principally to roots. Reductions in root biomass and reductions in mycorrhizal status are viewed as important areas for further study based on results from the current investigations.

Continuous measurements of photosynthesis in canopies of large trees are feasible based on exploratory experiments with an open-flow gas-exchange system. Such studies may provide a basis for in situ measurements of tree responses to ozone under ambient exposure regimes.

Collectively these studies indicate that adverse growth responses of loblolly pine seedlings to ambient levels of atmospheric ozone are likely but will be strongly dependent on genetic variation associated with seed source. Responses to ambient levels of acid deposition are likely to be much more complex and may involve growth stimulation, particularly in height. Ozone--acid rain interactions appear likely at acidity levels substantially above current ambient levels in the Southeast. At the highest levels of acid rain and ozone, the influence of combined exposures was antagonistic rather than additive or synergistic.

The more obvious growth responses observed in the field compared with those in laboratory studies suggest that substantial emphasis be placed on field work in the future. The influence of continuous low-level exposures occurring as a background between ozone additions in the field should be examined much more closely in future studies. In addition, major emphasis should be placed on evaluating responses of loblolly pine to chronic exposures at ambient and near ambient exposure doses where measurable adverse effects were observed in these studies.

1. INTRODUCTION

1.1 PROJECT OVERVIEW

This report summarizes results of the first year of field and laboratory studies on growth responses of loblolly pine seedlings to ozone. The project was undertaken in 1986 with the support of the U.S. Department of Agriculture/U.S. Environmental Protection Agency (USDA/EPA) Forest Response Program and was initiated as part of the Southern Commercial Forest Cooperative.

The scientific basis for this research was the need to evaluate individual and interactive effects of ozone and acid deposition on the growth and physiology of loblolly pine. The types of effects produced, their relationship to exposure dose, their mechanistic basis, and the extent to which they varied across genetic lines are all important areas of information needed for the evaluation of potential effects of regional atmospheric pollutants on growth of loblolly pine in southern commercial forests. An integrated program of field and laboratory research was initiated in April 1986 to focus on four principal objectives:

1. Quantify differences in growth responses of 53 half-sib loblolly pine families to the individual and interactive effects of simulated acid rain and ozone in the field.
2. Characterize the physiological basis of observed responses in field and laboratory studies.
3. Compare and contrast results obtained with similar experimental protocols in field and laboratory approaches.
4. Develop experimental protocols for quantifying physiological and growth responses of large trees in the field.

Collectively, these experiments explored the whole-plant physiological basis of pollutant effects under diverse pollutant regimes.

1.2 BACKGROUND

Evidence of regionally synchronous changes in growth and vigor of some species of forest trees in both Europe and in the eastern United States has accumulated rapidly in the past 5 years (McLaughlin 1985). The types of changes, their timing, and their regional distribution suggest that regional-scale influences such as climate or atmospheric pollution may be playing a role as either predisposing or triggering factors. In Europe, the diversity of species affected, the large area involved, and the existence of gradients in damage in proximity to known sources of atmospheric pollution have led to a general consensus among European scientists that atmospheric pollution is playing a role in these changes. In the United States, the most obvious changes in radial growth rate have been noted in red spruce growing at high elevations in the Appalachian Mountains (Johnson and Siccama 1983). At lower elevations in East Tennessee, recent reductions in radial increment in shortleaf pine have been noted (Baes and McLaughlin 1984). These reductions coincide temporally with shifts to slower growth by red spruce at high elevations. In addition, the analysis of forest inventory data from permanent survey plots by the U.S. Forest Service has revealed an unexplained shift to slower growth rates (a 30-50% reduction during the 30 years) for pines in the Southeast (Sheffield and Cost 1987).

The causes of these changes are poorly understood. In Europe, SO₂ is clearly involved in some industrial areas, but in the broader regional environment, principal theories of action involve (1) acid-precipitation-induced mobilization of trace elements such as aluminum to levels that are toxic to root growth and leaching of nutrients in the soil (Ulrich et al. 1980) and (2) physiological stress associated with ozone and acid rain impacts on foliage (Krause et al. 1983).

In the United States, fewer species appear to have been affected. Because symptoms are less diverse, the case for pollution as a causal factor in observed change is less clear. However, a number of factors

support the concern about a possible role of ozone in the patterns of reduced growth. These include (1) the occurrence in the ambient atmosphere of maximum atmospheric ozone concentrations that substantially exceed short-term damage thresholds (McLaughlin 1985), (2) the widespread distribution of ozone at potentially phytotoxic concentrations (NAS 1977), (3) the appearance of visual symptoms of ozone damage on foliage of native herbs and trees in the field, and (4) the documentation by the National Crop Loss Assessment Network of yield reductions in crop species exposed to ambient levels of ozone and associated gaseous pollutants in the field (Heck et al. 1984).

The previously discussed changes in productivity of southern pine forests have generated considerable concern that an extremely important economic resource may be at risk because of atmospheric pollution. A principal initial focus of this concern has been on responses of loblolly pine (the most important commercial species) to ozone, which is the most clearly documented phytotoxic pollutant at ambient concentrations. Potential interactions of ozone with acidic deposition across the region were a second area of emphasis. Two major areas of research have been delineated in the developing Southern Commercial Forest Research Program: (1) documentation of changes in growth patterns in the field and (2) characterization of the genetic variability and associated physiological mechanisms of resistance to ozone and acid rain on the basis of controlled field and laboratory studies.

Laboratory studies under controlled environmental conditions offer some significant experimental advantages in understanding the interactions of genetic variability, pollutant exposure level, and other environmental variables in the responses of tree seedlings to pollutant stress. There are also limitations in the extent to which results can be extrapolated to the responses of seedlings and particularly larger trees under field conditions. For this reason, laboratory screening studies must advance the conceptual understanding of the physiological basis of responses to principal environmental variables that may modify responses in the field. In this capacity,

such studies can help identify significant features of both pollutant dose and plant response. This identification can lead to improved understanding and more effective implementation and evaluation of field research. The research described in this report represents a merging of both laboratory and field approaches.

1.3 RESEARCH STRATEGY

This research focused on five principal hypotheses:

1. The physiological attributes that have led to the selection of test families of loblolly pine for superior growth under a wide range of environmental conditions will result in significant genetic differences in sensitivity of loblolly pine seedlings to pollutants and will be expressed as differences in response of total growth, relative growth of roots and shoots, and physiology.
2. Ambient levels of ozone are more phytotoxic to loblolly pine than are current ambient levels of acid deposition in the Southeast in low elevation forests.
3. Effects of ozone and acid deposition on loblolly pine growth will occur both as a result of reductions in the amount of carbon fixed and in the allocation of that carbon.
4. The point of maximum sensitivity for individual and interactive effects of ozone and acid deposition will be the root-rhizosphere system.
5. Background levels of ozone during exposure respites will have an important influence on response of seedlings to ozone during exposures.

These hypotheses have been tested by comparative analysis of growth responses of 53 half-sib families of loblolly pine in the field and a subset of 8 of those families in the laboratory. Growth

measurements have been coupled with physiological measurements of a subsample of these families to evaluate a mechanistic basis for observed responses.

1.4 TECHNICAL APPROACH

The four objectives of this project were addressed by implementing closely related field and laboratory studies designed to incorporate many common cultural and experimental protocols both within the studies at ORNL and across collaborating sites within the Southern Commercial Forest Cooperative. Laboratory studies focused on testing physiological and growth responses of eight common denominator (CD) families (for intersite comparisons) to ozone at three levels using the approximate ambient rainfall pH level (mean pH 4.38 with a median pH of 4.5) as a common background irrigant. Field studies used a 36-plot research site incorporating 3 open plots with no chambers and 33 open-top chambers to examine individual and interactive effects of ozone and simulated acid rain. An aerial view of this test facility is shown in Fig. 1.1.

The treatment combinations employed in this three-replicate design allowed the following responses to be tested for the 8 CD families and the 45 additional families:

1. Ozone alone -- Comparisons involved nonchambered, and chambered plots at near 15 ppb (charcoal filtered), ambient (nonfiltered); and ambient plus 40, 80, or 160 ppb ozone. All plots were exposed to simulated rainfall at pH 4.5, with natural rain being excluded. Total plots = 18.
2. Acid rain alone -- Three pH levels (median values of 3.3, 4.5, and 5.2) were tested using the charcoal-filtered chambers to evaluate a pure acid rain effect. Total plots = 9.
3. Ozone x acid rain interaction -- The 3 x 3 factorial design involving rainfall pH levels of 3.3, 4.5, and 5.2 and ozone levels

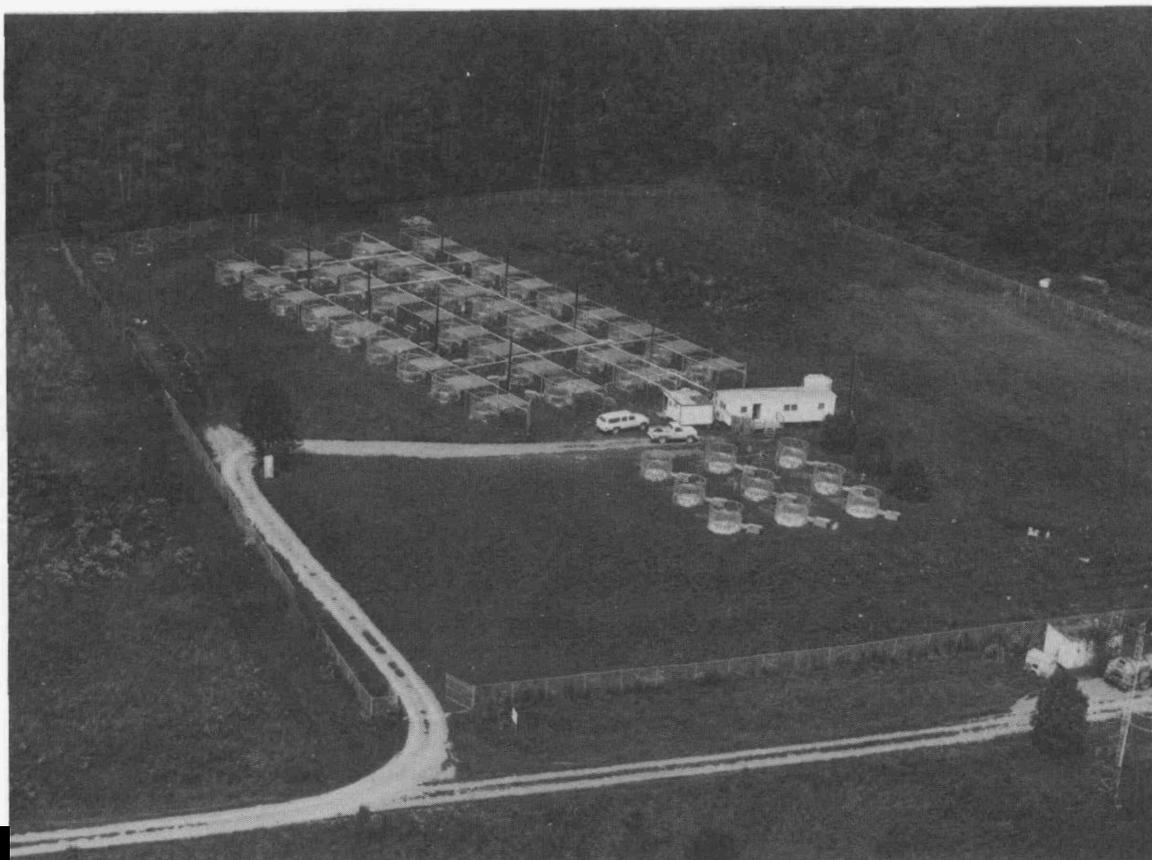


Fig. 1.1. Aerial view of the field research facility facing North. The 36 plots used in these studies are located in 3, 12 block (3 rows of 4 chambers each) segments extending from southeast (Block I) to northwest (Block III). Control and data acquisition systems were located in the largest trailer east of the center of the view. Larger trees used in canopy gas exchange measurements are southeast of the main trailer, and the data acquisition system for those measurements is located in the small trailer west of the main trailer.

of CF (\bar{x} = 15 ppb), ambient + 80 ppb, and ambient + 160 ppb tested interactive effects of these pollutants. Total plots = 27.

4. Chamber effects -- The influence of the open-top chambers on growth was evaluated by comparing open plots and nonfiltered chamber plots, both at pH 4.5. Total plots = 6.

1.5 EXPERIMENTAL DESIGN

The following outline provides a brief overview of experimental features of the first year of laboratory and field studies that have been directed heavily toward screening. It should be noted that the timing and choices of measurement parameters were predicated upon increasing the number and significance of comparisons between laboratory and field studies. Additional details on methodology are provided in the sections that address system operation and results of analyses of growth and physiological responses. Additional information on quality assurance considerations with parameter measurement protocols is included in Appendix E.

1. Laboratory Studies

A. Exposure Conditions:

- 6 CSTR chambers, artificial light, humidity control.
- Acid rain -- pH 4.3 rain applied as a common background (2.2 cm-wk⁻¹).
- ozone - 0, 160, 320 ppb.
- Duration - 6 h/d⁻¹ x 4 day•week⁻¹ x 12 weeks.
- Dosage levels - 0, 23, 46 ppm x L.

B. Cultural:

- 8 families (in common with collaborating laboratories).
- 8 seedlings per family per CSTR chamber x 2 chambers per concentration.

C. Sampling and Analysis Schedule:

<u>Parameters measured</u>	<u>Sampling schedule</u>				
	<u>(Weeks)^a</u>				
	0	3	6	9	12
Biomass a. Destructive sampling	X(8) ^b		X(8)		X(8)
b. D ² H	X(8)	X(8)	X(8)	X(8)	X(8)
Carbon allocation			X(3)		X(3)
Photosynthesis (Licor)	X(8)		X(8)		X(8)
Nutrient status					X(2)
Light response (Siemens)			X(2)		X(2)
Mycorrhizae			X(2)		X(2)

^aAfter the initial 10-12 weeks of preconditioning growth.

^bThe number of families sampled is shown in parentheses.

2. Field Studies

The 36 plots were distributed across 12 treatment conditions as shown below. Only open plots had no chambers. All plots had rainfall exclusion devices.

Acid rain	Ozone treatment					
	Open	A ^a	CF ^a	A + 40ppb	A + 80ppb	A + 160 ppb
5.2				X	X	X
4.5	X ^b	X	X	X	X	X
3.3				X	X	X

^aA = ambient; CF = charcoal filtered; open plots received only ambient ozone.

^bX represents three chambers.

A. Exposure Conditions:

- 33 open-top (USDA chambers) and 3 open plots.
- Acid rain - median pH 3.3, 4.5, 5.2; natural rain excluded.
- ozone - Ambient (open), filtered (control), ambient chamber, ambient + 40 ppb, ambient + 80 ppb, ambient + 160 ppb.
- All ozone added as square wave to fluctuating ambient levels.
- Duration - ozone added for $6 \text{ h} \cdot \text{d}^{-1} \cdot 4 \text{ d} \cdot \text{week}^{-1} \cdot 12 \text{ weeks}$.
- Interactions - 3 x 3 factorial with 3 levels of acid rain x CF (filtered), ambient + 80 ppb, and ambient + 160 ppb; all other ozone levels with pH 4.5 rainfall.

Projected dosage levels^a: The following seasonal dose levels were anticipated based on projected ozone additions and approximate regional mean ozone exposures for the study site.

	Open	Chambered Plots				
		CF ^b	A ^b	A + 40 ^b	A + 80 ^b	A + 160 ^b
Baseline (ppm·h)	65		65	65	65	65
Episodic (ppm·h)	12	0	12	20	30	60
Total (ppm·h)	77	0	77	85	95	125

^aCalculations were based on a background level of 0.04 ppm and a 7-h maximum growing season average of 0.06 ppm. episodic ambient addition would be $12 \text{ weeks} \times 7 \text{ d} \cdot \text{week}^{-1} \times 7 \text{ h} \cdot \text{d}^{-1} \times 0.02 \text{ ppm} = 12 \text{ ppm} \cdot \text{h}$.

^bA = ambient; CF = Charcoal filtered; Chambered, non-filtered plots A + 40, A + 80, and A + 160 indicate additions to ambient in parts per billion.

B. Family Selection:

- 8 families in common with laboratory studies plus one additional intersite family.
- 44 new families.
- 8 seedlings per family per chamber for 9 common families.
- 4-6 seedlings per family per chamber for 44 families.

C. Sampling and Analysis Schedule:

<u>Parameters measured</u>	<u>Sampling Schedule</u>		
	<u>(Weeks)^a</u>		
	0	6	12
Biomass a. Destructive	X (10)	X (8)	X (10)
b. D ² H	X (all)	X (20)	X (all)
Carbon allocation			X (4)
Photosynthesis (Licor)	X (10)	X (3)	X (4)
Nutrient analysis			X (2)
Light response (Siemens)		X (2)	X (2)
Mycorrhizae	X (9)	X (2)	X (3)

^aMeasured on a subsample of families; the number of families is shown in parentheses.

3. Cultural and Plant Measurement Protocols

The following protocols were used to grow seedlings and to measure growth and physiology.

Cultural practices - Seedlings were grown in 7.5 x 8 x 27-cm-deep containers in a 3:1 vermiculate peat mixture for 12 weeks prior to exposure. Nutrients for laboratory seedlings were supplied biweekly with a standard nutrient solution, while field grown seedlings were fertilized initially with a pelletized slow-release fertilizer (17-6-10 with micronutrients). See Sect. 3.

Growth measurements - Stem diameter was measured below the cotyledonary scar, and height was measured to the base of the apical bud. Seedlings were measured initially and at the end of the experiment, with up to three interval measurements being obtained for more intensively sampled subsets. See Sect. 3.

Photosynthesis - Three different techniques were used to measure photosynthesis: (1) Licor 6000 measurements in the greenhouse under artificial light using the terminal 60 mm (± 20 mm) of shoot; (2) Siemens Sirigor (open flow under controlled light and temperature); and (3) a gross measure of relative uptake of CO_2 during ^{14}C tagging studies. See Sects. 4 and 5 and Appendix C for a comparison of techniques.

Carbon metabolism - In these studies seedlings were exposed to $^{14}\text{CO}_2$ in the greenhouse followed by subsequent subsampling of tissues at day 1 and day 7 to determine patterns of movement of photosynthate between plant parts. Analysis of starch in the root systems was used as a measure of changes in storage reserves. See Sect. 5.

Mycorrhizal assessment - These studies assessed the percentage of mycorrhizal infection of root systems at harvest ovulation of fine root; coarse root fraction was done for all seedlings on which carbon metabolism measurements were taken. See Sect. 6.

4. Control and Distribution of Pollutants (See Sect. 1)

Ozone generation and control - Ozone was delivered to open-top chambers in the field using a series of three pressurized manifolds in which air streams ozonized by an ultraviolet ozone generator were mixed with carrier air to add 40, 80, or 160 ppb of ozone to ambient air in appropriate chambers. Control of the rate of delivery of ozone was effected by a

computer-based, shared-time monitoring and control system operating in a feedback control loop (McEvers et al. 1988). Ozone was monitored in all plots by a series of three Dasibi Model 1003PC fluorescent ozone monitors. Monitoring data were stored on computer and subsequently reduced and summarized by automated procedures (McEvers et al. 1988). This system was developed jointly by this project and a related research project funded by the Electric Power Research Institute and the Tennessee Valley Authority.

In the laboratory, ozone generation was accomplished by ultraviolet irradiation as in the field; however, bottled-oxygen was used as the source gas for the laboratory exposure. Control of ozone concentrations was performed manually to achieve desired set points of 160 and 320 ppb. Plants were returned to the greenhouse and charcoal-purified air on the three days between exposures.

Acid deposition control and application - All 36 field chambers were equipped with rainfall addition/exclusion devices to exclude natural rain events and allow a systematic addition of rain stimulant twice each week. Rainfall acidities were controlled by addition to deionized water of stock solutions (5000x) containing background concentrations of ions typical of rainfall in eastern Tennessee. Acidities were adjusted to provide final pH values of approximately 5.2, 4.5, and 3.3 by adding a 2:1 (molar ratio) mixture of $\text{SO}_4:\text{NO}_3$ to deionized water and stock ions in 500-gal mixing vessels. Simulated rainfall was provided to a depth of 1.1 cm with each rain event, giving a total of about 2.2 cm/week. Rainfall addition in the laboratory studies was implemented by the same mixing chemistry; however, all additions were at median pH 4.3 using the rainfall simulation system described by Shriner et al. 1977.

5. Exploratory Research on Whole Canopy Exposure/Response System

In an exploratory project, a canopy-level, open-flow photosynthesis system was developed and tested as a technique for measuring gas exchange of larger trees under ambient conditions in the field. This work consisted of developing a system of small, inexpensive, lightweight cuvettes that could be placed within a tree canopy in the field to measure gas exchange processes on a real-time basis. An IBM data logger coupled with an infrared differential gas analyzer was used in this system.

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2. POLLUTANT DOSE

N. T. Edwards and M. B. Adams

2.1 OZONE FUMIGATION -- FIELD SITE

The 36 field plots utilized in these studies were exposed to 6 different levels of ozone. Three "Open" plots were not covered by chambers and were exposed to ambient ozone levels. The remaining 33 plots were covered by open-top chambers and were exposed to ozone concentrations as follows: ambient (Amb) concentrations that were nonfiltered (NF), amb + 40-ppb ozone (Amb + 40), amb + 80-ppb ozone (Amb + 80), amb + 160-ppb ozone (Amb + 160), and ambient air filtered through charcoal filters (CF). Three chambers received NF, three received Amb + 40, nine received Amb + 80, nine received Amb + 160, and nine received CF. Details of the ozone-generation subsystem are illustrated in Figure 2.1. The NF, the Amb + 40, and the "Open" plots received artificially mixed pH-4.3 rain. The other ozone fumigated plots and the CF plots were watered with pH-3.3 rain, pH-4.5 rain, or pH-5.2 rain (i.e., three different pH levels per ozone concentration). Ozone concentrations at all plots were monitored using a computer-controlled, shared-time sampling and monitoring system that sampled each plot for 70 seconds three times each hour throughout the experiment. Details of the ozone data-acquisition subsystem are illustrated in Figure 2.2.

2.1.1 Instrument, Chamber, and Sample Line Calibrations

A Dasibi Model 1003PC Ozone Analyzer, calibrated by Environmental Protection Agency designated equivalent method EQOA-0577-019 (reference standard traceable to the National Bureau of Standards) was used to calibrate a Protocal CSI 3000 ozone generator, which in turn was used to calibrate the three Dasibi Model 1008-AH Ozone Analyzers used to monitor the ozone concentrations during this experiment. The former calibration was performed just prior to the beginning of the experiment and again on September 9, 1986. The latter calibrations were performed

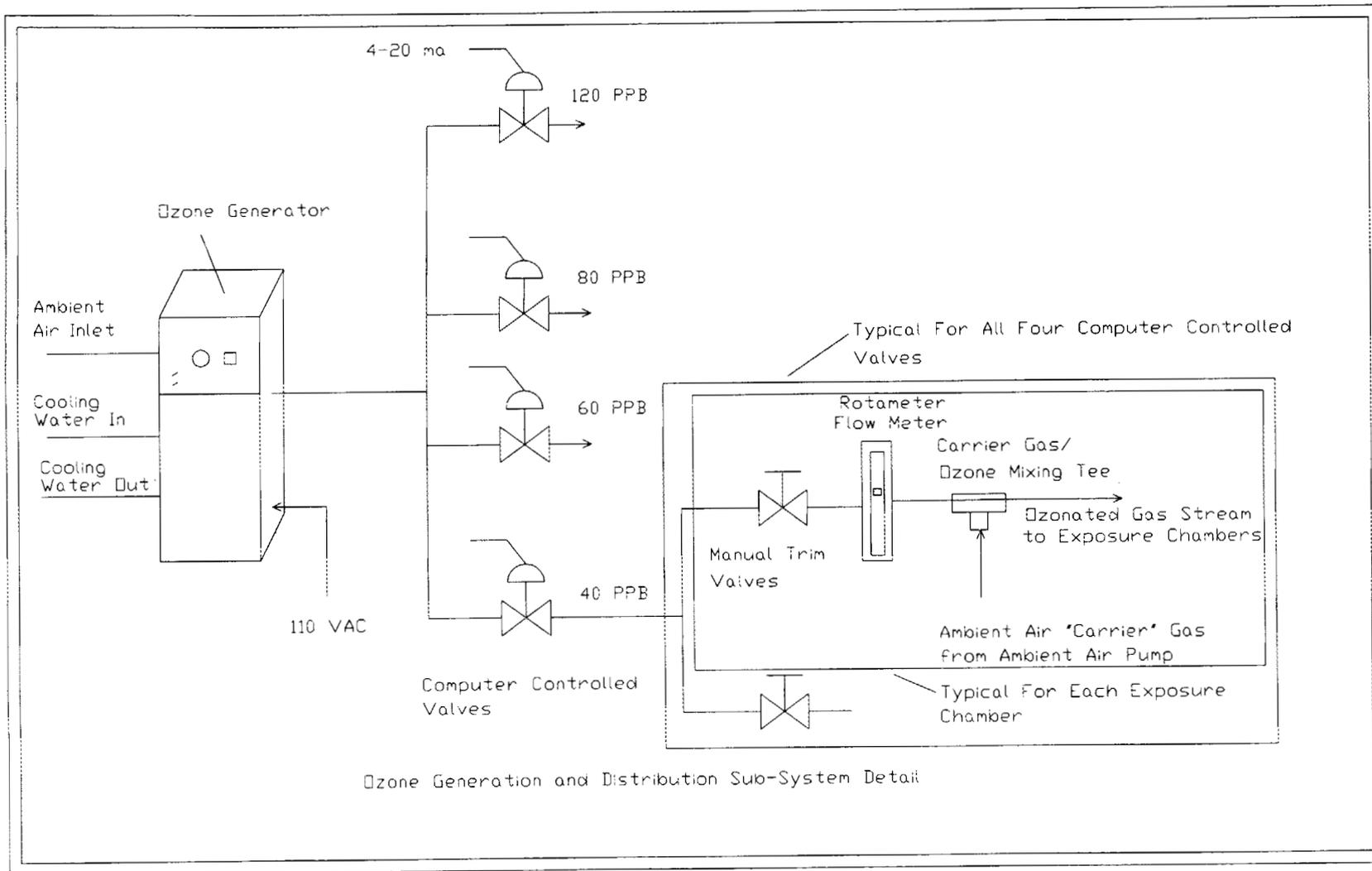


Fig. 2.1. Details of ozone-generation subsystem.

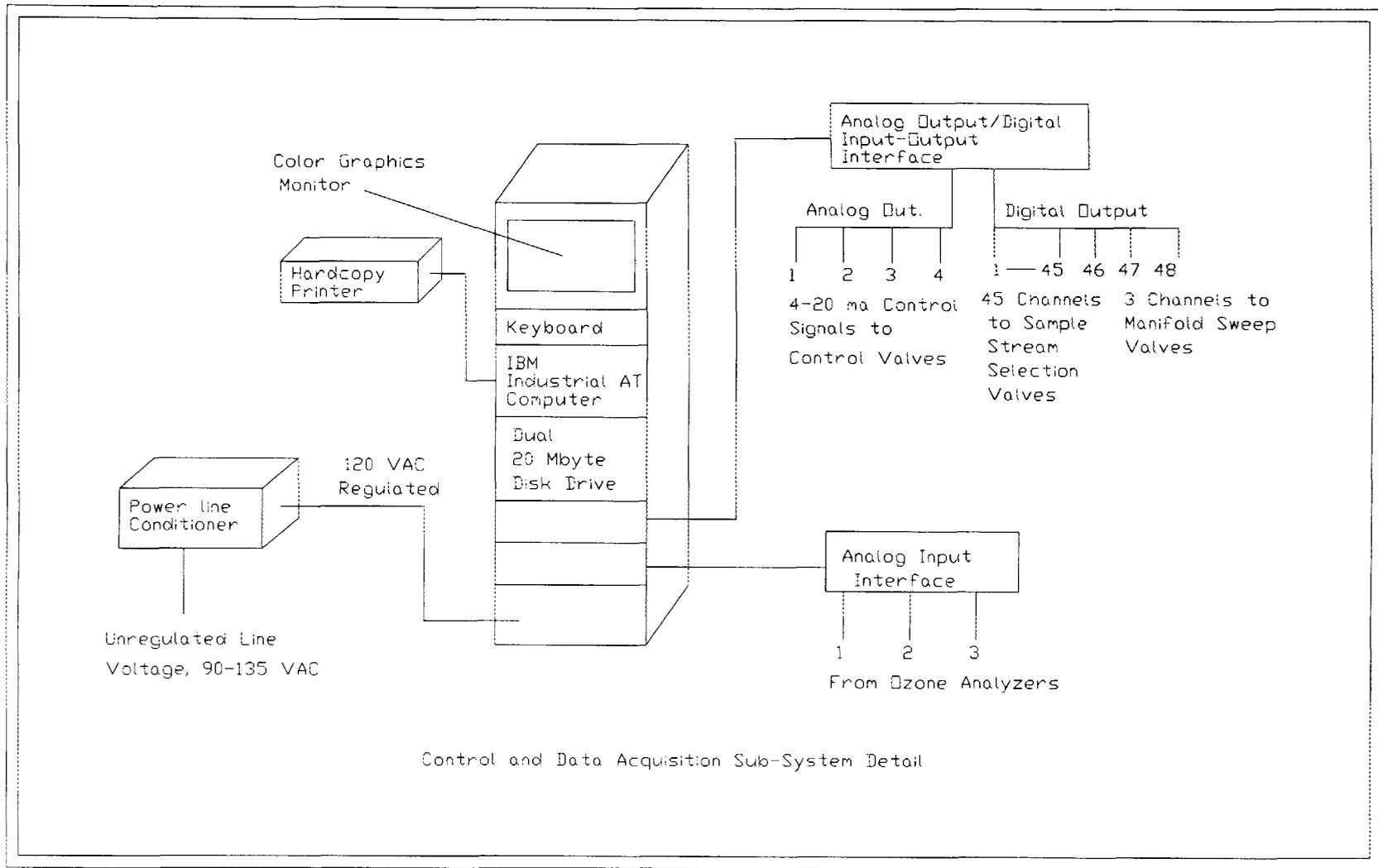


Fig. 2.2. Details of ozone data-acquisition subsystem.

at the beginning of the experiment and at weekly intervals during October 1986 (Table 2.1).

Horizontal ozone concentration variability within chambers was determined by comparing ozone concentrations drawn from 13 locations within a single chamber. The filtered ends of 13 sample lines were systematically positioned in the chamber at seedling canopy height. Ozone in the air pumped through these sample lines was sequentially analyzed at 5-min intervals. The control valves were set to deliver a fixed concentration of ozone. The results are depicted in Fig. 2.3. The coefficient of variation was 7%.

Ozone loss rates on sample lines (Teflon tubing 6-mm diam x 60-m long and fitted with Teflon dust filters) were determined by measuring ozone generated in known concentrations by a Protocol CSI 3000 generator and introduced into the ends of the sample lines located in each chamber. Loss rates averaged 14.4% with a 36% coefficient of variation (Table 2.2).

2.1.2 Times and Durations of Ozone Fumigations

Ozone exposure were begun on August 7, 1986 immediately after all plants were placed in the plots, measured and fertilized, exposures were continued intermittently until the final plant harvest on November 10, 1986. For the first 5 d exposures were run continuously (24 h/d), but at only half-concentration levels as a precautionary measure during initial operation of the new ozone systems. Ozone exposures were then interrupted until August 19, 1986 for adjustments on the control system. During this interval plants received either ambient air or in the case of CF treatments charcoal filtered air. Through the rest of August, exposures were continued at half-concentrations levels 6 h/d, 5 d/week.

Full-concentration ozone exposures were begun September 1 and continued intermittently until November 10. During September and October, plants were exposed to ozone an average of 6 h/d during 15 d each month for a total of 90 h/month. In November plants were exposed to ozone 6 h/d during 4 d. Exposures were done between 9:30 A.M. and 3:30 P.M., Monday through Thursday, except when the ozone system was

OZONE CONCENTRATIONS IN CHAMBER 27

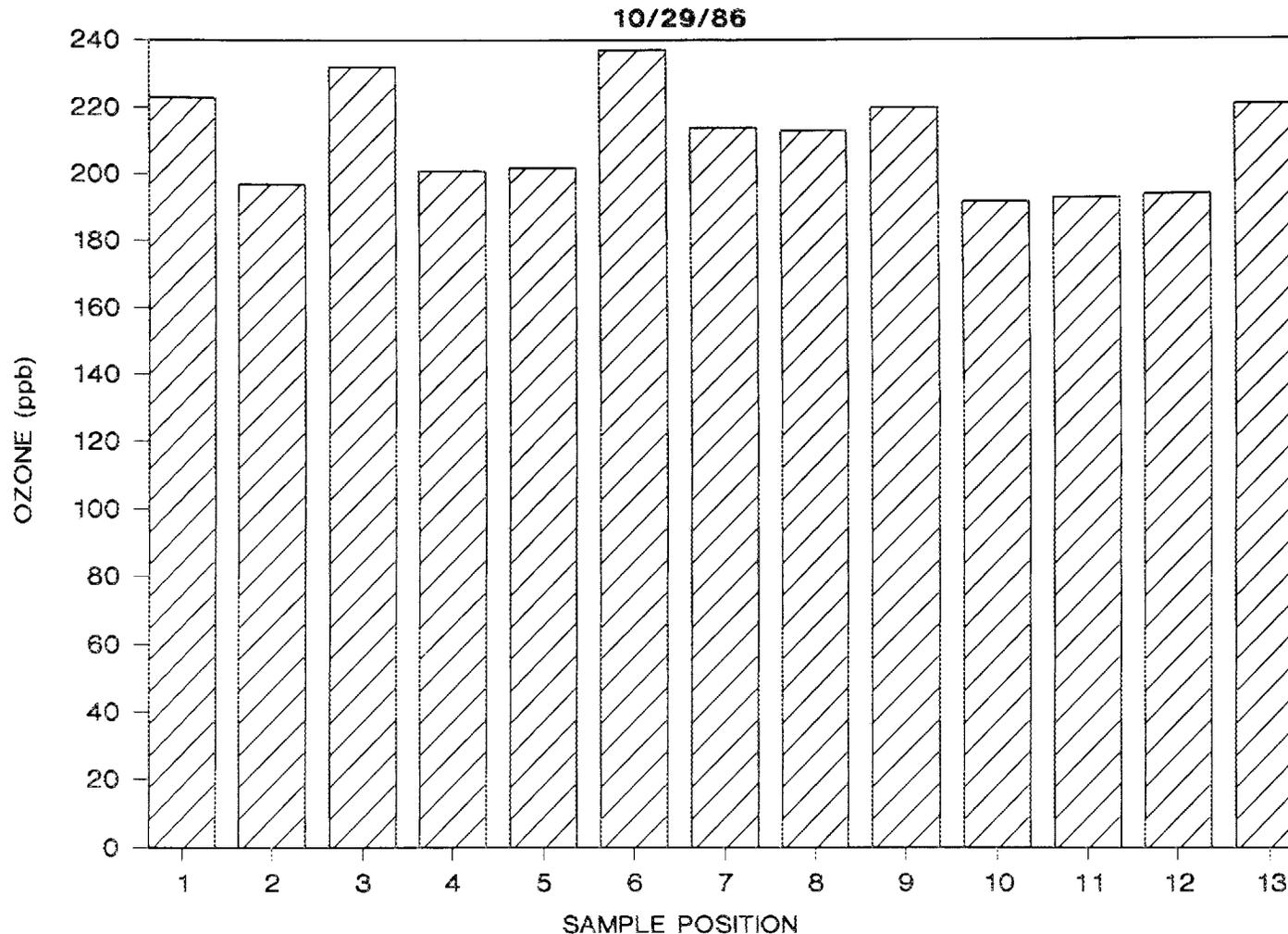


Fig. 2.3. A comparison of ozone concentrations at 13 evenly spaced sample collection points at a height of about 2 ft in chamber 27. Measurements were taken sequentially at 5-min intervals. The dispensing system was set to deliver ozone at a constant rate throughout the test period. Sample positions 1, 3, 4, 6, 7, 9, 10, and 12 were nearest the walls of the chamber, while the other four positions were nearer the center. Position 1 was directly in front of the blower.

Table 2.1. Calibrations of ozone analyzers, 1986

Date	Photocal settings (ppb)	Dasibi analyzer readings (ppb)	Analyzer ID #
10/9	1	1	58005
	0	0	58006
	0	-1	58007
	200	194	58005
	201	199	58006
	196	190	58007
10/17	0	-4	58005
	0	1	58006
	0	-2	58007
	199	190	58005
	203	196	58006
	205	196	58007
10/24	0	-1	58005
	0	-2	58006
	0	-1	58007
	199	195	58005
	201	201	58006
	200	198	58007

Table 2.2. Ozone sample line calibrations, October 13, 1986^a

Chamber #	Photocal output (ppb)	Analyzer reading (ppb)	Line delivery efficiency %	Loss on line %
10	100	88	88.00	12.00
11	99	83	83.84	16.16
12	99	82	82.83	17.17
14	100	84	84.00	16.00
15	99	80	80.81	19.19
17	101	95	94.06	5.94
21	102	95	93.14	6.86
22	99	85	85.86	14.14
24	101	83	82.18	17.82
27	101	97	96.04	3.96
30	101	91	90.10	9.90
31	100	82	82.00	18.00
32	100	81	81.00	19.00
33	101	84	83.17	16.83
36	101	83	82.18	17.82
37	101	87	86.14	13.86
39	99	73	73.74	26.26
40	100	85	85.00	15.00
41	102	93	91.18	8.82
42	99	81	81.82	18.18
43	100	90	90.00	10.00
Mean	100.24	85.81	85.57	14.43
St dev	0.97	5.78	5.21	5.21

^aOzone from the Protocal CSI 3000 was introduced into the ends of the sample lines in the individual chambers at the concentrations indicated in the table.

turned off for calibration or repair. Reduced ozone treatments were continuous throughout the experiment (i.e., charcoal filters were left in place all the time).

2.1.3 Monitored Ozone Concentrations and Calculated Dosages

Monitored average, minimum, and maximum ozone concentrations in the various treatment combinations during controlled exposures at half concentration and full concentration are shown in Figures 2.4 and 2.5, respectively. Figure 2.6 shows average, minimum, and maximum ozone concentrations by ozone treatment level during the entire experiment (i.e., 24 h/d for the entire 96 d of the experiment). Numerical data for these figures are provided in Table 2.3.

Ozone dosage is summarized by treatment in Table 2.4. The daytime ozone dose was calculated assuming 12 h (0800-2000) of daylight per day. The respite dose was calculated as that occurring during all remaining hours. The CF chambers received the smallest daytime dose, 19353 (average ppb x daylight h exposed). The Open, NF, Amb + 40, Amb + 80, and Amb + 160 chambers dosages exceeded the CF dosage by factors of 1.7x, 1.9x, 2.1x, 3x, and 4.1x, respectively. Note that the dosages are given as uncorrected and corrected. The corrected dosage assumes an average 14.4% loss of ozone in the sample lines between the chambers and the analyzers. The importance of respite dose to the total dose received in these experiments is readily apparent in Table 2.4. Respite dose amounted to approximately 67% of the total received in NF chambers, and ranged from 50% (A40) to about 30% of the total supplied in chambers in which ozone was added to ambient levels.

2.2 OZONE FUMIGATION -- LABORATORY STUDIES

Ozone was generated in the laboratory studies using an Ozone Research and Equipment Corporation Model SP 38-O ozone generator incorporating ultraviolet irradiation of oxygen supplied from a compressed gas supply tank. Ozone generated from this system was distributed to inlet ducts of each Continuously Stirred Tank reactor (CSTR) chamber using manually adjusted flow valves. Ozone monitoring

OZONE CONCENTRATIONS BY TREATMENT

DURING HALF CONC. EXPOSURE

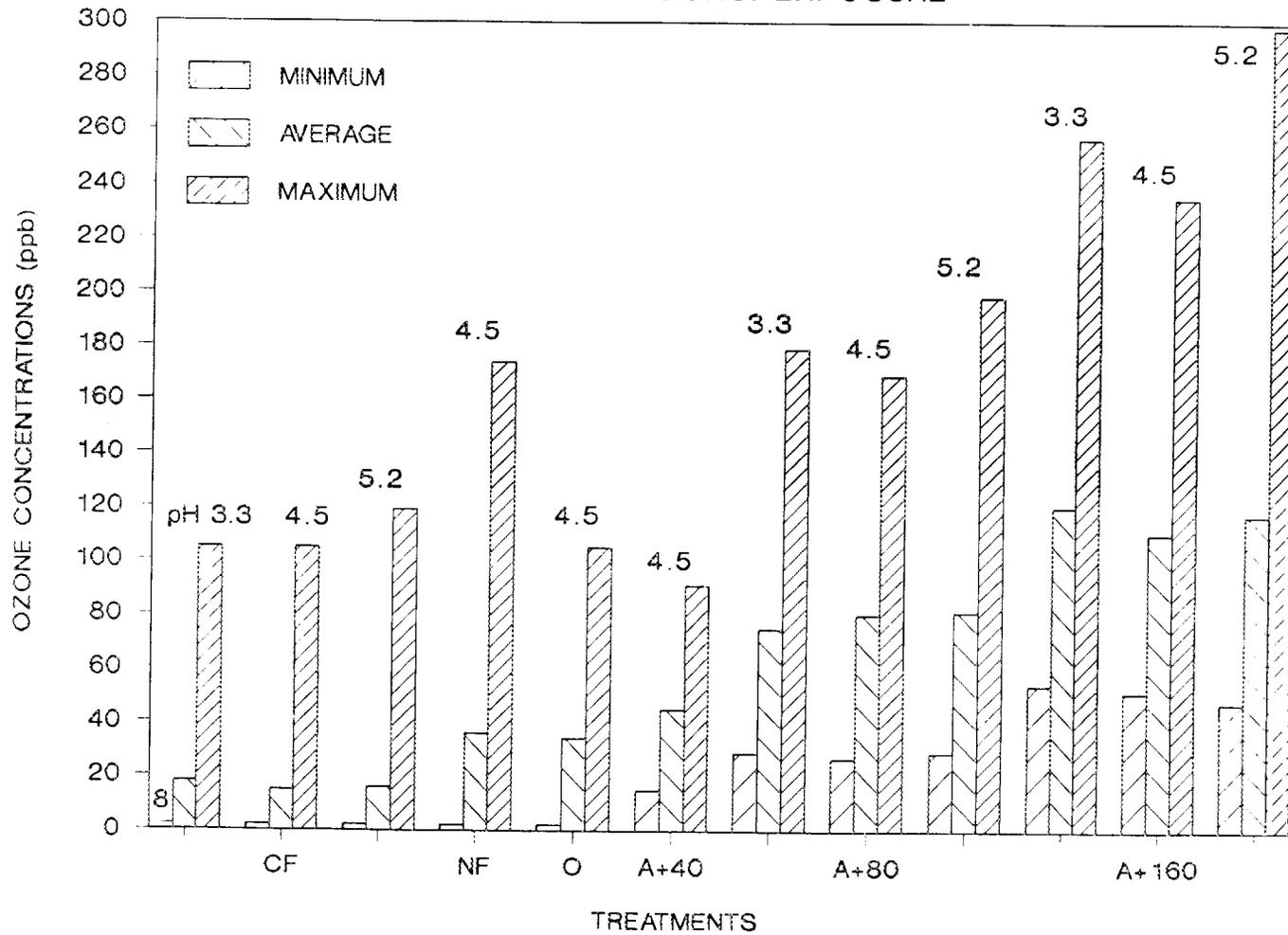


Fig. 2.4. Average (12h, 0800-2000h), minimum, and maximum ozone concentrations during half concentration exposures at different rain pH levels. Rain pH is shown above the bars. CF = charcoal filtered; + 40 NF = nonfiltered (ambient); O = open plots; A + 40 = ambient ppb ozone; A + 80 = ambient + 80 ppb ozone; and A + 160 = ambient + 160 ppb ozone.

OZONE CONCENTRATIONS BY TREATMENT DURING FULL CONC. EXPOSURE

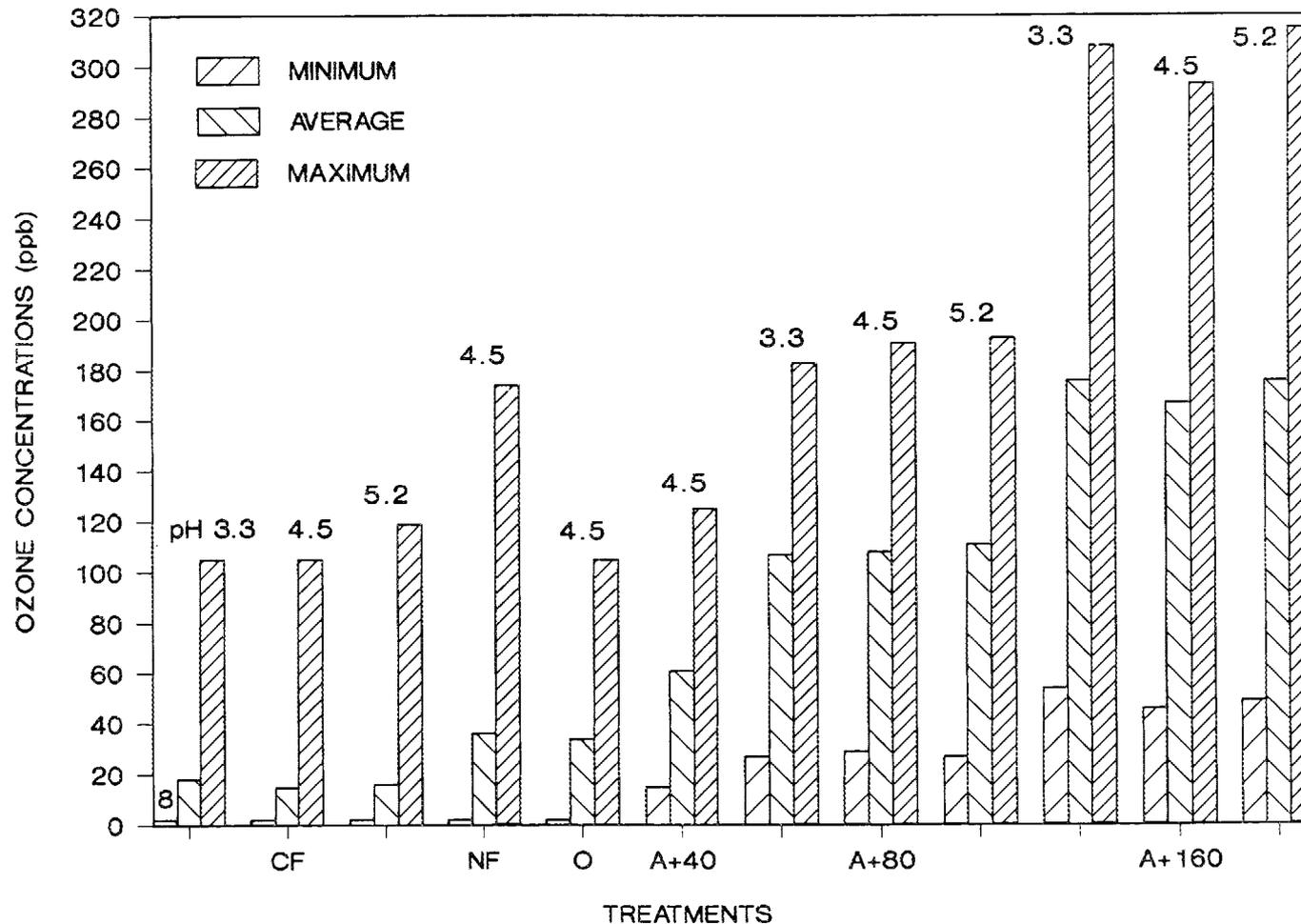


Fig. 2.5. Average (12h, 0800-2000h), minimum, and maximum ozone concentrations during full concentration exposures at different rain pH levels. Rain pH is shown above the bars. CF = charcoal filtered; NF = nonfiltered (ambient); O = open plots; A + 40 = ambient + 40 ppb ozone; A + 80 = ambient + 80 ppb ozone; and A + 160 = ambient + 160 ppb ozone.

OZONE CONCENTRATIONS BY TREATMENT

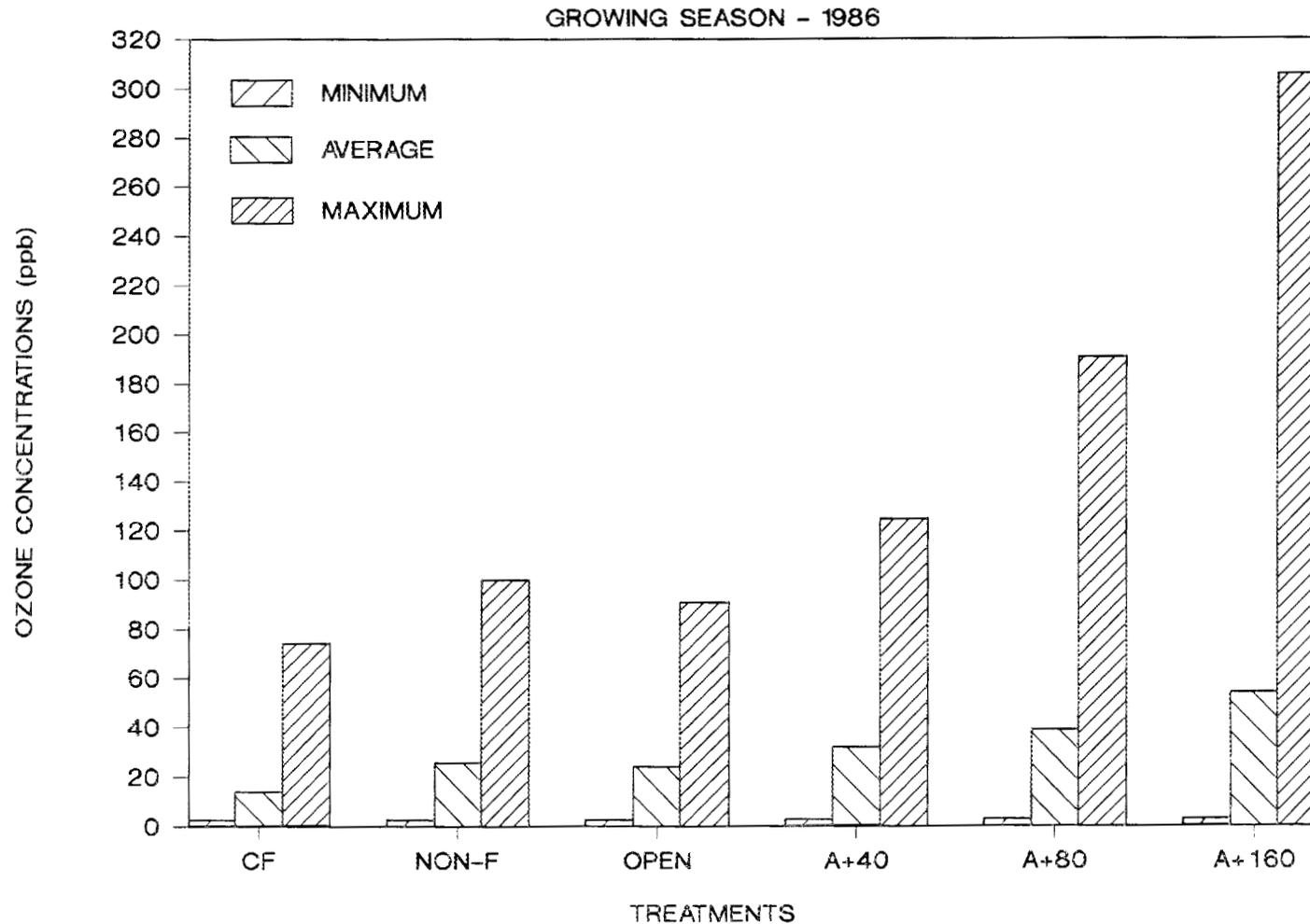


Fig. 2.6. Average (12h, 0800-2000h), minimum, and maximum ozone concentrations in treatment plots from August 7 to November 10, 1986. Values for times when chambers were not fumigated are included. CF = charcoal filtered; NF = nonfiltered (ambient); Open = open plots; A + 40 = ambient + 40 ppb ozone; A + 80 = ambient + 80 ppb ozone; and A + 160 = ambient + 160 ppb ozone.

Table 2.3. Ozone averages by treatment, Summer 1986

Treatment	<u>During growing season</u>											
	1	2	3	4	5	6	7	8	9	10	11	12
	CF,3.5	CF,4.3	CF,5.0	NON-F,4.3	OPEN,4.3	A+40,4.3	A+80,3.5	A+80,4.3	A+80,5.0	A+160,3.5	A+160,4.3	A+160,5.0
Mean	13.98	14.1	14.13	25.94	23.91	32.03	37.47	39.83	39.19	53.76	52.79	54.26
Std dev	8.47	9.31	9.35	17.01	16.82	23.43	35.31	36.88	37.37	60.94	57.92	62.66
Max	68.5	78.2	75.8	100.2	90.46	124.69	183.4	190.7	198	308.1	293.4	315.4
Min	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
	<u>During exposure period only (full concentration Sept. 1 - Nov. 10, about 6h/day for 34 days)</u>											
Mean	18	15	16	36	34	61	107	108	111	176	167	176
Std dev	8	10	10	18	17	21	32	32	32	45	41	52
Max	105	105	119	174	105	125	183	191	193	308	293	315
Min	2	2	2	2	2	15	27	29	27	54	46	49
	<u>During half concentration exposure (August -- 5 days continuous, 9 days -- 6h/d)</u>											
Mean	18	15	16	36	34	45	75	80	81	120	110	117
Std dev	8	10	10	18	17	15	21	25	24	28	26	31
Max	105	105	119	174	105	91	179	169	198	257	235	298
Min	2	2	2	2	2	15	29	27	29	54	51	47

Table 2.4. Mean Ozone dosage characteristics per treatment for day time exposures (12h, 0800-2000h)

	CF	Open	Ambient	A + 40	A + 80	A + 160
Daytime Experiment duration (h)	1,152	1,152	1,152	1,152	1,152	1,152
Daytime exposure at 1/2 conc.(h)	114	114	114	114	114	114
Daytime respite at 1/2 conc.(h)	186	186	186	186	186	186
Daytime exposure at full conc.(h)	204	204	204	204	204	204
Daytime respite at full conc.(h)	648	648	648	648	648	648
Mean ozone during half conc.(ppb)	18	34	36	45	79	116
Mean daytime ozone during respite half conc.(ppb)	13	22	24	21	23	24
Mean ozone during full conc.(ppb)	18	34	36	61	109	173
Mean daytime ozone during respite full conc.(ppb)	13	22	24	21	23	24
Dose (ppb x h) during exposures	5,724	10,812	11,448	17,574	31,242	47,516
Daytime respite dose (ppb x h)	10,842	18,348	20,016	17,514	19,182	20,016
Total daytime dose (ppb x h)	16,566	29,160	31,464	35,088	50,424	67,532
Corrected total daytime dose (ppb x h/0.856)	19,353	34,065	36,757	40,991	58,907	78,893
Experiment duration day + night (h)	2,304	2,304	2,304	2,304	2,304	2,304
Mean ozone conc. (ppb)	14	24	26	32	39	54
Total dose (ppb x h)	32,256	55,296	59,904	73,728	89,856	124,416
Corrected total dose (ppb x h/0.856)	37,682	64,598	69,981	86,131	104,972	145,346

was accomplished with a sequential sampler that automatically cycled between chambers providing one measurement of three-min duration every 30 min for each chamber during the 6-h exposure period. A Monitor Labs Model 4510 chemiluminescent ozone analyzer was used to measure ozone concentrations at the exhaust port of each chamber. Data were recorded on a chart recorder and reduced by hand. Chamber temperature and relative humidity were measured at hourly intervals and humidity was increased by steam addition as needed to maintain levels above 60%.

The ozone monitor was calibrated once before, six times during, and one time after the 12-week study interval. The ozone monitoring instrument was found to be quite stable as shown in Table 2.5.

A summary of the monitored ozone, temperature, and humidity levels in the CSTR chambers is included in Table 2.6. The measured concentrations represent a running mean of one 3-min sample per hour for those days including the approximately 25-min period of increase to steady state conditions. Resultant mean concentrations reproduced the desired setpoint concentrations within $\pm 5\%$. Typical peak concentrations exceeded these mean values by $\leq 10\%$ as a technician was always nearby to manually adjust control valves. One approximately 2-h exception to this range was produced when, due to an instrument malfunction associated with loss of ethylene pressure in the supplyline, the ozone monitor produced erroneously low readings and it is estimated that chamber concentrations may have exceeded set points by a factor of 2 on that occasion. This occurred during the second week of exposure and produced some visual injury but no apparent effects on growth or physiology. A maximum of 14% of foliage area was injured in family 6 and a minimum of 4% occurred for family 3 at the highest ozone level. Maximum injury for the ambient + 160-ppb treatment was estimated at 2.5% of foliage area affected.

2.3 SIMULATED RAIN CHEMISTRY

2.3.1 Field Site

All 36 chambers were equipped with rainfall exclusion/addition systems as shown in Fig. 2.7. Ambient rainfall was detected with a

Table 2.5. Dates and results of calibration tests with the Monitor Labs Model 4510 ozone analyzer used in laboratory studies

Date	Setpoint concentration (PPb)	Monitor reading (PPb)	Comments
7/23/86	886	860-900	Range for all six chambers ^a
9/4/86	300	305	Readjust span and gain
9/18/86	300	300	No change
10/2/86	300	300	No change
10/10/86 ^b	0	0	Zero stable
	193	193(205)	Span was
	240	240(255)	Changed
	307	307(322)	2.43 to 2.28
10/16/86	307	307	No change
10/23/86	307	307	No change
11/20/86	320	300(293)	Zero unchanged,
	151	145(138)	Span increased
	0	0	2.28 to 2.36

^aInitial calibration was done through the sample lines between chambers and the monitor. Subsequent calibration was directly into the monitor based on the absence of a line effect.

^bReadings are as found except for those on 10/10 and 11/20. The "as found" values for those dates are included in parenthesis as estimates based on gain sensitivity of the instrument amplifier and recorded gain adjustments required to obtain reported readings.

Table 2.6. Ozone, temperature, and humidity data from CSTR exposure chambers averaged for 10 d distributed over the 12-week study interval

<u>Ozone Setpoint</u>				
Date	160 ppb	320 ppb	Temperature(°C)	Humidity(%)
<u>Half concentration exposures</u>				
8/22/86	81.3	158	34.9	59.9
8/28/86	80.3	161	32.3	63.3
Mean	80.6	160	33.6	61.6
<u>Full concentration exposures</u>				
9/4/86	156	319	35.1	54.0
9/15/86	153	304	33.0	60.0
9/23/86	149	301	35.0	62.1
10/1/86	123	298	34.1	68.3
10/9/86	152	306	35.0	61.0
10/15/86	156	309	33.7	53.7
10/27/86	162	299	33.8	50.8
11/6/86	163	317	33.8	55.7
Mean	152	307	34.1	58.9
<u>Estimated Total Dose(ppm•h)</u>				
	40	81		

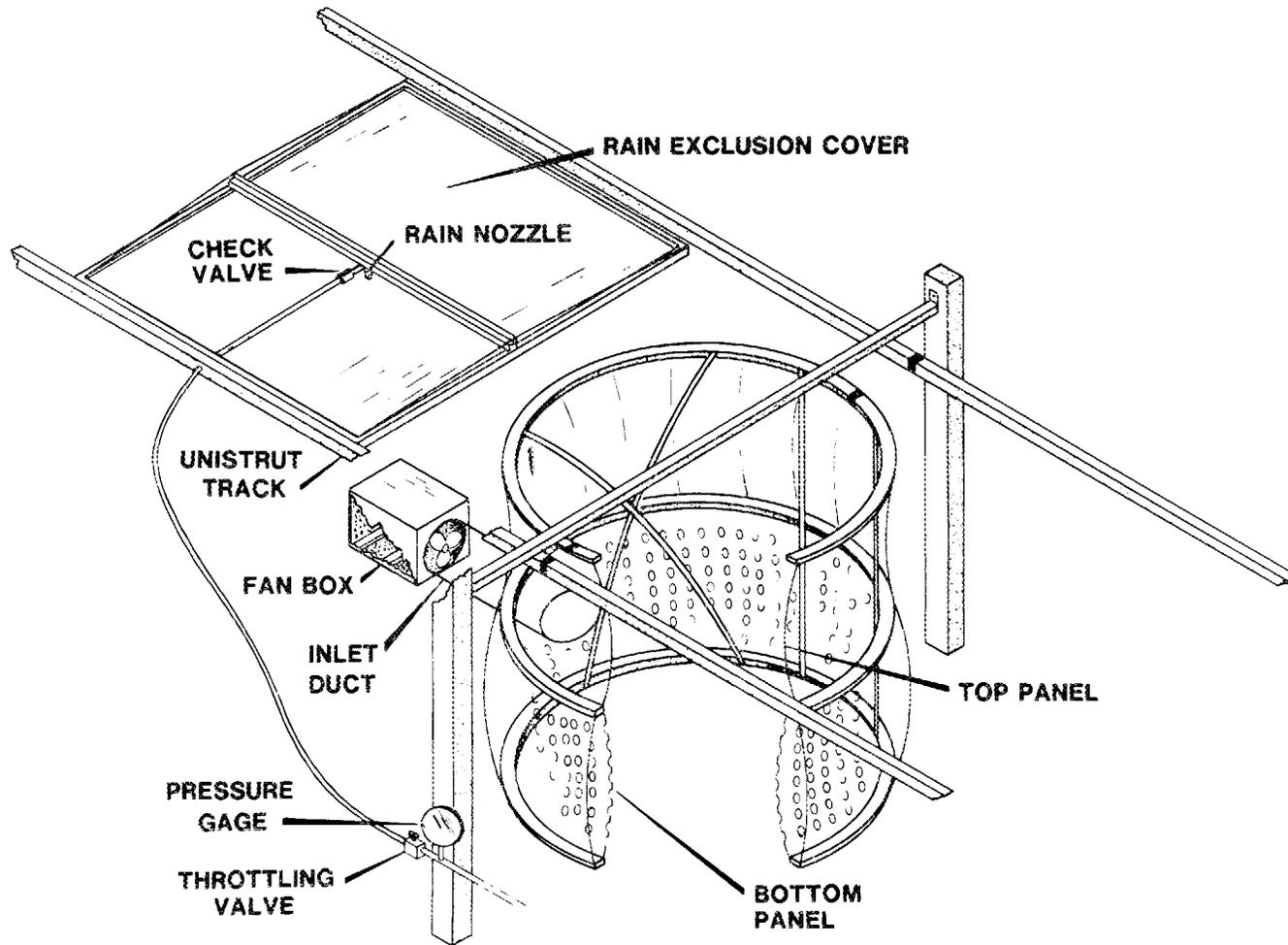


Fig. 2.7. Details of an open-top chamber, rain exclusion cover, and simulant-dispensing apparatus (from Johnston et al. 1986).

rain sensor (Wong Laboratories, Cincinnati, OH 45209). When the rainsensor became wet, a signal was sent to a controller unit which caused door openers to pull the covers into place over the chambers. When the rain event ended, as the rain sensor dried, the door openers were reactivated in the reverse direction, off of the chambers.

The rain simulant dispensing system consisted of three components: (1) the water supply, (2) the simulant mixing tank, and (3) the rain simulant delivery system (Figure 2.8). Process system water (non-chlorinated) was pumped into a 5000-gallon tanker, which was driven to the field site. Water was then pumped from the tanker through a pair of deionizing columns (Culligan Water Systems, Knoxville, Tennessee) into 2000-L polyethylene storage tanks. The deionizing columns were replaced when the resistance of the water was less than 1 megohm, as measured with an in-line resistance meter located downstream from the columns. The storage tanks were located in a covered, 1.5-m deep trench to minimize temperature fluctuations, and were painted silver to limit algal growth in the simulant solutions.

After the storage tanks were filled with deionized water, the rain simulant solutions were mixed to approximate rainfall chemistry of pH 5.0, 4.3, and 3.5, as described by Irving (1985). (See Table E-2, Appendix E for ionic concentrations.) Stock solutions were mixed to 5000x for each rain simulant. The stock solutions were injected, using a peristaltic pump, into a water stream pumped from the rain simulant storage tank past the mixing station, and back to the storage tank. Additions continued until the pH of the solution approached the desired level. Mixing of the simulant solution was achieved by pumping water from the bottom of the tanks and subsequent return to the top.

"Rain" was applied twice a week from August 14, through October 31, 1986, for 30 min each time (approximately 1.25 cm per application). Solutions from the storage tanks were pumped to the field plots where they were distributed by stainless steel wide-angle, full-cone spray nozzles mounted beneath the exclusion covers so as to be approximately over the center of each plot.

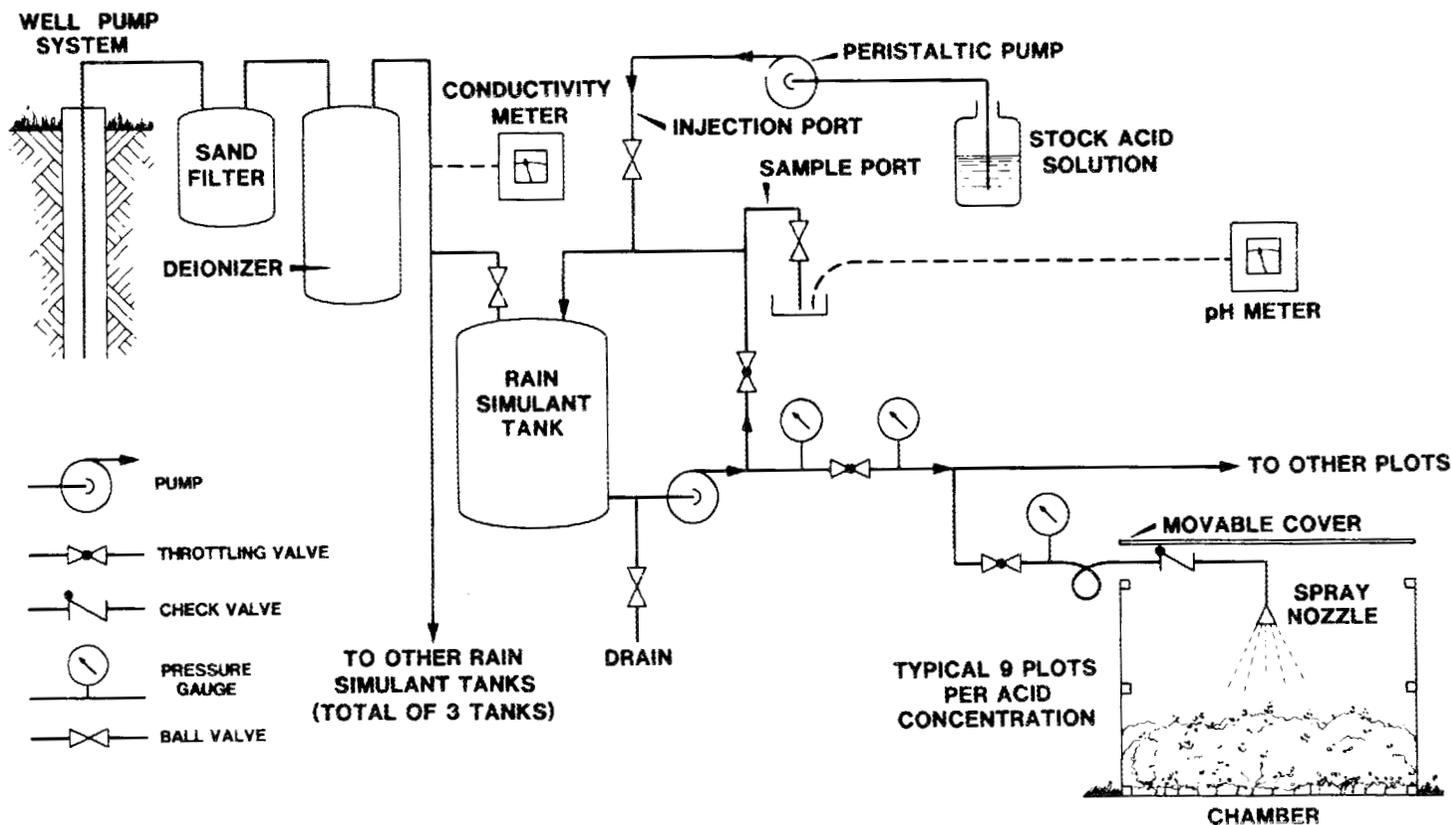


Fig. 2.8. Schematic of the water purification, pH adjustment, and simulated rain delivery system (from Johnston et al. 1986). For this experiment the system was modified such that water entering the deionizer was pumped from a tanker, which hauled dechlorinated water from the ORNL ESD aquatics laboratory.

During the mixing of stock solutions with deionized water, small samples (approximately 50 mL) were taken from the mixing stream and the pH of the sample measured. Addition of stock solution was either continued or ceased as needed. The pH of the final solution was checked several times at 2 to 5-min intervals to be sure a stable pH had been achieved prior to application. The pH meter used for this was calibrated against certified buffer solutions of pH 7.0 and 4.0.

During a "rain" event, the delivery system on each of the 36 plots was checked for proper line pressure and to be sure the nozzle was operating properly. Four 50-mL glass beakers were placed in four chambers (randomly selected) of each pH level prior to the rain event. The four samples per chamber were composited and the pH of the bulk sample measured. Samples of rain received in the chambers were tested once during each "batch" of rain simulant (once per week).

Overall statistics for the 13-week exposure period are given in Tables 2.7. Three different "rain" treatments were achieved, though the mean pH did vary somewhat. The mean pH of the 4.3 treatment was slightly higher than the desired value, while the pH values of the 5.0 and 3.5 treatments were slightly lower than desired. Figure 2.9 shows the distribution of rain pH for each of the three treatments. Because the mean values are strongly influenced by extreme values from only a very few rain events, the median pH values were deemed more representative of rain "exposures". Median pH values produced were 3.3, 4.5, and 5.2. These values are used throughout the remainder of this report to describe pH exposures for the field experiments. (See Appendix E, "Project Quality Assurance" for further characterization of rain data).

2.3.2 CSTR Study

Rain simulant of pH 4.3 was applied twice weekly (1.25 cm per approximately 30-min application) using a rain simulator (Figure 2.10; For a more complete description, see Shriner et al. 1977.). The simulant was made from a stock solution (the same as described above for pH 4.3) that was mixed to a 50x concentrate, then diluted in the

Table 2.7. Mean rain simulant H^+ concentration and pH
by treatment -- field study

Treatment Target (pH)	Mean of [H^+]	Standard error	Mean of pH rain events	Standard error	Median pH	Range
5.0	4.63	0.097	5.11	0.118	5.2	3.8 - 6.3
4.3	4.38	0.063	4.56	0.093	4.5	3.6 - 6.2
3.5	3.26	0.037	3.33	0.073	3.3	2.9 - 4.0

Rain Simulant pH Distribution 1986

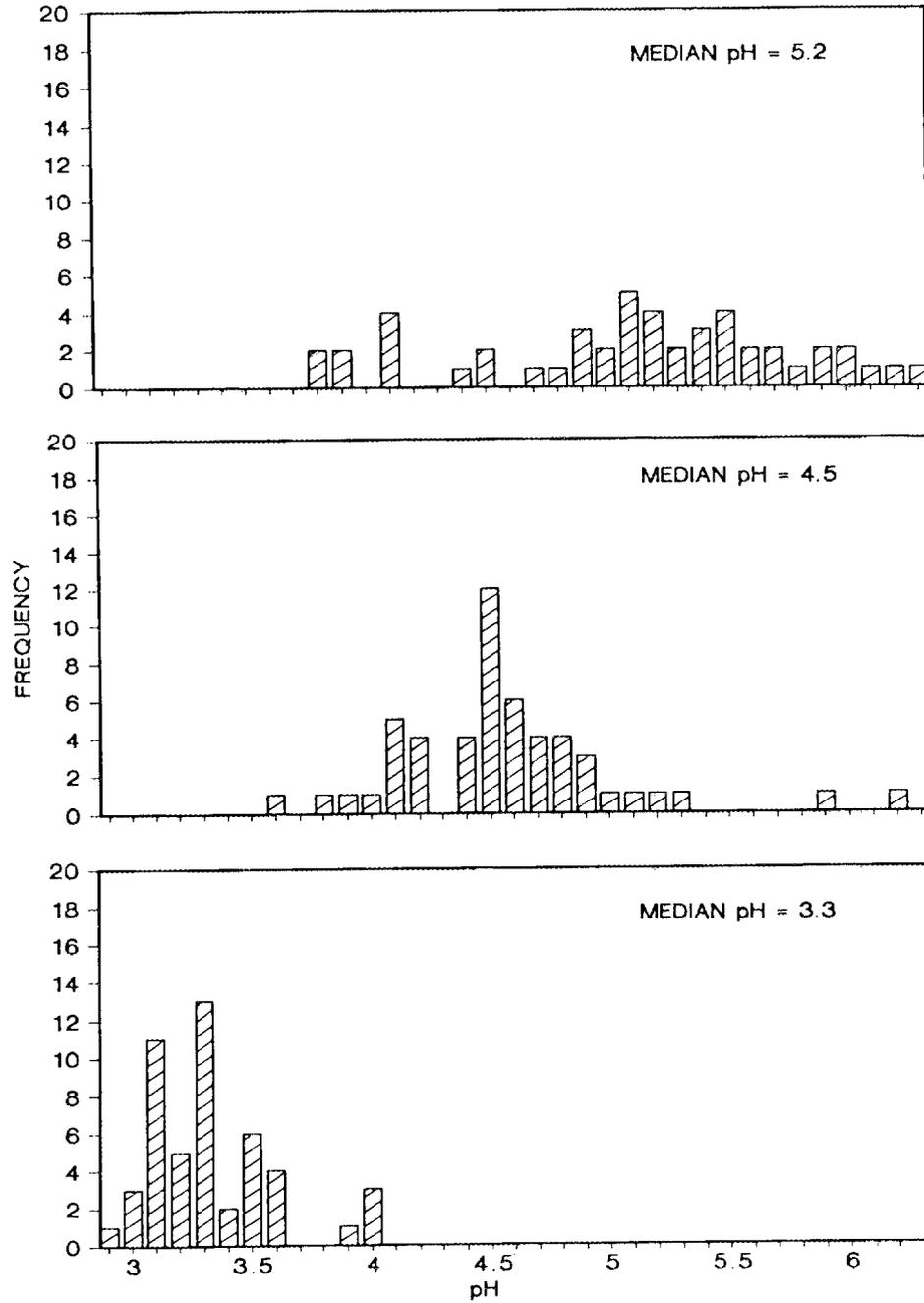
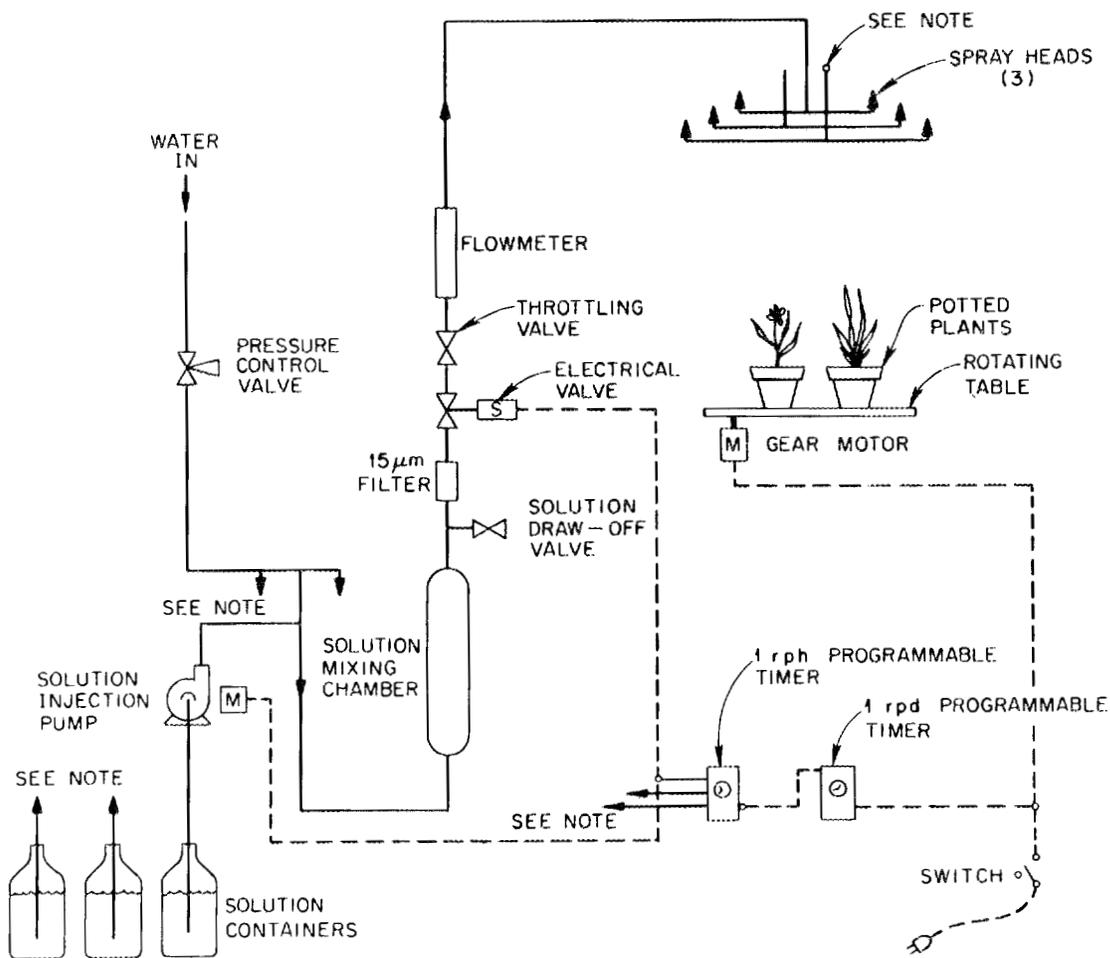


Fig. 2.9. Frequency distribution of Ph of rain simulant by different rain pH treatments.



NOTE: THE SYSTEM CONSISTS OF THREE IDENTICAL SOLUTION DISTRIBUTORS. FOR CLARITY, ONE IS SHOWN

Fig. 2.10. Schematic of the simulated rain delivery system used in the CSTR study.

rain simulator through mixing with distilled water. Mixing pumps were calibrated prior to rainfall application by drawing small aliquots from the stream and checking the pH with a calibrated pH meter. Two beakers were placed on each of the two rain tables prior to one "rain" event per week, and the contents of the two beakers were combined after the rain event for measurement of pH by table. The overall mean rainfall pH for the two tables were 4.18 and 4.37, with a median pH of 4.3. Plants were randomly located on the two tables over time so that any variations caused by differences between the two tables and their respective systems would be minimized.

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3. ANALYSIS OF GROWTH RESPONSES TO OZONE AND ACID RAIN

P. A. Layton, S. B. McLaughlin, N. T. Edwards,
E. G. O'Neill, and W. K. Roy

A major objective of the first year of research in the Southern Commercial Forest Research Cooperative (SCFRC) was to test for the responses of loblolly pine species to acid rain and ozone. To address this objective, heavy emphasis was placed on characterizing responses across a wide range of commercially important families of loblolly pine available from the seed orchards of forest industries throughout the region. A list of 209 ortets from which more than 1000 open-pollinated seed were available was initially identified by the SCFRC. A subset of 10 families was chosen for concentrated effort and as such were common to more than one research site. Three of these families from the coastal plain of North Carolina, were chosen for their acceptability at the Duke Forest site where intensive field research was initiated. The other seven common families were distributed across loblolly's commercial range. Other randomly chosen families were distributed to the three laboratories [Oak Ridge National Laboratory (ORNL), U.S. Department of Agriculture (USDA)/North Carolina State University, and Texas A&M University] engaged in these initial studies in an attempt to screen at least 100 families. The research plan, reached through discussions between collaborating laboratories and management of the SCFRC, was designed to provide a basis for comparison of the 10 common families across all test sites and methodologies. At the same time, the additional families from a regionally representative cross section were distributed to each laboratory in accordance with their capacity to incorporate them into their projects and facilities.

ORNL performed two different but related studies: a laboratory study and a field study. The laboratory study included the families common to all three projects. It involved exposing seedlings in a continuously stirred tank reactor (CSTR) to charcoal-filtered (CF) air and air containing ozone at 2 levels, 160 ppb and 320 ppb. The field

study actually included two experimental components. The first was a comparison of loblolly families to five different regimes of ozone: CF, ambient air, ambient +40 ppb (A40), ambient +80 ppb (A80), and ambient +160 ppb (A160). The second was a factorial experiment exposing loblolly pine families to three levels of ozone (CF, A80 and A160) and rain at 3 pH levels (median pH values of 3.3, 4.5, and 5.2).

Because the ORNL site had a large field facility, a total of 55 families was supplied to this site in March, 1986. The list shown in Table 3.1 includes the 10 common families and 45 families unique to the ORNL site. While seed of all families were germinated, germination success was low for two families (1 and 22), and they were not included in either of the studies. Survival of seedlings of two additional families (54 and 55), was poor, reducing some of our overall analyses to a total of 51 families. Because of the dimensions of our laboratory exposure chambers and poor germination, we used only 8 of the 10 common families for these studies. In the field, we were able to include 9 of the 10 common families, and growth analyses were performed on these 9, plus either 42 or 44 unique families.

The growth data collected in this study were of two types:

- (1) seedling diameter and height were obtained for all seedlings at least twice, at the initiation and at the end of the experiments, and
- (2) biomass data were collected for all common families grown in the field and in the laboratory. Diameter and height data were used to estimate seedling volume because of the large number of seedlings in this study and the desirability of obtaining repeated measurements on the same seedlings. Common families in the field were measured after 0, 6, and 12 weeks, whereas seedlings grown in the laboratory were measured at 0, 3, 6, 9, and 12 weeks.

In analyzing these data we attempted to examine several dimensions of observed growth patterns: (1) comparisons among families to describe the range in magnitude of their observed responses; (2) comparisons among different indices of growth to evaluate their utility in describing whole-plant growth patterns and their use as indicators of the dimensions of growth response; and (3) comparisons

Table 3.1. Identifying numbers and seed sources origins
for half-sib families examined in these studies

ORNL Code ^a	Family Source	Code	State	County
1	NCSU ^b (1974)	1-68	Georgia	Gwinnett
2	NCSU (1987)	24-4	Alabama	Wilcox
3	NCSU (1987)	25-74	Florida	Marion
4	NCSU	7-34	S. Carolina	Georgetown
5	WGFTIP ^c	1061003	Texas	Liberty
6	WGFTIP	1341002	Arkansas	Lafayette
7	WGFTIP	1131012	Louisiana	Livingston
8	Weyerhaeuser	8-80	N. Carolina	Gates
9	Weyerhaeuser	8-130	N. Carolina	Beaufort
10	Weyerhaeuser	8-103	N. Carolina	Onslow
11	Georgia Kraft	15-91	Georgia	Chattahooche
12	IPCO ^d	7-58	S. Carolina	Marion
13	NCSU (1974)	1-532	S. Carolina	Chesterfield
14	NCSU (1974)	1-527	S. Carolina	Union
15	NCSU (1974)	12-5	Alabama	Cleburne
16	NCSU (1974)	20-521	Virginia	Lunenburg
17	NCSU (1981 Rust)	20-504	Virginia	Northumberland
18	NCSU (1981)	15-47	Georgia	Gilmer
19	NCSU (1981)	23-28	Mississippi	Greene
20	NCSU (1981)	15-1	Georgia	Monroe
21	NCSU (1981)	1-529	S. Carolina	Newberry
22	NCSU (1981)	19-2	Mississippi	Tishomingo
23	NCSU (1981)	22-14	Florida	Dixie
24	NCSU (1981)	5-63	S. Carolina	Allendale
25	NCSU (1981)	19-17	Mississippi	Monroe
26	NCSU (1981)	5-64	S. Carolina	Berkeley
27	Union Camp	5-64	Georgia	Greene
28	Union Camp	12-13	Alabama	Tallapoosa
29	Union Camp	10-37	Georgia	Camden
30	Union Camp	5-19	Georgia	Lincoln
31	Union Camp	10-506	Alabama	Covington
32	University of Florida	1-81	Florida	Nassau
33	WGFTIP	4031001	Texas	Nacogdoches
34	WGFTIP	1121040	Louisiana	Grant
35	WGFTIP	1021024	Texas	Cherokee
36	WGFTIP	1711077	Arkansas	Cleburne
37	WGFTIP	1341026	Arkansas	Cleveland
38	WGFTIP	1121015	Louisiana	Desota
39	WGFTIP	1051009	Texas	Newton
40	WGFTIP	1131032	Louisiana	Allen
41	WGFTIP	1341094	Arkansas	Ashley
42	WGFTIP	1711002	Arkansas	Howard

Table 1. (continued)

ORNL Code ^a	Family Source	Code	State	County
43	WGFTIP	1341012	Arkansas	Clark
44	WGFTIP	1061019	Texas	San Jacinto
45	Weyerhaeuser	8-146	N. Carolina	Pitt
46	West Virginia Co.	3-512	Alabama	Walker
47	West Virginia Co.	11-143	Virginia	Pittsylvania
48	West Virginia Co.	11-709	Tennessee	Henderson
49	West Virginia Co.	1-60	Alabama	Marshall
50	West Virginia Co.	6-33	N. Carolina	Durham
51	West Virginia Co.	20-524	Virginia	Dinwiddie
52	West Virginia Co. (1985)	11-41	S. Carolina	Dorchester
53	Weyerhaeuser	2-16	N. Carolina	Bertie
54	Weyerhaeuser	8-67	N. Carolina	Craven
55	Weyerhaeuser	17-43	Alabama	Clarke

^aFamilies 2, 3, 4, 5, 6, 8, 9 and 10 were used in laboratory studies. All families except 1 and 22, which had poor germination, were used in the field. Families 54 and 55 had poor survival and were not included in the growth analyses of the ozone only experiment because of the irregular replication.

^bNorth Carolina State University.

^cWestern Gulf Forest Tree Improvement Program.

^dInternational Paper Company.

between results of laboratory and field studies as a test of methodological differences involving both growing conditions and exposure levels.

3.1 METHODS

Seed stratification, germination, and seedling growth protocols were developed by the SCFRC and used by all three projects. Seedlings used in this study were grown for ORNL by Phyton Technologies, a commercial plant propagation laboratory in Oak Ridge, Tennessee. Seeds were stratified at 5°C for 6 weeks, planted on April 10, 1986, and germinated in trays in a 3:1 vermiculite-peat mixture. After three weeks they were transplanted to the Marx V containers (3 x 3.5 x 11 in. outside dimension) containing a 3:1 vermiculite-peat mixture as specified by the SCFRC. Seedlings were grown in a CF, evaporatively cooled greenhouse for approximately 12 weeks. Spore pellets of Pisolithus tinctorius (Pers.) Coker & Couch provided by the SEFRC were used to inoculate the seedlings and, thereby, attempt to standardize the initial ectomycorrhizal status. At the beginning of the experiments, root colonization by the fungus averaged $39.9 \pm 18.1\%$ for the 9 common families. Further discussion of this can be found in Sect. 6. Nutrients and irrigation were applied to the seedlings during first 12 weeks as prescribed by the SCFRC. By the end of July 1986, the seedlings were sufficiently large to begin both field and laboratory experiments.

3.1.1 Field Study

On July 29-31, 1986, seedlings were loaded into 40- by 30-in. wooden pallets and transported to the field site. These facilities and the protocols used in ozone and acid rain applications are described in Sect. 2. The pallets each contained up to 48 seedlings (4 rows of 12 each) with 6 pallets being placed in each of the 36 chambers or open plots (Fig. 3.1). The 36 test plots, were allocated into 3 blocks of 12 each. Seedling arrangement was spatially stratified with each family being represented with a seedling pair in at least 2 of the 6 pallets in each chamber. Common families were represented in 4 of the

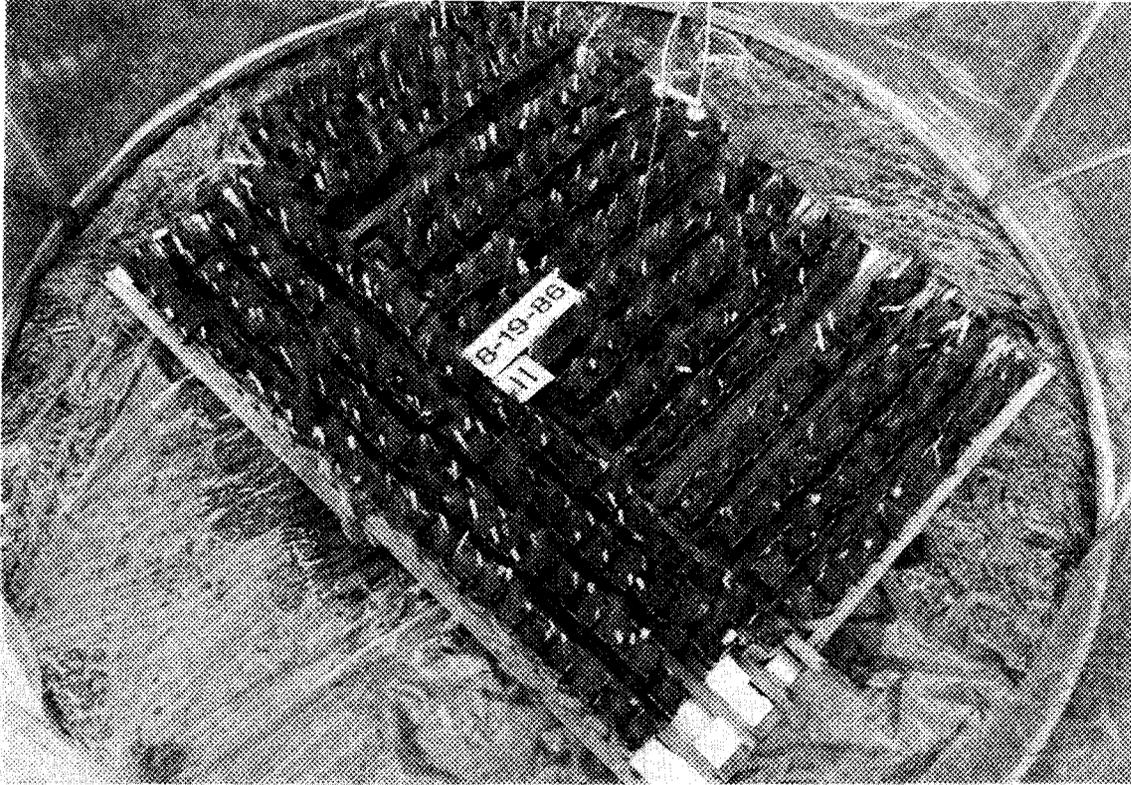


Fig. 3.1. Containerized seedlings from 44 to 53 loblolly pine families were placed in a wooden pallet per chamber for exposure to ozone and acid rain. Each pallet contained 48 plants coded by family, treatment, block, and replicate.

6 pallets, but they appeared only in the 9 treatments (27 chambers) comprising the acid rain x ozone factorial portion of the experiment (3 rain pH levels x 3 ozone levels) as shown in Table 3.2. The 44 unique families appeared in all treatments, although families 54 and 55 were only in 2 blocks of treatments. The arrangement of seedlings within pallets was slightly different for the three treatments (9 chambers) not included in the factorial design, with seedling pairs being represented in three of the six pallets, and consequently, there were at least six seedlings per family per chamber. Pallets were numbered and the arrangement of the six pallets within each chamber was the same across all chambers. Pallets were rearranged twice during the 12-week exposure period to minimize edge effects of pallet x row position within the chambers. The design described required over 9,000 trees. SCFRC protocol called for seedling fertilization with a liquid fertilizer every three weeks. The large number of seedlings in the field study dictated a change in this procedure. Each seedling was fertilized with 11 g of a pelletized slow-release fertilizer (17-6-10 NPK, Sierra brand). Fertilizer was added to the surface of the soil of all pots on August 4, 1986, just before the initial measurement of height and diameter. A comparison study made between this and the SCFRC recommended liquid-fertilizer method will be discussed in another section.

Seedlings were irrigated with 1 cm of artificial rain at the appropriate pH (Sect. 2) at least twice weekly. Median pH values for the artificial rain were 5.2, 4.5, and 3.3, and the corresponding mean pH values were 4.63, 4.38, and 3.26. Discussions in this section will refer to median pH values. Natural rainfall events were excluded by covering the chambers.

Measurements of diameter were made using digital calipers oriented to measure diameter along the pallet row axis and positioned approximately 1-2 mm below the cotyledonary leaf scar. Seedling height was measured with a plastic ruler to the nearest 0.5 mm based on the distance from the pot rim to the base of the apical bud. The pot rim was used as a constant reference rather than the surface of the potting

Table 3.2. The experimental design for the field study demonstrates how the ozone and ozone X acid rain experiments are connected

Median acid rain pH levels	Ozone levels ^a					
	Open plots	Ambient	Charcoal filtered	Ambient +40 ppb	Ambient +80 ppb	Ambient +160 ppb
5.2			X		X	X
4.5	X	X	X	X	X	X
3.3			X		X	X

^aX represents 3 chambers per experimental treatment.

medium because the potting medium surface is subject to shrinking and swelling changes and had surface irregularities associated with both the distribution of the potting medium and the fertilizer pellets added to that surface. Seedling volume was estimated as the square of diameter times the height.

To accomplish the measurement of this large number of seedlings in a short period of time, teams of measurers were used. Measurement variability across six observers was determined by each measuring ten standard plants. As indicated by the coefficient of variability for mean height and diameter, this variability was approximately 3.1% for height and 4.5% for diameter. Appendix E contains details of this procedure.

Six-week measurements were made on September 19-22, 1986, on a subset of the seedlings. Half of the common family seedlings were harvested at that time and brought back to the laboratory for further study and biomass measurement. This process was similar to that described for the final harvest.

Final measurements were made on November 13-14, 1986, after which seedlings to be harvested (those representing common families) were moved to the CF greenhouse for physiological measurements (completed within 1 week) and then into cold storage. Biomass separation and measurements varied in intensity, with the simplest involving separation of seedlings into roots and shoots. For those seedlings no physiological measurements were taken. The most intense measurements included a biomass breakdown that distinguished components in three classes: (1) immature terminal needles, primary and secondary needles, and stem tissues included in measurements of gas exchange; (2) subsamples (three) of needles removed for time lapse studies of ^{14}C metabolism; and (3) fine and coarse root samples. The most detailed biomass separation was reserved for seedlings for which both ^{14}C metabolism and photosynthetic gas exchange measurements were obtained.

3.1.2 Laboratory Study

Seedlings from eight of the common families were brought to ORNL from Phyton. Family 7 did not have enough seedlings to be included in

both the field and laboratory study, so it was excluded in the laboratory. Wire baskets were especially constructed for the lab study, and two seedlings from each of the eight families were placed in each wire basket. The baskets were designed so that four would fit into a CSTR chamber for exposure. For description of the CSTR system at ORNL refer to Taylor et al. (1983) and Fig. 3.2. Ozone concentration was determined using an ozone analyzer (Monitor Labs Ozone Analyzer, Model 8410). Temperatures in the chambers were recorded at hourly intervals and generally ranged from 32 to 36°C. Photosynthetic photon flux density within the chambers at a height of 32 cm ranged from 240 μE at the outside edge of the chamber to 610 μE at the center. Relative humidity was monitored also and ranged from 50 to 70%. During the first two weeks of exposure to ozone, it was decided to halve the target levels of 160 ppb and 320 ppb. This decision was based on visible injury observed on seedlings in the field at 40 ppb and 80 ppb above ambient levels during their first few days of exposure. When no further injury was noted, levels were increased up to the target levels. For the rest of the experiment the target levels were maintained. See in the Appendix E for a more complete description of variation in chamber conditions.

Seedlings were placed in the CSTRs four days a week and exposed to ozone for 6 h/d. After two exposure days they were returned to the CF greenhouse overnight for irrigation on an artificial rain table. Light levels in the chambers were too low to maintain the seedlings; therefore, on the three days each week when they were not exposed to ozone they were kept in a CF greenhouse and were irrigated again.

Baskets of seedlings were rotated in the chamber each day. Each week baskets were moved to a new chamber to ameliorate chamber effects. Two baskets of seedlings formed a replication and were kept together throughout the experiment.

Seedlings were irrigated twice each week with approximately 1 cm/event of an artificial rain at pH 4.3. Fertilization of seedlings was conducted every two weeks using 140 mL of a standard nutrient solution specified by the SCFRC protocol. A special group of seedlings

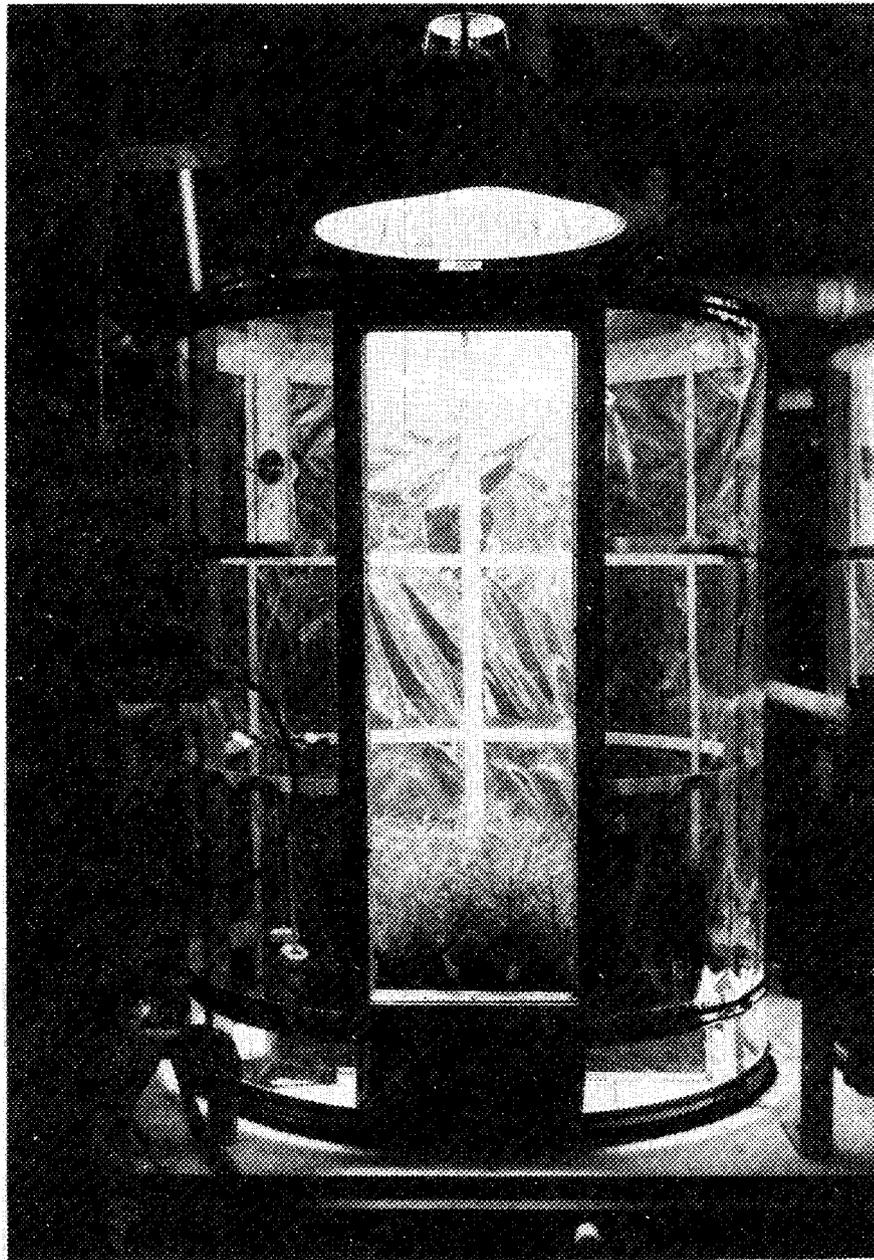


Fig. 3.2. Continuously stirred tank reactors each contained 64 seedlings representing 8 common families at the beginning of the laboratory experiments. Half of the seedlings were harvested after the first 6 weeks of exposure.

was not exposed to ozone; they were left in the greenhouse throughout the duration of the experiment. Half of these seedlings were fertilized with the slow-release fertilizer used in the field study and half with the liquid fertilizer used on the laboratory study seedlings. The seedlings were measured periodically for comparison of the two fertilizer methods (Appendix E).

Seedlings in this experiment were measured in the same manner as those in the field experiment; however, all seedlings were measured every three weeks.

3.2 RESULTS AND DISCUSSION

3.2.1 Field Studies

3.2.1.1 Growth Indicators

Because the size of these experiments precluded obtaining weight data for all seedlings, we compared the four growth parameters [height, diameter, weight, and volume (the square of diameter times height)], to determine how well each of the three dimensional measurements compared with seedling weight. Fig. 3.3 shows comparative values of these 4 parameters for approximately 15 seedlings per family; this represents each of the 9 common families grown in CF air in the field. The data show that the magnitude of fluctuations in total seedling weight among families was typically similar in direction for the three dimensional parameters, with diameter being the most consistent indicator of both the magnitude and direction of differences in weight among families. Both height and volume overestimated the differences in seedling weight among families, particularly for Families 4 and 6. While we typically have examined growth responses to pollutant exposure using all three dimensional variables, it appears that diameter may be the most appropriate indicator of weight changes when nondestructive sampling is desired. In general, the height of loblolly pine is a more highly heritable trait than diameter, diameter being affected to a greater extent by environment.

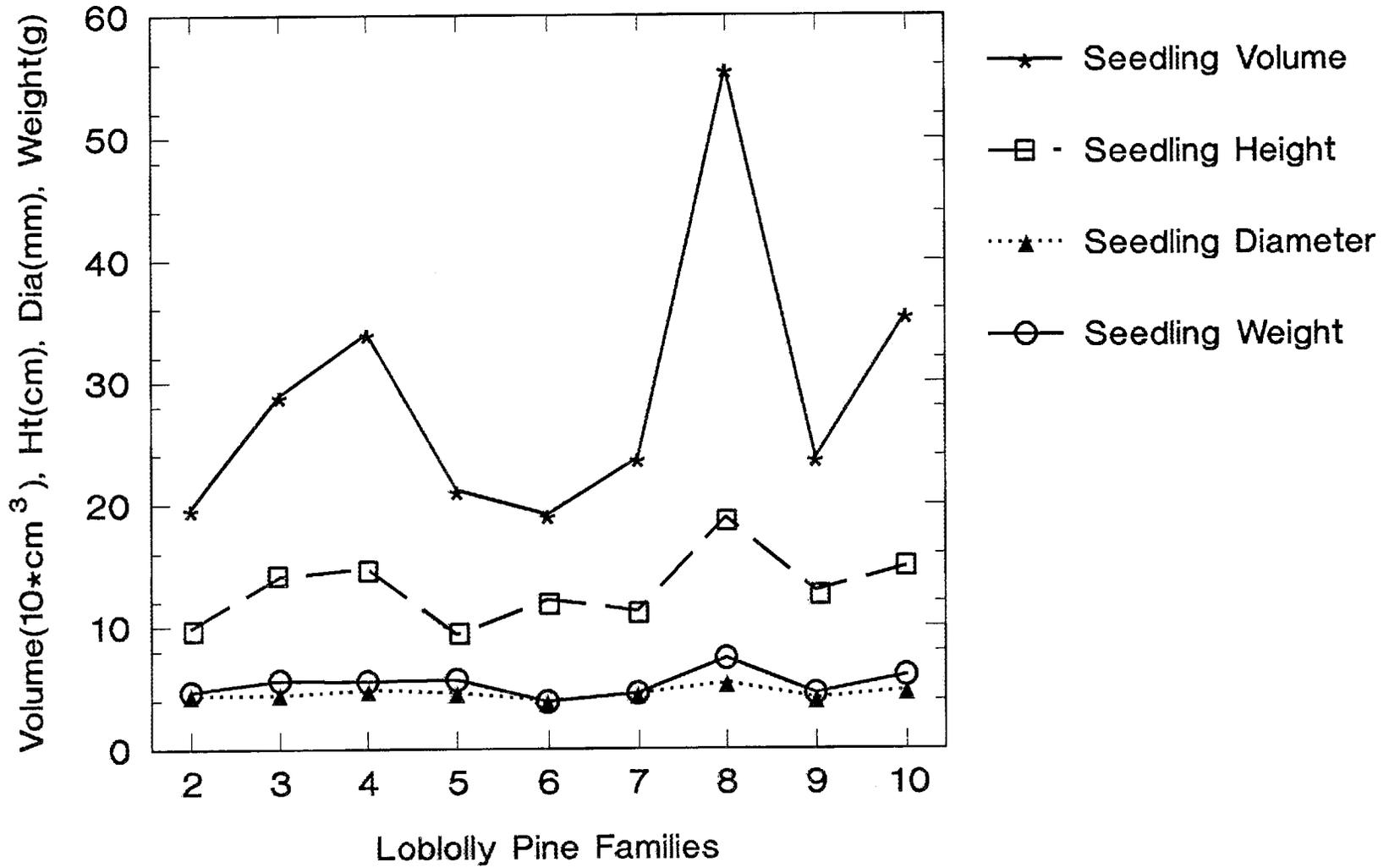


Fig. 3.3. Comparison of four growth parameters for nine common families grown in CF air in the field.

3.2.1.2 Growth Trends

Another consideration in examining growth responses is the overall pattern of growth observed over time and across conditions in the laboratory and field portions of these studies. The growth of seedlings was quite good with a mean increase in diameter of 300%, a 50% increase in height, and an 800% increase in volume. There was a large amount of variation among families in volume growth (measured as D^2H) (Fig. 3.4). Growth was also approximately linear throughout the entire 12-week period for both laboratory and field-grown seedlings. In the field growth was typically greater, and the effects of adding ozone were more well-defined than in the laboratory (Fig. 3.5).

3.2.1.3 Growth Responses to Ozone

Summary data for height, diameter, and volume growth for approximately 4000 seedlings (representing 42 families) that were grown in the field in six ozone treatment levels and one acid rain level (median pH 4.5) are noted in Table 3.3. Growth changes of individual seedlings over the 12 weeks of the study were the basis of the average growth data for these analyses. Data reported in Table 3.3 show that compared to growth in charcoal-filtered air reductions in height, diameter, and volume growth of 26, 5, and 14%, respectively, occurred in ambient air; the response to ambient air was quantitatively the most significant response observed in any of the treatments. Seedlings in open plots grew the largest of all treatments, indicating that there was an adverse chamber effect on growth (Fig. 3.6).

Because differences insensitivity to ozone among families was a major emphasis for this research, comparisons across families in response to ambient, A40, and A160 exposures are shown in Figs. 3.7-3.9. Differences in growth in height (a) and diameter (b) for the three treatments are plotted respectively in Figs. 3.7, 3.8, and 3.9 using the CF treatment as a reference point. Thus, data represent a fractional change in growth from that measured in the CF treatment (i.e., $-0.25 = 25\%$ reduction). The consistency of the response of families to ambient air is readily apparent in Fig. 3.7

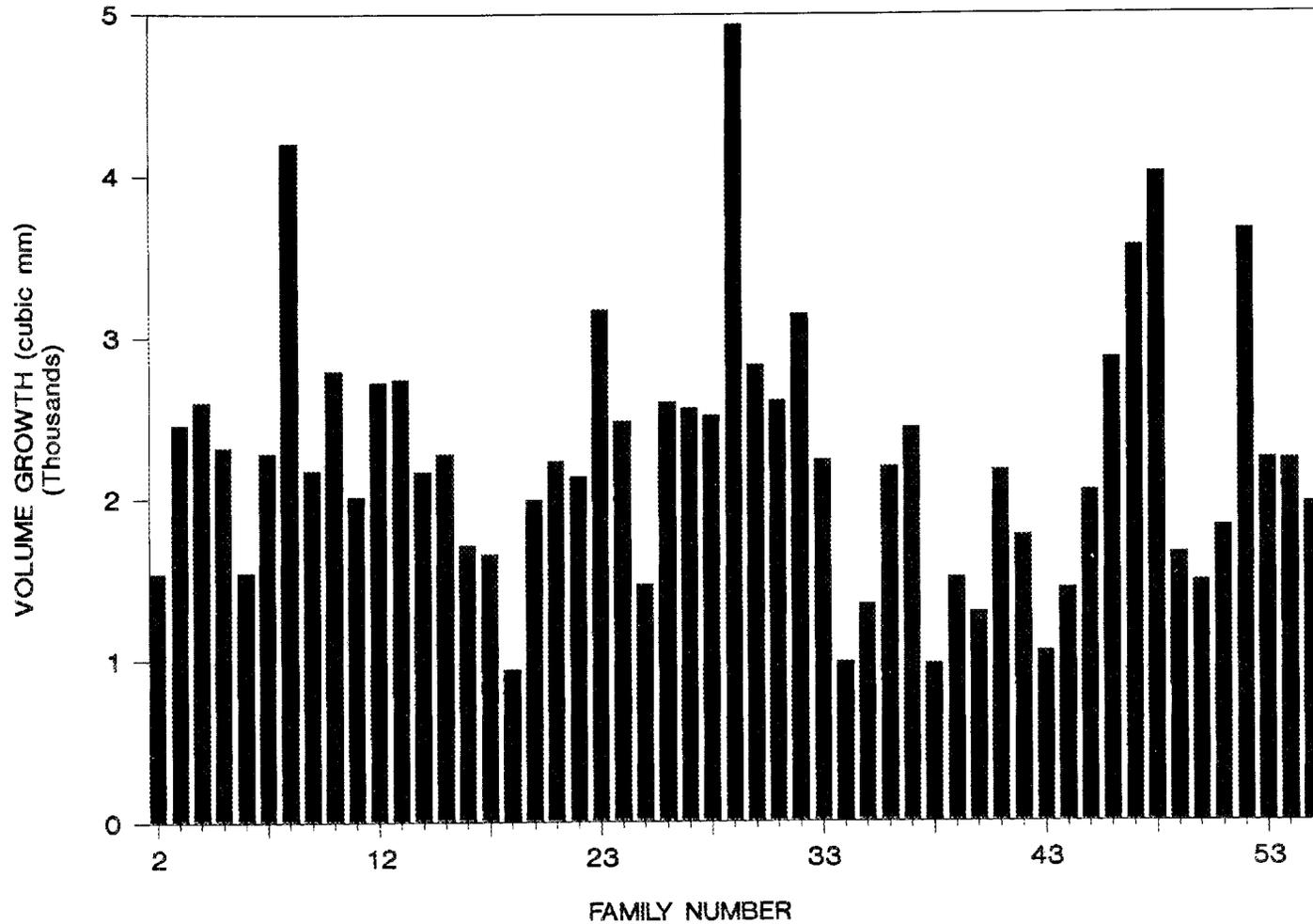


Fig. 3.4. Mean estimated total volume growth of 53 loblolly pine families during a 12-week period. Volume calculations were based on the square of seedling diameter times seedling height. These families were grown in 5 ozone regimes in the field and irrigated with artificial rain (pH 4.5).

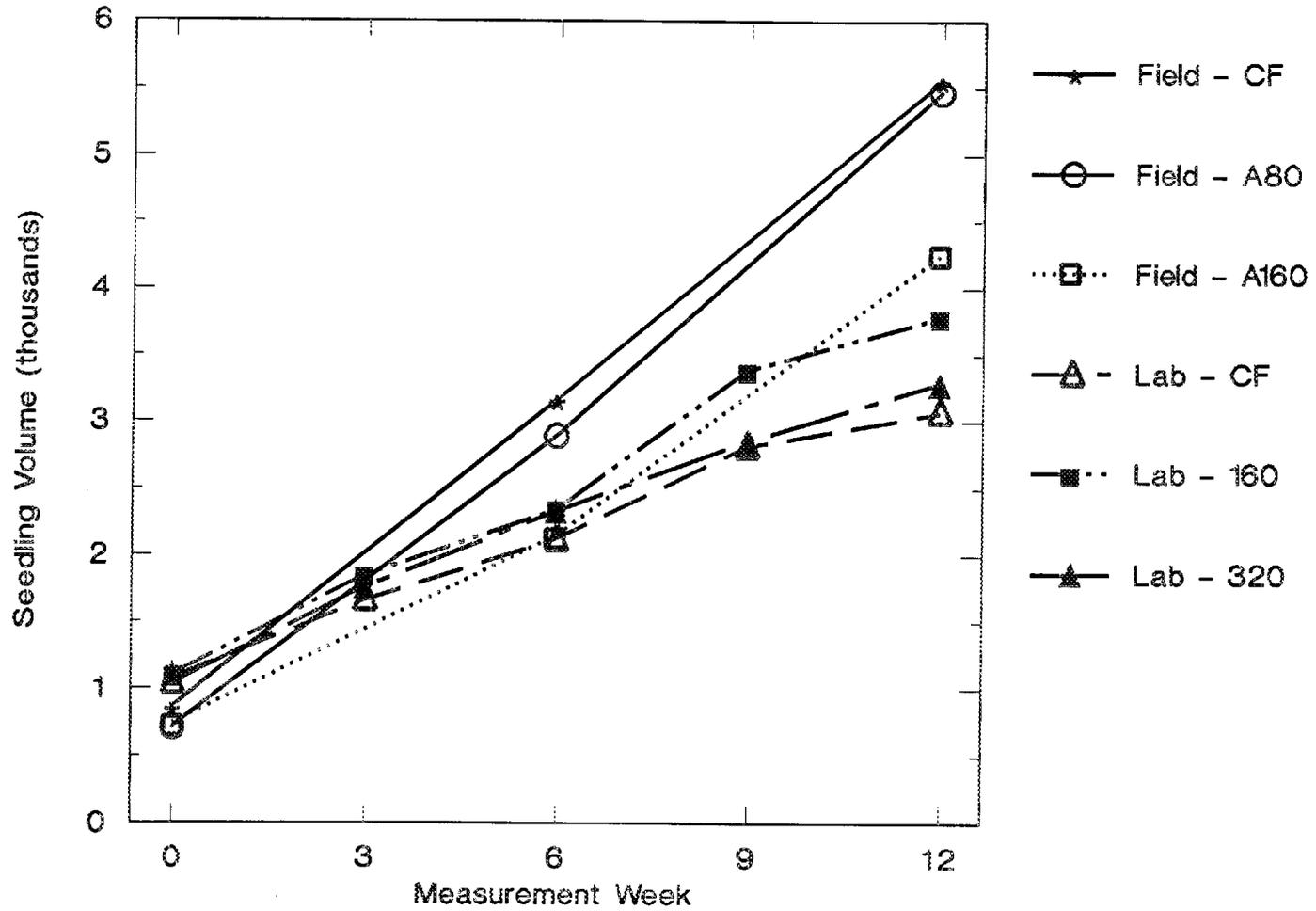


Fig. 3.5. Volume growth of loblolly pine Family 8 in response to ozone in the field and laboratory.

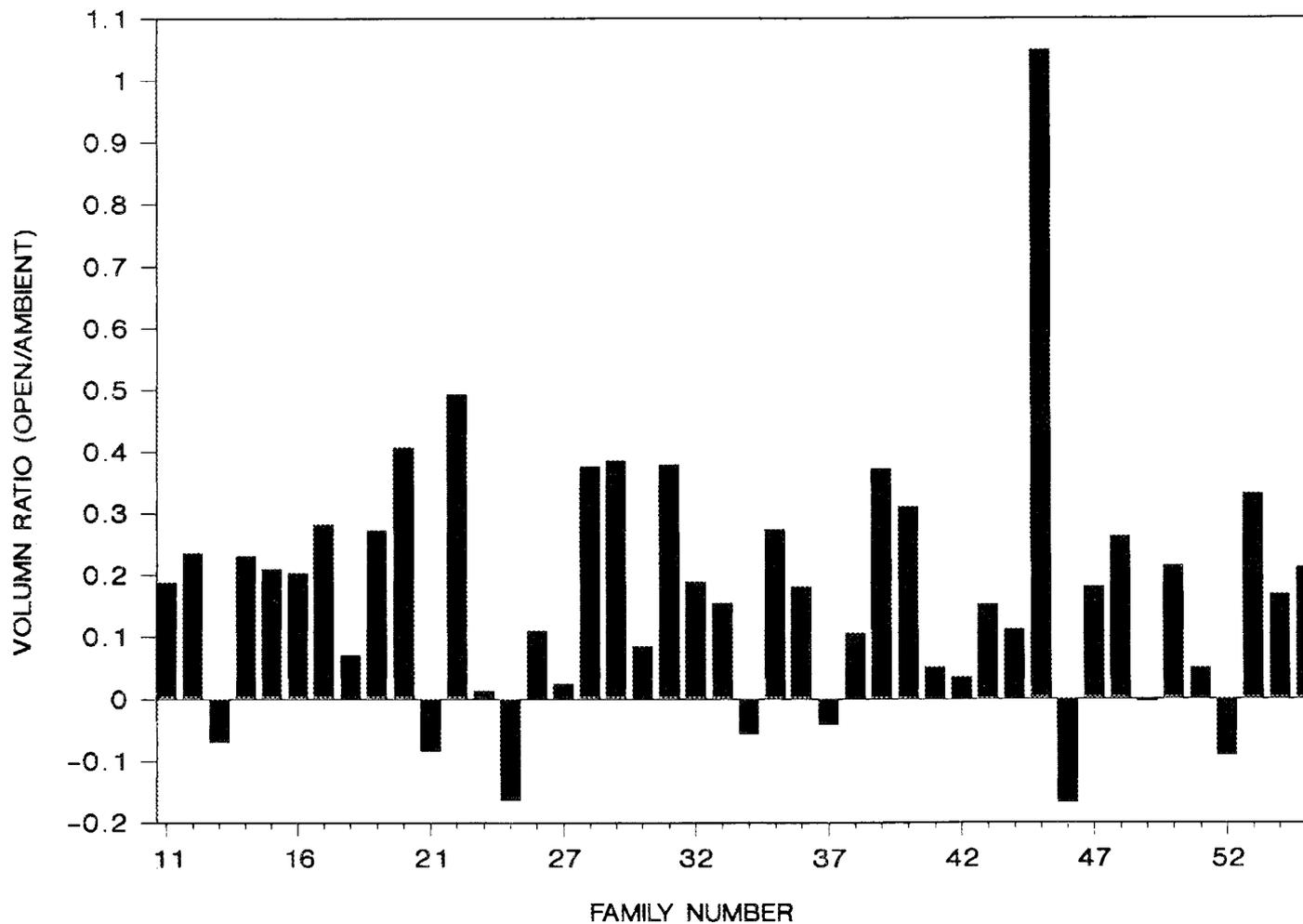


Fig. 3.6. A comparison of 44 loblolly pine families for the ratio (minus 1.0) of their volume growth in open plots to that in chambered plots with ambient ozone levels. A value of 0 indicates equal growth in open plots and chambered plots, and 0.20 indicates 20% more growth in open plots (all trees irrigated with pH 4.5 artificial rain).

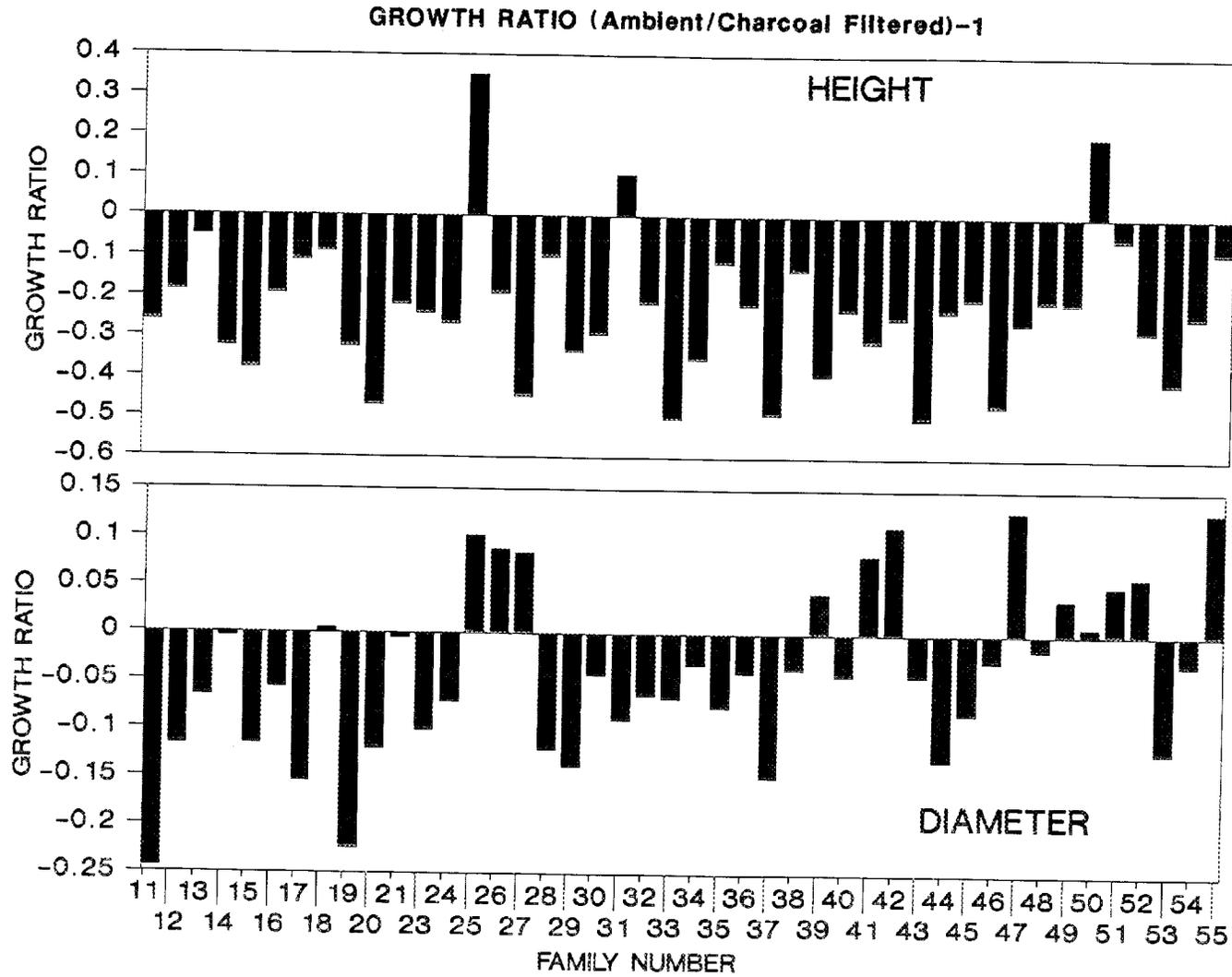


Fig. 3.7. A comparison of 44 loblolly pine families for the ratio (minus 1.0) of their height and diameter growth in ambient to that CF chambers. A value of 0 indicates equal growth rates, and -0.20 indicates 20% better growth in charcoal filtered air (all seedlings irrigated with pH 4.5 artificial rain).

OZONE EFFECTS BY FAMILY

(Ambient +40 vs Charcoal Filtered)

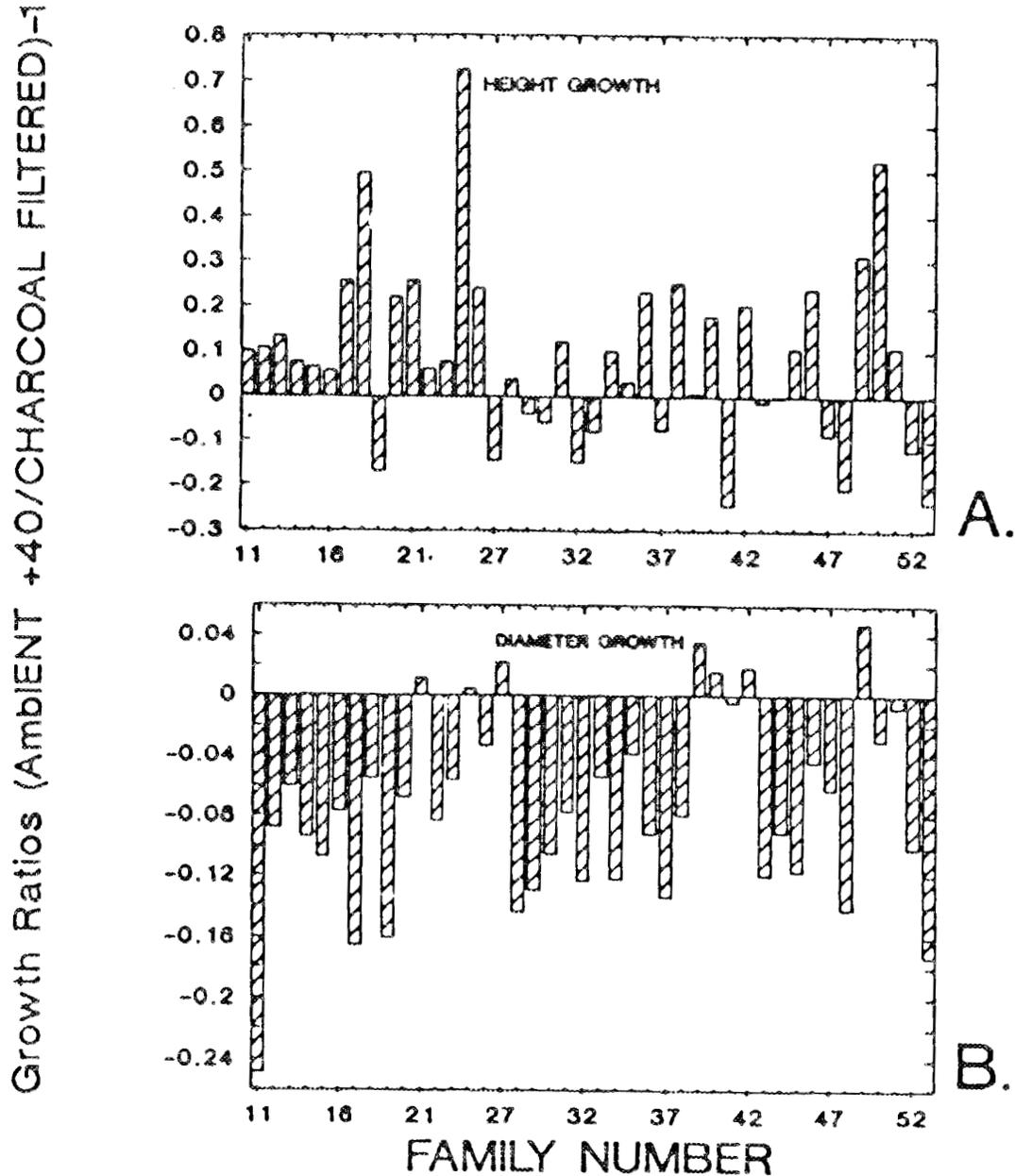


Fig. 3.8. A comparison of 44 loblolly pine families for the ratio (Minus 1.0) of their height and diameter growth in ambient +40 ppb ozone to that in charcoal filtered chambers, where a value of 0 indicates equal growth rates, and a value of -0.20 indicates 20% better growth in charcoal filtered air (all seedlings irrigated with pH 4.5 artificial rain).

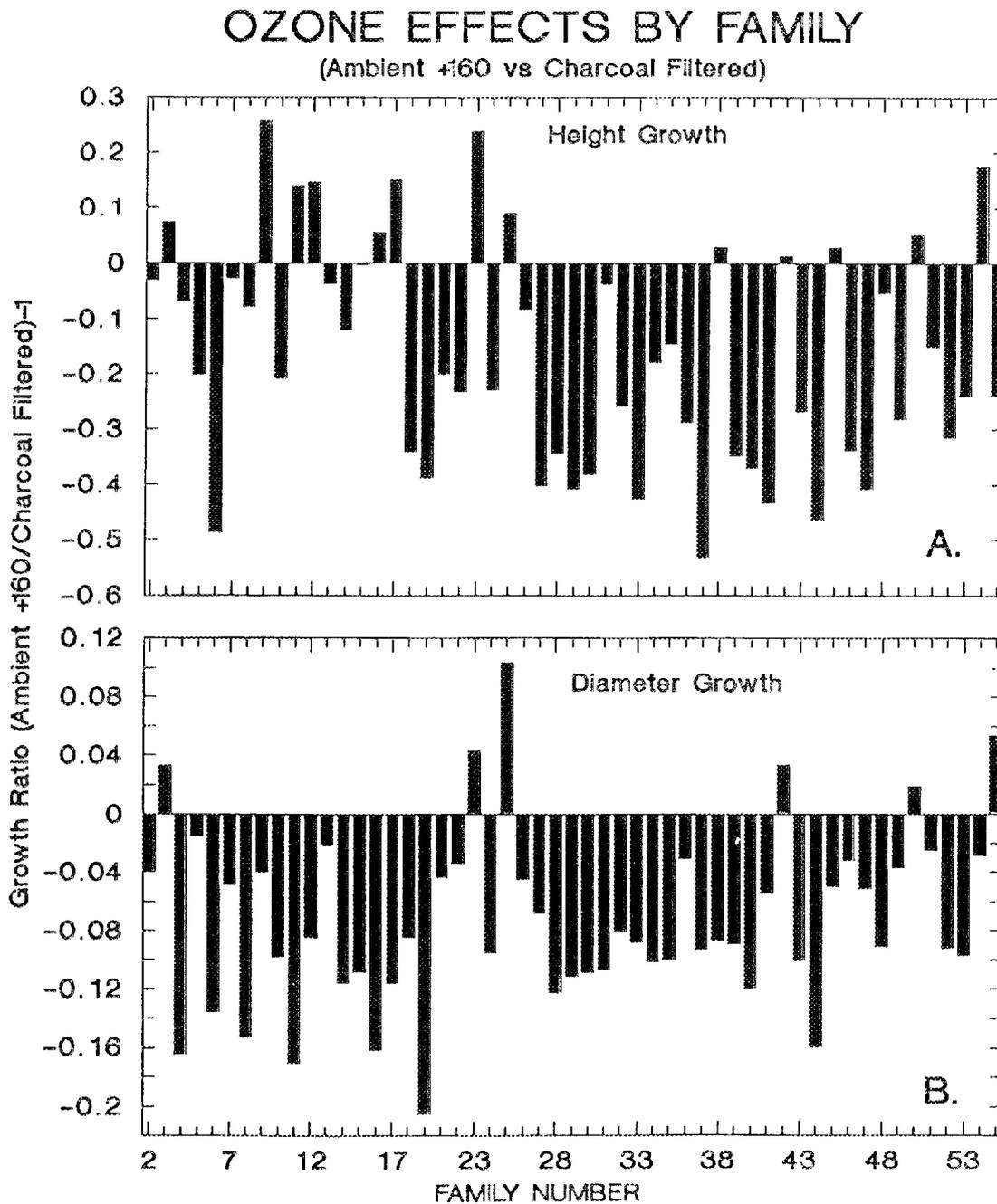


Fig. 3.9. A comparison of 44 loblolly pine families for the ratio (minus 1.0) of their height and diameter growth in ambient +160 ppb ozone to that in charcoal filtered chambers. A value of 0 indicates equal growth rates, and a value of -0.20 indicates 20% better growth in charcoal filtered air (all seedlings irrigated with pH 4.5 artificial rain).

Table 3.3. Mean height, diameter, and volume growth of 42 loblolly pine families across ozone treatments during the 13-week field study.

Volume treatment	Ozone dose (ppb h x 10 ³)	Change	Height	Diameter	Volume ^a
			(mm)	(mm)	(mm ³)
Charcoal filtered (CF)	19	a	46.32	2.75	2495
Ambient chamber (AC)	38	b	34.40 -26 ^b	2.62 -5	2140 -14
Ambient open (AO)	34	b	47.77 +3	2.77 +1	2687 +8
Ambient +40 ppb (A40)	41	b	48.10 +4	2.53 -8	2382 -5
Ambient +80 ppb (A80)	59	b	40.39 -13	2.59 -6	2212 -11
Ambient +160 ppb (A160)	79	b	36.68 -21	2.53 -8	2189 -12

^aHeight, diameter, or volume at the end of the experiment (November 7) minus initial values determined on August 2, 3, and 4. Volume was indicated by the parameter D²H.

^bPercent change for a parameter compared to the value of that parameter for the CF treatment.

with 39 of 42 families contributing to the 26% mean reduction in height noted in Table 3.3. The maximum reduction noted was about 50%. Diameter reductions included a maximum of 25% and were less consistent across families with 30 of 42, families responding negatively.

As the level of ozone increased to A40, diameter reductions became more consistent across families (36 of 43) but height growth responses shifted to more positive values (Fig. 3.8) (the mean response was +4%). Further increases in the ozone level resulted in a shift to negative height responses [Fig. 3.9(a)], while diameter reductions remained fairly constant at -8% [Fig. 3.9(b)].

The shape of the ozone dose response surface for effects on diameter growth for four families (Fig. 3.10) illustrates both similarities and differences in the levels, response patterns, and consistency of ozone responses among families. These results formed the basis of the ozone x family interaction noted in Table 3.4 and indicated that further analysis of the basis for differences among families was warranted. A major consideration in these analyses has been exploring the genetic basis (seed source origin) and physiological basis (growth characteristics) of these differences.

3.2.1.4 Statistical Analysis

With these response patterns in mind, the statistical analysis of the growth data can be viewed in better perspective. Analyses of observed differences in height, diameter, and volume growth over the entire study interval (Table 3.3), were performed using an analysis of covariance. The statistical model took the form:

$$Y_{ijkl} = B_i + O_j + (B \cdot O)_{ij} + F_k + (O \cdot F)_{jk} + rC_{ijkl} + e_{ijkl} ,$$

where B = Block, O = Ozone, F = Family, C = covariate, and e = error.

The experimental design was a split plot with blocks and ozone levels as main effects. The main plot was the large chamber to which one of five ozone levels was applied. Within each chamber there were up to 53 families, although only 42 families were considered in this

OZONE EFFECTS ON DIAMETER GROWTH

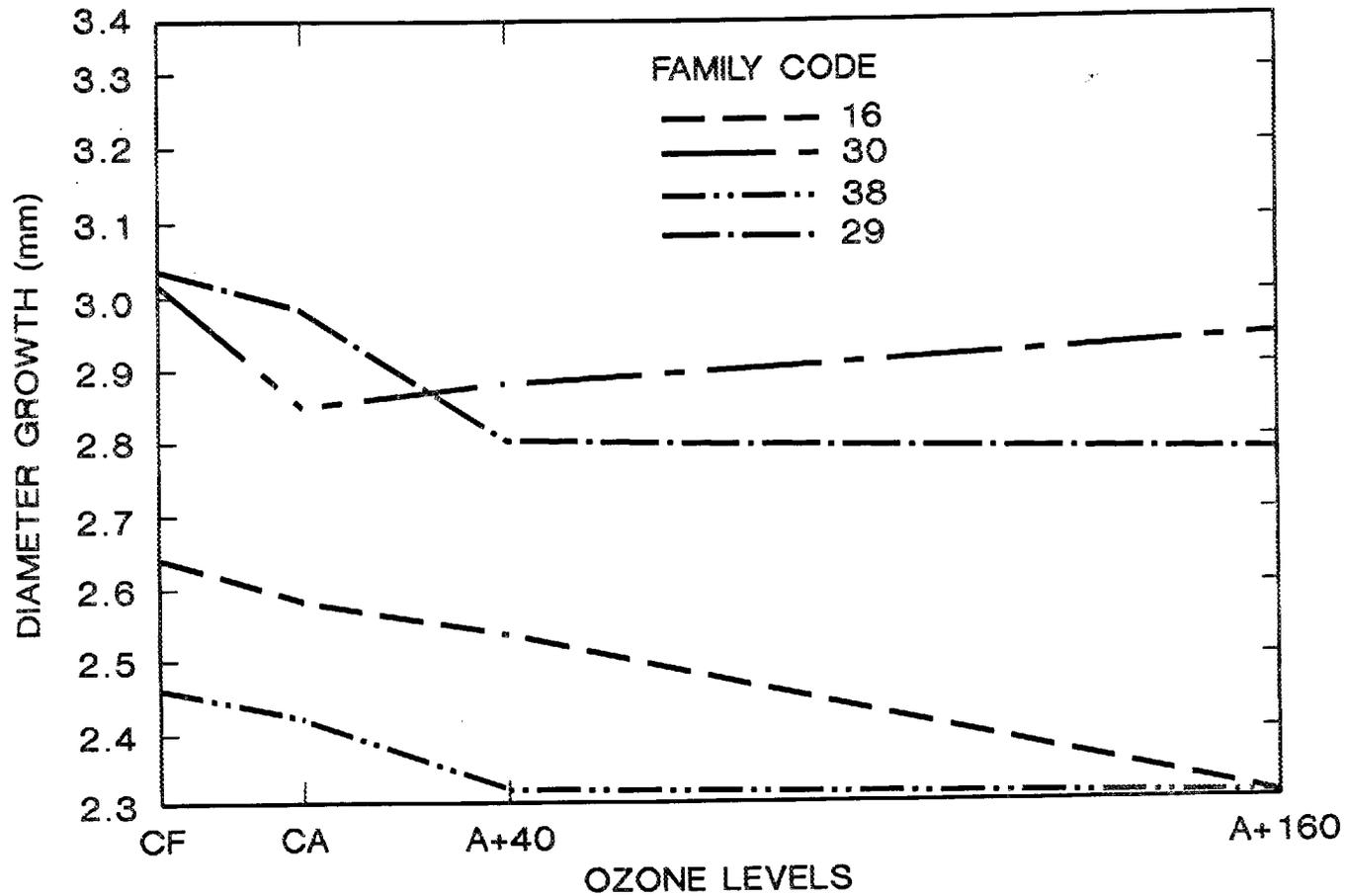


Fig. 3.10. Diameter growth trends of 4 loblolly pine families grown in the field in 4 ozone regimes during a 12-week period. Mean ozone concentration for CF, CA, A40, and A160 during controlled exposure were 18, 36, 61, and 173 ppb respectively during full concentration exposure (see Table 2.4).

Table 3.4. Analysis of covariance of the change in height, diameter, and volume growth of loblolly pine families during a 12-week period in a field study of the effects of 5 ozone levels

Source	df	Type III SS	F value	PR>F
<u>Height difference</u>				
Block (B)	2	5,820	0.39	0.8333
Ozone (O)	4	95,510	1.53	0.2815
B • O	8	124,785	37.08	0.0001
Family (F)	41	624,735	28.29	0.0001
O • F	164	88,324	1.28	0.0109
Initial height	1	105,861	251.64	0.0001
Error	3,046	1,281,422		
<u>Diameter difference</u>				
Block (B)	2	20	1.00	0.4097
Ozone (O)	4	17	0.42	0.7906
B • O	8	81	45.64	0.0001
Family (F)	41	130	10.69	0.0001
O • F	164	49	1.35	0.0023
Initial diameter	1	17	77.32	0.0001
Error	3,077	689		
<u>Volume difference</u>				
Block (B)	2	50,511,838	0.79	0.4849
Ozone (O)	4	58,181,275	0.46	0.7657
B • O	8	254,648,076	41.68	0.0001
Family (F)	41	850,998,370	17.46	0.0001
O • F	164	194,986,933	1.56	0.0001
Initial volume	1	608,864,107	797.21	0.0001
Error	3,004	2,294,273,250		

analysis because of design imbalances introduced by including 11 of the 53 families. Nine of these were the common families that appeared only in the factorial portion of the experiment, and the other two were families 54 and 55, which had poor survival. The height, diameter, and volume of each seedling at the beginning of the experiment were used as covariates in the analysis. The dependent variables, height, diameter, or volume difference, were calculated by subtracting the initial height, diameter, or volume of the seedling from the final height, diameter, or volume. In these analyses, block and family were considered random effects. The covariate, initial height, diameter, or volume, had a substantial impact on the growth change. This would be expected because there were only 12 weeks between the initial and final measurements and the compounding effects of seedlings size are important for smaller rapidly growing seedlings. To satisfy questions concerning the validity of the highly significant covariates, a test for heterogeneity of slopes was performed as described by Freund and Littell (1981). There was no evidence from this test to suggest that the covariate was inappropriate. The analyses presented in Table 3.4 were for chamber plots only. There were significant differences in growth between the open plots and the chamber plots supplied with ambient air (Fig. 3.6). An analysis of height-growth differences between these two treatments was performed using the above model, confirming the statistical significance of this difference ($p \leq 0.01$). However the purpose of these experiments was to test ozone effects, not effects caused by chambers; consequently, open plots were eliminated from all further analyses. The power of this analysis to detect differences was very low.

As can be seen in the analysis of covariance (Table 3.4), the ozone by family interaction was highly significant for all three measurements. Because this interaction was significant, an interpretation of the main effects was not possible. Therefore, because of the large number of families and their genetic backgrounds, it was important to look at analyses of ozone differences (the main effect) on a family-by-family basis. The analysis of covariance

results for each family can be found in Appendix B. The model for these analyses was more simple and included the effects of block, ozone, and the covariate ($y_{ijk} = B_i + O_j + (B \cdot O)_{ij} + rC_{ijk} + e_{ijk}$). Block was a random effect, and ozone was fixed. All 53 families were analyzed in this manner. Families 2 to 10 were exposed to 3 levels of ozone, and families 11 to 55 were exposed to 5 levels. Mean height and diameter changes for each family treatment combination can be found in Appendix A.

The variances in these analyses were large and therefore in general only statistical difference between treatment means of greater than one standard deviation could be detected (ie 22 mm). In the height analyses, the block effect was significant ($p \leq 0.05$) only three times. In 20 of the 53 families, the ozone effect on height was significant ($p \leq 0.05$). The influence of initial height as a covariate was significant ($p \leq 0.05$) in 30 of the 53 families. In the analysis of diameter growth, block effects were significant 18 of 53 times, ozone effects 9 of 53 times, and initial diameter 11 of 53 times. Since, height is generally considered a more heritable trait than diameter, it is logical that the relationship between initial vs final measurements was stronger. This is supported by the fact that the number of families with significant initial height effects was almost three times greater than those with significant initial diameter effects.

For the nine common families analyzed, only one family had significant ($p \leq 0.05$) height-growth response to ozone exposure. In the case of diameter growth, three of the nine families responded significantly ($p \leq 0.05$) to the ozone exposures.

3.2.1.5 Growth Response to Acid Rain and Ozone

A summary of the mean height, diameter, and volume growth data for 53 families included in the factorial acid rain by ozone interaction portions of this study is shown in Table 3.5. The interactive effects of acid rain (levels 5.2, 4.5, and 3.3) and ozone (levels CF, A80, and A160) on growth over the entire study interval are characterized across each treatment combination by comparing growth under each treatment

Table 3.5. Interactive effects of acid rain and ozone on mean height, diameter, and volume growth of 51 loblolly pine families

Median acid rain pH	Growth	Ozone treatment		
		CF	A80	A160
<u>Height (mm)</u>				
5.2	a	38.8	35.4	38.1
			-9	-2
4.5	a	46.4	42.0	37.8
		+20	+8	-3
3.3	a	31.4	35.4	42.0
		-19	-9	+8
<u>Diameter (mm²)</u>				
5.2	a	2.59	2.65	2.42
			+2	-7
4.5	a	2.74	2.61	2.53
		+6	+1	-2
3.3	a	2.53	2.70	2.59
		-2	+4	0
<u>Volume (mm³)</u>				
5.2	a	2307	2557	2147
			+11	-7
4.5	a	2508	2279	2210
		+9	-1	-4
3.3	a	2097	2396	2286
		-9	+4	-1

^aΔ% expresses percent change from values for CF chambers at pH 5.2.

combination to that of seedlings in the CF plot exposed to pH 5.2 rainfall. From these data there appears to be a substantial and typically antagonistic interaction between acid rain and ozone, with the greatest inhibition of growth occurring under CF conditions at pH 3.3. Increasing the ozone concentration typically reduced the growth impact of acid rain compared to that observed under CF air. Conversely, ozone impacts were always greater at higher rainfall pH values than at pH 3.3. These data also indicate that the principal effects of ozone were on height growth. The range of responses typically observed with acid rain x ozone combinations is shown more clearly with the diameter growth data for Families 7 and 8 (Figs. 3.11 and 3.12). These responses included both almost linear decreases with increasing ozone (Family 7, pH 4.5 and 5.2) and threshold responses (Family 8, pH 4.5 and 5.2) and both convex and concave bimodal responses at the pH 3.3 level. The uniqueness of the response surface observed at the lowest pH level was apparent with both families.

An examination of the variability in responses of height growth to acid rain across the 53 families included in this factorial experiment is shown in Fig. 3.13. When comparisons are made under CF conditions between growth at pH 5.2 and at either pH 3.3 [Fig. 3.13(a)] or pH 4.5 [Fig. 3.13(b)], the bimodal nature of the response surface is apparent. Substantial differences among families were also quite apparent. From these comparisons it can be seen that 36 of 53 families responded positively to a pH decrease from 5.2 to 4.5, and 43 of 51 responded with poorer height growth as the pH level was lowered to pH 3.3.

3.2.1.6 Statistical Analysis

With these response patterns in mind, the statistical analysis of the growth data can be viewed in better perspective. Analyses of observed differences in height and diameter growth over the entire study interval (Table 3.5), were performed using an analysis of covariance. The statistical model took the form:

$$y_{ijklm} = B_i + O_j + R_k + (B \cdot O)_{ij} + (O \cdot R)_{jk} + (B \cdot O \cdot R)_{ijk} + F_l + (O \cdot F)_{jl} + (R \cdot F)_{kl} + (O \cdot R \cdot F)_{jkl} + r^C_{ijklm} + e_{ijklm}$$

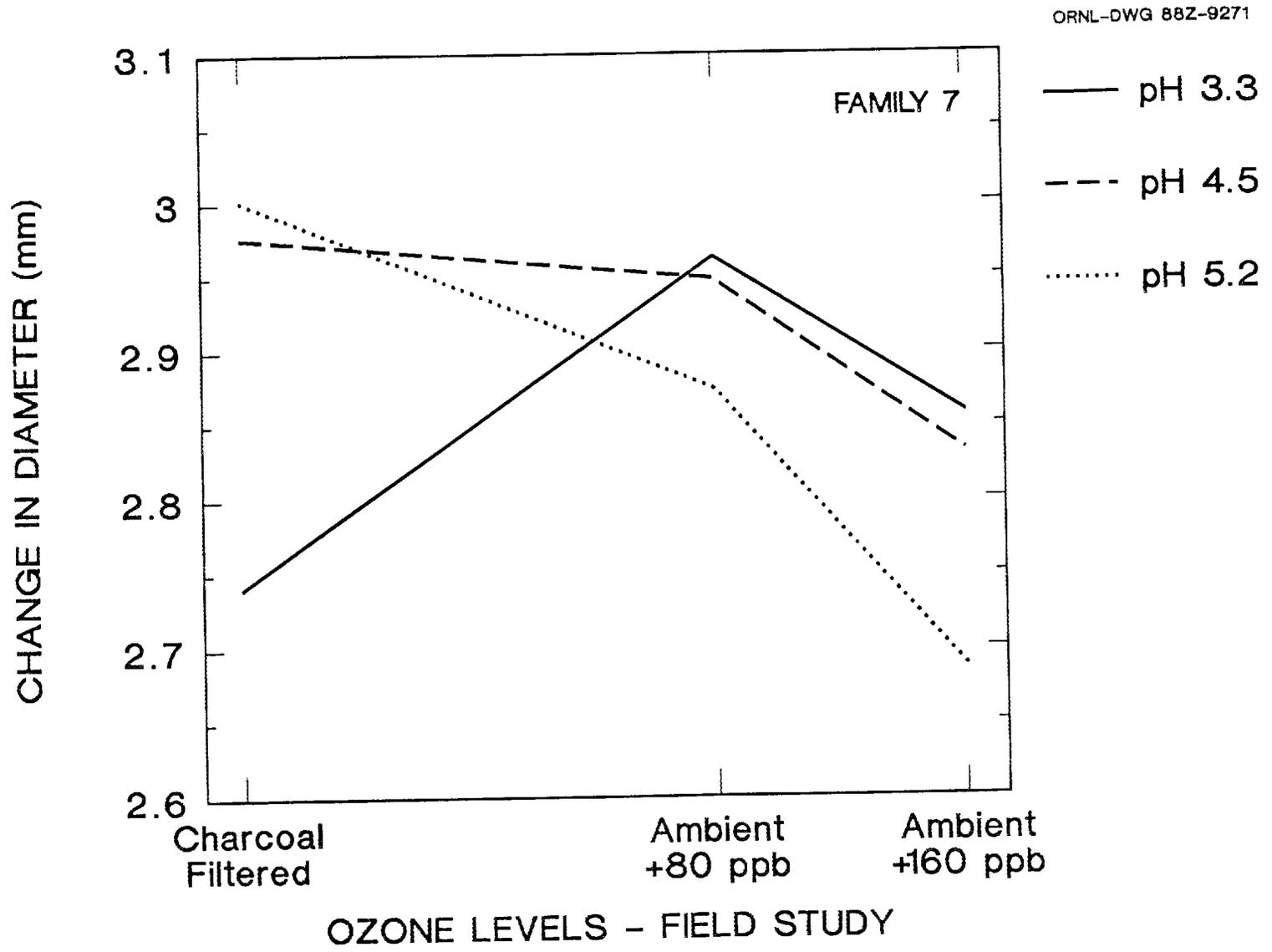


Fig. 3.11. Mean diameter growth of loblolly pine Family 7 exposed to three levels of ozone and acid rain in a field study.

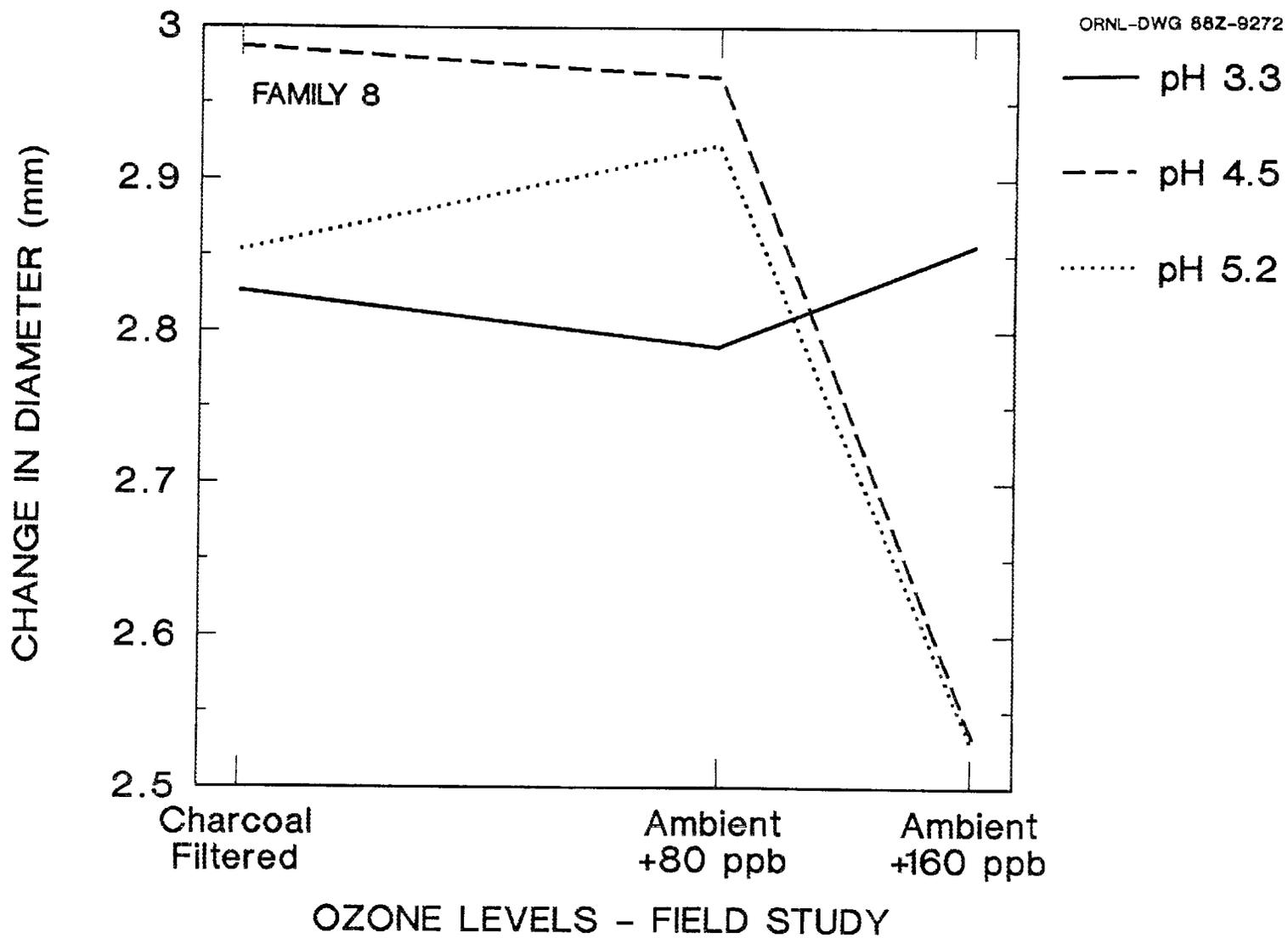


Fig. 3.12. Mean diameter growth of loblolly pine Family 8 exposed to three levels each of ozone and acid rain in a field study.

ACID RAIN COMPARISON

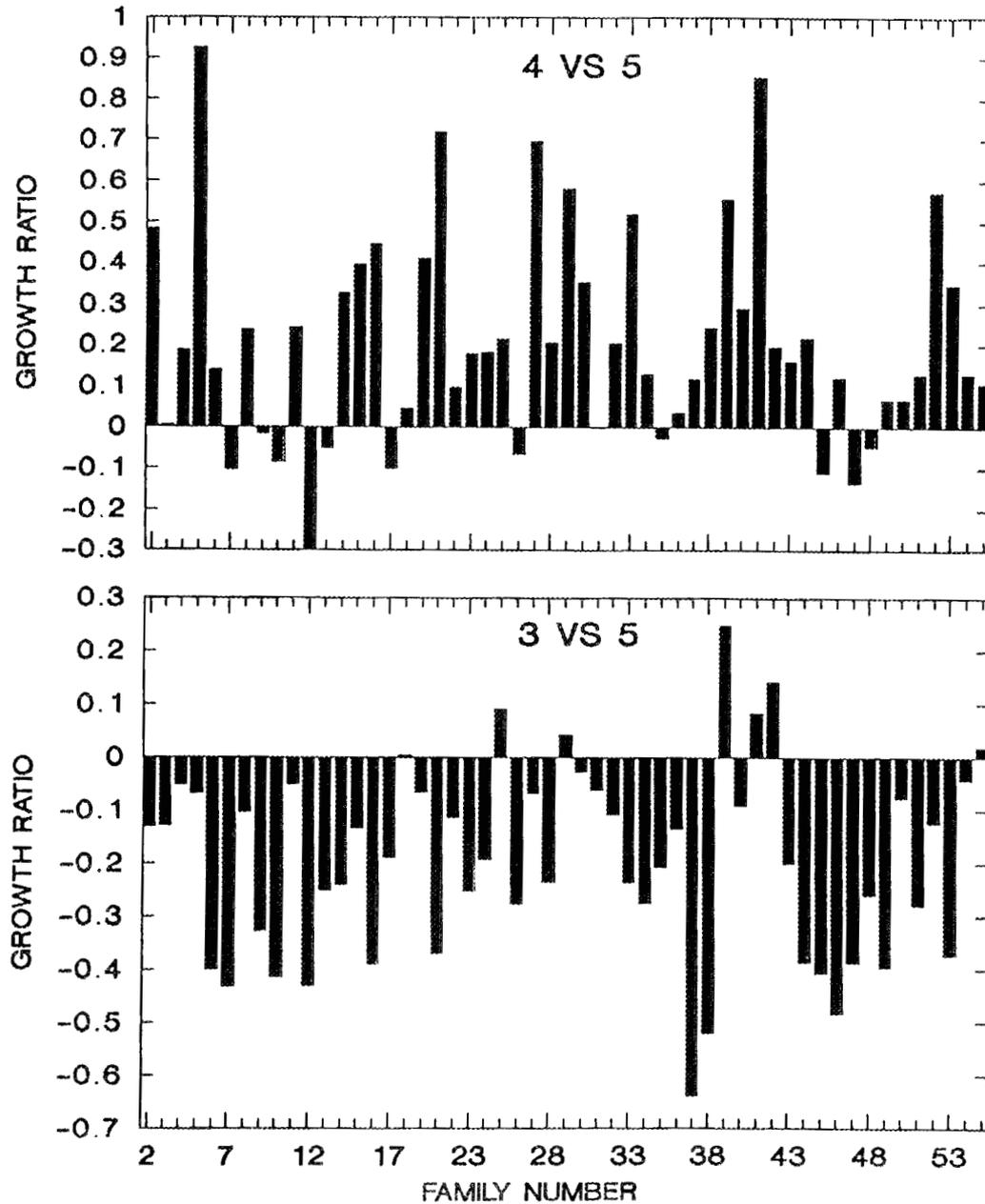


Fig. 3.13. A comparison of 53 loblolly pine families for the ratio (minus 1.0) of their height growth at pH 3.3 or pH 4.5 to that at pH 5.2. A value of 0 indicates equal growth rates, and a value of +0.20 indicates 20% better growth in the lower pH rain.

where B = Block, O = Ozone, R = Rain, F = Family, C = the covariate, and e = error.

The factorial experimental design was a split plot with blocks, ozone levels, and rain pH levels as main effects. The main plot was the large chamber to which one of the three ozone levels and one of the three rain pH levels were applied. All 53 families were considered in these analyses.

The height and diameter of each seedling at the beginning of the experiment were used as covariates in the analysis. The dependent variables, height, or diameter difference were calculated by subtracting the initial height or diameter of the seedling from the final height or diameter. In these analyses, both block and family were considered random effects. The covariate was tested in a manner similar to that described previously for the ozone analyses and, as before, there was no evidence from these tests to suggest that the covariates were inappropriate. Unfortunately, computer memory was not large enough to include all 53 families in the same analysis. Therefore, two analyses were conducted for each variable: one for families 2 to 25 and another for families 26 to 55. Pseudo-f tests were performed to determine significance (Hicks 1973).

Statistical analysis of these data (Tables 3.6 and 3.7) using ANCOVA procedures indicated that there was a significant family effect on relative growth rates. There was not, however, a significant ozone x family interaction as was found in the previous analysis of 5 ozone treatments. The ambient and A40 treatments which many times had the lowest and highest treatment means, respectively, in the ozone alone analysis were not included in this factorial experiment. Separate analyses for each family were performed (Appendix B). A summary of the numbers of families for which height or diameter differences were significant for both the factorial and previously discussed ozone-only studies is provided in Table 3.8. Family mean heights and diameters at each treatment level are in Appendix A.

When applied to the test of ozone effects, these data reflect the substantial influence of differences in statistical power of the

Table 3.6. Analysis of covariance of the change in height growth of 53 loblolly pine families during a 12-week period in a factorial field experiment using 3 levels each of acid rain and ozone

Source	df	Type III SS	F value
<u>Families 2-25</u>			
Block (B)	2	15,577	4.25 ^a
Ozone (O)	2	5,140	0.44
Rain (R)	2	18,543	2.52
R • O	4	12,679	1.73
B • R	4	14,719	2.01
B • O	4	23,270	3.17
B • R • O	8	14,666	4.35 ^a
Family (F)	22	457,242	41.68 ^b
O • F	44	19,425	0.88
R • F	44	17,808	0.81
R • O • F	88	43,882	1.18
Initial Height	1	98,135	233.00 ^b
Error	2,451	1,032,321	
<u>Families 26-55</u>			
Block (B)	2	2,687	0.45
Ozone (O)	2	6,064	0.24
Rain (R)	2	13,505	0.54
R • O	4	59,128	4.11 ^a
B • R	4	21,726	1.78
B • O	4	26,673	2.23
B • R • O	8	23,902	6.04 ^a
Family (F)	29	633,064	44.13 ^b
O • F	58	30,090	1.05
R • F	58	28,792	1.00
R • O • F	116	49,651	0.87
Initial Height	1	70,735	142.98 ^b
Error	3,365	1,664,704	

^aSignificant at the 5% level.

^bSignificant at the 1% level.

Table 3.7. Analysis of covariance of the change in diameter growth for 53 loblolly pine families grown in a 12 week field study in a factorial design using 3 levels each of acid rain and ozone

Source	df	Type III SS	F value
<u>Families 2-25</u>			
Block (B)	2	18.839	2.36
Ozone (O)	2	10.445	1.31
Rain (R)	2	1.440	0.18
R • O	4	12.825	0.80
B • R	4	2.793	0.18
B • O	4	4.548	0.29
B • R • O	8	31.880	16.50 ^a
Family (F)	22	89.113	16.70 ^a
O • F	44	9.162	0.86
R • F	44	9.230	0.87
R • O • F	88	16.842	0.79
Initial diameter	1	2.630	10.89 ^a
Error	2490	601.220	
<u>Families 26-55</u>			
Block (B)	2	9.486	1.27
Ozone (O)	2	11.742	1.58
Rain (R)	2	3.407	0.45
R • O	4	9.472	0.63
B • R	4	4.399	0.29
B • O	4	1.685	0.11
B • R • O	8	29.774	14.99 ^a
Family (F)	29	179.826	19.50 ^a
O • F	58	14.744	0.80
R • F	58	20.649	1.12
R • O • F	116	36.931	1.28
Initial diameter	1	11.926	48.05 ^a
Error	3426	850.349	

^aSignificant at the 1% level.

Table 3.8. Results of statistical analysis of differences in height and diameter growth associated with a 12-week exposure of field-grown loblolly pine seedlings to ozone only or ozone and acid rain

Data set	Effect ^a	Parameter	Numbers of families at significant level ^b		
			≥90	≥95	≥99
Ozone alone	Ozone	Diameter	16	9	2
		Height	27	20	8
Ozone x acid rain	Ozone	Diameter	15	10	3
		Height	5	2	1
	Acid rain	Diameter	10	6	1
		Height	13	6	2
	Ozone x acid rain	Diameter	13	6	2
		Height	16	11	3
Charcoal filtered vs ambient chambers	Ozone	Diameter	5	4	3
		Height	7	7	6

^aAnalyses of ozone alone effects were conducted across 5 ozone levels (CF, AC, A40, A80, and A160). Ozone x AR effects were similarly tested within a 3 x 3 factorial of 3 ozone levels (CF, A80, and A160) and 3 acid rain levels (pH 5.2, 4.5, and 3.3). All comparisons were tested with ANCOVA.

^bNumbers of families involved in the three data sets were 44, 53, and 44, respectively.

comparisons involved because the number of significant effects decreased as statistical comparisons were focused on successively smaller subsets of the larger experimental design. The percentage of families with significant differences (using a less rigorous significance level $\underline{p} \leq 0.1$) was highest in the ozone-only data set (36% height, 61% diameter), intermediate in the factorial experiment (28% height, 9% diameter) and lowest in an analysis (not presented here) of ambient air vs CF air in the ozone-only experiment (11% height, 16% diameter). The latter comparison provided the largest differences in growth relative to the CF baseline (Table 3.3), with a 26% mean reduction in height growth and consistent responses across families; however, these differences were not statistically significant for most families due in large part to the low power of detection.

There was no evidence of significant interactions for rain x family or rain x ozone x family. There was, however, a significant block x rain x ozone effect. Part of this interaction effect may have resulted from the way in which seedlings were originally placed in chambers within blocks. Analysis of variance of the initial measurements indicated that a small but statistically significant ozone x block effect was present even at the beginning of the experiments before treatments were applied. It is important to remember when interpreting those data that statistical significance was found for very small height differences. Overall mean initial diameters across families for the three blocks in the ozone-only experiment were 1.79, 1.77, and 1.89 for and 1.81, 1.77, and 1.82 mm for the acid rain x ozone study for blocks I, II, and III respectively. Initial heights were 78.3, 82.3, 84.0 and 85.3, 90.6, and 88.0 for these same comparisons. When dealing with large data sets grown under such condition and statistically with many degrees of freedom, very small differences can be found to be significant. Block effects were not found to be significant in the analysis of ozone effects (see Table 3.4).

Acid rain effects, both individually and in interaction with ozone, were statistically significant ($p \leq 0.1$) for approximately 20% of the 53 families tested. In general, height responses to acid rain and diameter responses to ozone were more easily detected within the factorial analysis.

3.2.1.7 Differences in Sensitivity Based on Seed Source

As noted earlier, one focus of these initial studies was to examine the variation in sensitivity of loblolly pine across its commercial range. The seed sources selected by the SCFRC represented fast-growing, commercially planted sources from 209 counties distributed across 12 states within the southeastern region. To determine whether systematic differences occurred within this area, families were separated into Coastal Plain and Piedmont sources of origin. Results given in Table 3.9 summarize the average differences in response to ozone in height and diameter and the frequency of statistically significant differences of families from these two zones and for the nine common families. The growth data are based on change in growth after the final measurements at the 12-week harvest, and growth responses in ambient and A160 chambers are presented as a percentage of that observed in CF chambers. Tests of significance are based on the individual family ANCOVA tests of an overall ozone effect ($p \leq 0.1$).

Results indicate that the Piedmont families were affected comparably to those from the Coastal Plain in ambient air (approximately 25% in height and 3-5% in diameter reduction). As ozone was increased to the A160 level, height reduction of Piedmont families was comparable to that in ambient air, but for Coastal families, the height response was reduced by approximately 0.5 to 12.0%. These differences in response to the higher ozone levels resulted in a much higher frequency of significance (64%) of the overall ozone effect among the Piedmont families. Diameter growth was not very different between regions and was at least 92% of the growth observed in CF chambers. Responses observed within the nine common families were less significant than those observed in either regional subgroup. Eight of

Table 3.9. A comparison of response to two ozone levels for loblolly pine families from the Coastal Plain and Piedmont areas of the southeastern United States and a summary of responses for common families tested in field chambers at ORNL

Seed source	Number of families	Mean growth response -- percentage of growth in CF air				Percentage of families with statistically significant responses to ozone levels (p ≤ 0.1)	
		Ambient		Ambient + 160 ppb		Height	Diameter
		Height	Diameter	Height	Diameter		
Piedmont	25	75.93	97.41	78.46	93.51	64.00	28.00
Coastal	28	77.65	94.52	88.62	92.16	39.2	32.1
Common	9	-	-	93.45	92.16	11.1	11.1

nine common families were Coastal Plain seed sources and their responses were included in that group's calculation.

A similar comparison was made to evaluate regional differences in response to acid rain as shown in Table 3.10. The growth data in this table are based on change in growth determined at the 12-week harvest and growth responses compared are for CF chambers with median rain pHs of 4.5 and 3.3 and (Table 3.10) as a percentage of growth observed in CF chambers with a rain pH of 5.2. The number of families with statistically significant differences ($p \leq 0.1$) in height in response to acid rain and the acid rain by ozone interaction is also given. Although there was a higher percentage of Piedmont families with statistically significant differences, the actual differences in growth between the seed sources in CF environments was small. Both height and diameter growth were consistently enhanced by a rain pH of 4.5 and depressed at pH 3.3.

3.2.1.8 Laboratory Studies

Height, diameter, and weight data

The laboratory studies with seedlings exposed in CSTR chambers provided the most complete data set because the height and diameter measurements were obtained on 5 dates, and all seedlings were harvested (one-half at 6 weeks and one-half at 12 weeks). As previously shown in Fig. 3.5, growth rates of seedlings in the CSTR experiments were generally slower than those under field conditions, and responses to added ozone were less significant. Comparisons of height, diameter, root-shoot ratio, and total weight responses to ozone of the eight families common to both CSTR and field experiments are shown in Table 3.11. These data indicate that height growth was the most variable response and was frequently positive, even at the 320 ppb level. Diameter responses were less variable among families; they were positive in 5 of 8 cases at 320 ppb. Responses in total plant weight were about equally divided between positive and negative at both 160 and 320 ppb and, on the average, were negligible across all families. The most consistent response observed, however, was the reduction in

Table 3.10. A comparison of responses to acid rain levels for loblolly pine families from the Coastal Plain and Piedmont areas of the southeastern United States and a summary of responses for the common families tested in field chambers at ORNL

Seed source	Number of families	Mean growth response percentage of growth in pH 5.2 rain				Percentage of families with statistically significant responses to rain pH levels ($p \leq 0.1$)	
		pH 4.5		pH 3.3		Height	Diameter
		Height	Diameter	Height	Diameter		
Piedmont	25	122.29	106.17	78.70	98.77	48	40
Coastal	28	119.83	106.49	81.22	96.80	39	32
Common	9	119.75	100.15	77.28	91.85	33	11

root-shoot ratio that occurred in about 75% of the families. This reduction occurred frequently when height or diameter responses were positive; therefore in many cases in the CSTR studies, it appeared that decreased root growth or a diminished balance between root and shoot growth may have been the predominant negative response to ozone additions under the CSTR exposure conditions.

Growth trends

Diameter growth trend data for Families 4 and 9, which showed the most negative responses, and Family 5, with the most noted weight stimulation by ozone exposure under CSTR conditions, are shown in Figure 3.14 (a), (b), and (c). Family 4 [Fig. 3.14(a)] grew more slowly with increasing ozone, while Family 5 [Fig. 3.14 (b)], as was previously shown with diameter trends for Family 8 (Fig. 3.5), showed the opposite response. Family 9 [Fig. 3.14 (c)] was the fastest growing family and had a response similar to Family 4. Some common features of the timing of these responses are apparent. For all families, the ozone-treated seedlings began to grow at an altered rate after three to six weeks of exposure. Differences generally increased after that time. These observed growth trends can be more meaningfully evaluated in relationship to the final harvest weights reported in Table 3.11. The diameter trends shown in Fig. 3.14 indicate generally good agreement with final seedling weight responses to ozone recorded in Table 3.11 for Families 4, 5, and 9. Overall, the observed growth response trends indicate that response thresholds do exist as a function of total time of exposure and that observed effects would likely have further increased with extension of the length of the experiment.

Statistical analysis

The model for analysis of covariance in the laboratory study was

$$y_{ijkl} = O_i + R(O)_{j(i)} + F_k + (O \cdot F)_{ik} + [F \cdot R(O)]_{kj(i)} + C_{ijkl} + e_{ijkl} ,$$

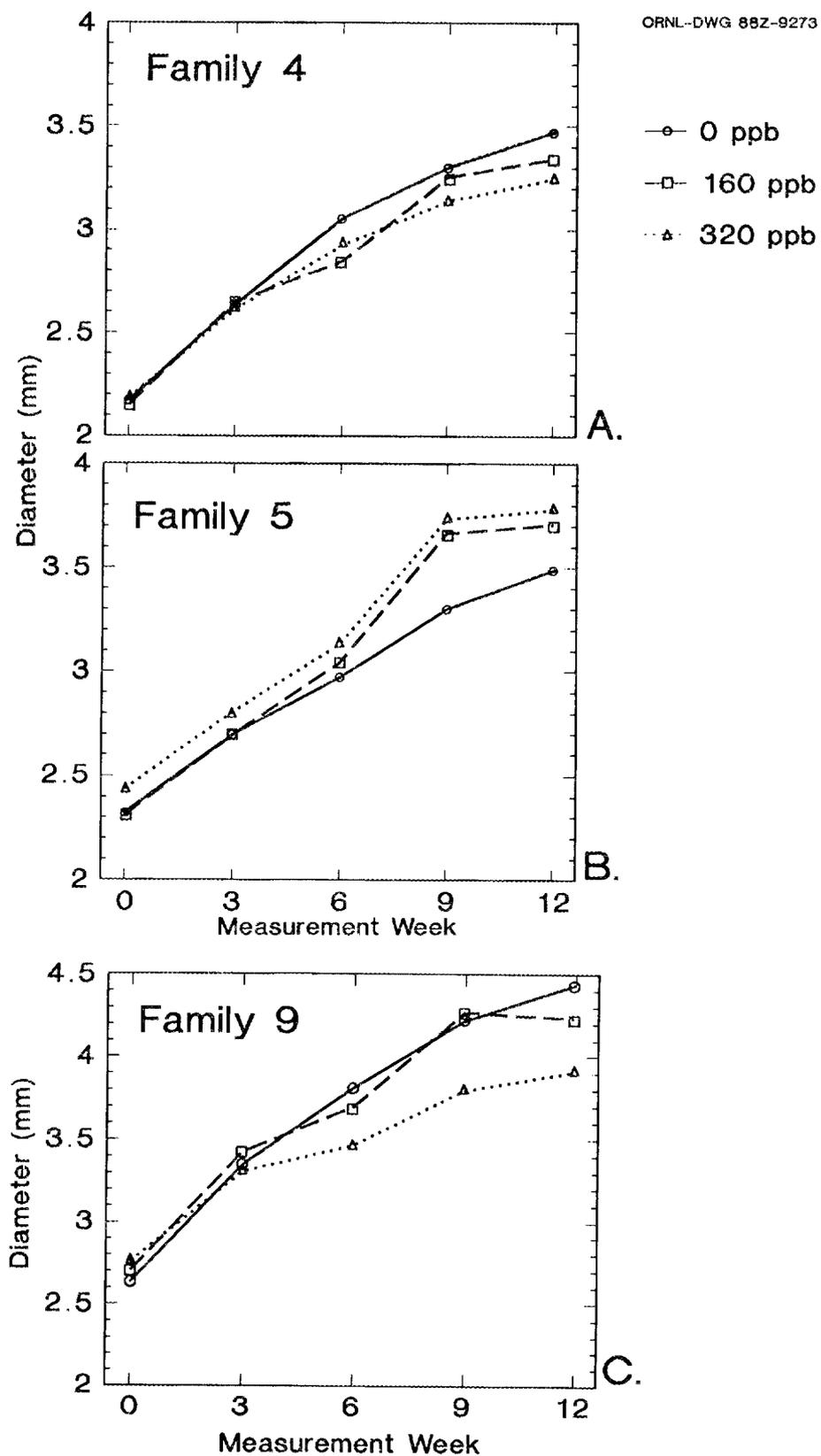


Fig. 3.14. Diameter growth of (a) Family 4, (b) Family 5, and (c) Family 9 seedlings grown in CSTR chambers with 0, 160, and 320 ppb ozone.

Table 3.11. Mean responses of 8 loblolly pine families exposed to ozone at 160 ppb or 320 ppb for 12 weeks in CSTR chambers. Data compare responses at each ozone level to responses from CF chambers

Family	Percentage Change from CF at Harvest							
	160 ppb				320 ppb			
	Height	Diameter	R:S ^a	Weight	Height	Diameter	R:S	Weight
2	+52	+11	-8	-6	+66	+10	-11	+7
3	-4	-1	+2	-3	+33	+4	-10	+9
4	-21	-1	-6	-13	-22	-18	-11	-16
5	-9	+16	-4	+10	+20	+1	+4	+11
6	+78	+10	-7	+4	+38	-8	-13	-5
8	+49	+28	-3	-3	+60	+12	-3	+1
9	-10	-21	-3	+3	-26	-30	+6	-6
10	-22	+11	-6	-5	+2	+5	-17	+4
Mean response	+14	+7	-5	-2	+21	-3	-7	+1

^aRoot:shoot ratio.

where O = ozone, R(O) = replication within ozone, F = family, C = covariate, and e = error.

Analysis of covariance of height and diameter growth data at harvest (Table 3.12) indicates that there was no significant overall ozone treatment effect, either individually or in interaction with families. Pseudo-f tests were performed (Hicks 1973), and the only significant variable was family. Separate tests of significance for individual families (Appendix B) revealed that only Family 9 showed a slightly significant negative response ($p \leq 0.09$) in diameter growth, and there were no significant responses in height growth. The powers of the tests were low and limited the ability to detect significant differences.

3.2.1.9 Field And Laboratory Comparisons

Because the principal objective of these studies was the comparison of testing methodologies, the differences in response of the common families between field and laboratory protocols was of major interest. While it was apparent from measured growth patterns that trees were more sensitive to ozone under field conditions, it was important to know whether such differences were caused by differences in the toxicity of applied dose due to the concentration patterns (kinetics of exposure or presence of ambient background levels during respites) or to differences in the growth patterns of the seedlings themselves.

To examine relative response patterns of the eight common families under the two test regimes, treatment effects on total plant weight at harvest (expressed as weight ratios of ozone-treated seedlings vs CF control) were compared for the two highest ozone treatments in each regime (Fig. 3.15). Responses to A80 (field) and 160 ppb (laboratory) are shown in Fig. 3.15(a), and responses to the highest field and laboratory concentrations (A160 and 320 ppb) are shown in Fig. 3.15(b). The patterns shown in these figures indicate that family-to-family variations in growth responses to ozone were generally similar between laboratory (CSTR) and field exposed seedlings. The observed pattern

Table 3.12. Analysis of covariance of the change in growth of loblolly pine families during a 12-week period in response to 3 levels of ozone

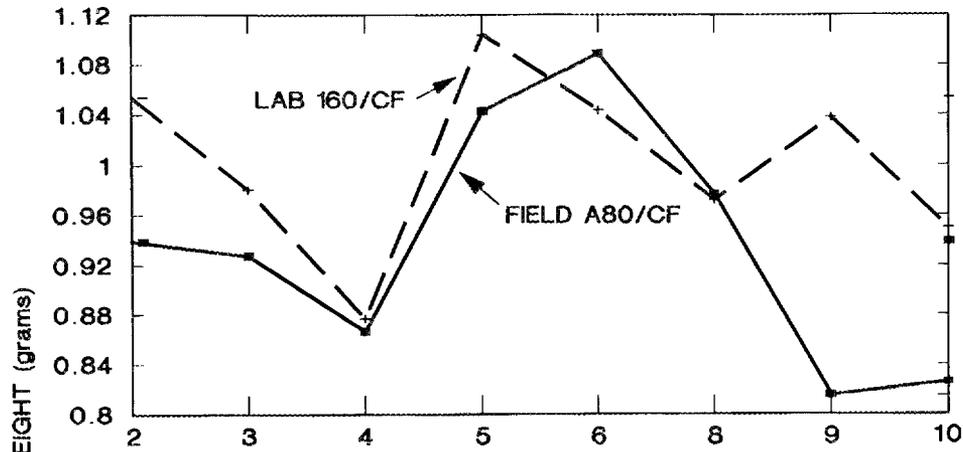
Source	df	Type III SS	F value
<u>Height</u>			
Ozone (O)	2	147	0.18
Rep(O) ^a	9	942	0.35
Family (F)	7	49,677	17.66 ^b
O • F	14	5,623	1.34
F • Rep(O) ^a	63	13,577	0.72
Initial height ^c	1	1,027	3.42
Error	89	26,702	
<u>Diameter</u>			
Ozone (O)	2	0.388	0.80
Rep(O)	9	1.824	1.80
Family (F)	7	6.733	6.35 ^b
O*F	14	2.120	1.34
F*Rep(O)	21	7.087	1.09
Dia ^b	1	2.310	22.39 ^b
Error	93	9.596	

^aRep(O) = replication within ozone treatments.

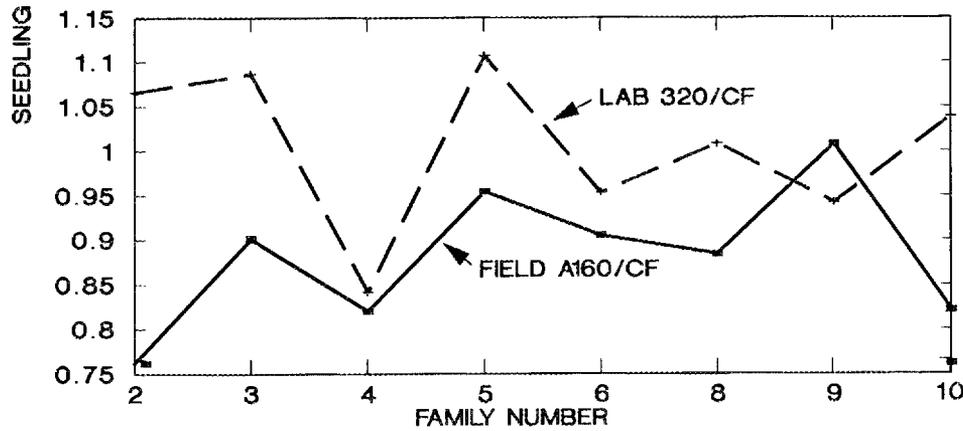
^bSignificant at the 1% level.

^cCovariate.

fig 15



Low Ozone Level



High Ozone Level

Fig. 3.15. Comparisons of the weight response to ozone of common loblolly pine families for the field and laboratory studies for (a) low- and (b) high-ozone levels.

supports similar relative sensitivity between these families in the field and laboratory situations, even though the degree of inhibition of growth was much less in the laboratory.

The most notable exception to this general pattern was Family 9, which was most sensitive to ozone under CSTR exposure conditions. It is interesting that this is the only family showing a faster growth rate in the CSTR chambers than in the field.

An examination of the sensitivity of seedling root-shoot ratios is included in Fig. 3.16 to evaluate similarity of ozone effects on whole plant allocation of dry matter. Comparative plots of responses to lower [Fig. 3.16(a)] and higher [Fig. 3.16(b)] levels of added ozone show that at lower ozone levels root-shoot ratios were on the average more consistently depressed in the laboratory. At higher ozone levels, response patterns among families were more comparable between the laboratory and field, indicating similar relative sensitivity at these generally lower root-shoot ratios. At the highest ozone level, root-shoot ratios of field-grown seedlings were shifted relatively more downward than those for laboratory-grown seedlings.

3.3 DISCUSSION

Statistical analysis of these data indicated that ozone affected growth of trees differently in the 53 families in both the level of response and the shape of the response surface. The variability was sufficiently large and the power of the tests to detect was low so that the response to ozone of loblolly pine as a species was not significant at the 95% significance level. However, there were notable trends related to seed source observed across families including a substantial and rather consistent reduction in mean height growth (26%) in ambient air, stimulation of growth at lowest ozone additions, further reduction of growth by further increasing ozone levels, and a generally greater sensitivity of height than diameter responses. Effects of ozone on height were significant for about 40% of the families, and significant diameter effects were found on approximately 15% of those families tested.

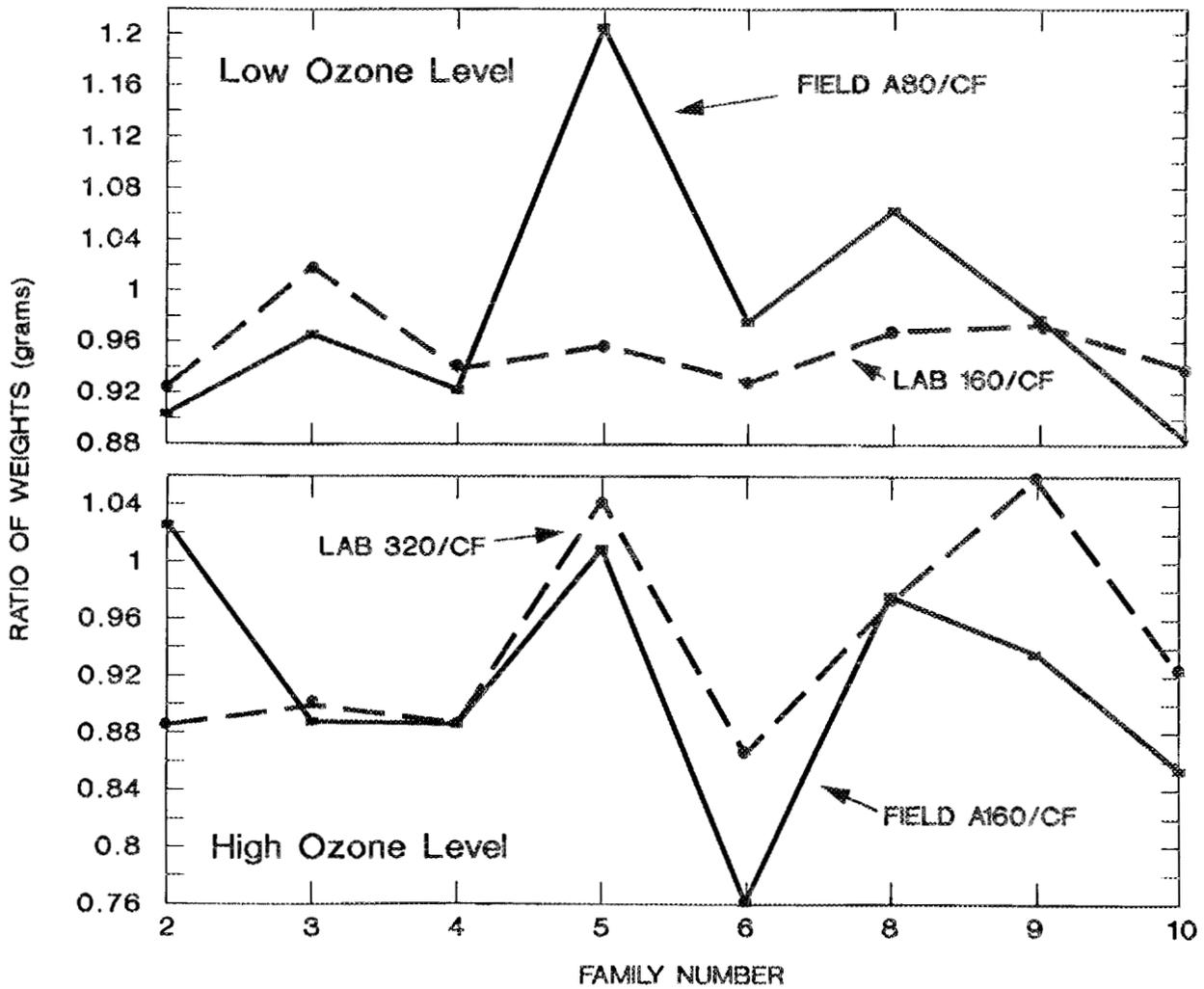


Fig. 3.16. Comparisons of the root:shoot ratio response to ozone of common loblolly pine families for the field and laboratory studies for (a) high- and (b) low-ozone levels.

The lack of a linear dose response surface and the evidence of stimulation of height growth by ozone addition at lower levels [Fig. 8(a)] cannot be explained at this time. Stimulation of growth by low levels of ozone has been reported occasionally in the literature (EPA 1984). In this study we hypothesize that the manner in which ozone was added, a rather abrupt increase to desired levels over a 45-min period, may have induced partial closure of stomates during the period of addition. The effects of such a closure would be twofold: (1) restriction of entry of ozone and CO₂ through stomates and (2) reduced loss of water in transpiration. The net effect of such a process would be dependent on the balance between the effects of any additional ozone influx at higher external ozone doses, reduced carbon influx in photosynthesis caused by stomatal stricture, and, possibly, beneficial effects of water conservation. The fact that there were typically 5 or more hours of daylight after the termination of ozone additions at around 1530 h suggests that post-fumigation compensatory photosynthetic uptake under generally lower ambient levels of ozone and more optimal temperature and moisture conditions might favor diminishment of the effects of added ozone. The period of time during which ozone was being added to test plants represents only approximately 25% of the total daylight hours such that hypothetically, even if stomates were completely closed during this time and reopened immediately after exposure, only 25% of gross photosynthetic production would be lost. In actuality, total closure and immediate return to full opening would be unlikely, and compensatory increases due to altered source sink relations (McLaughlin and Shriner 1980) would probably occur. Closure of stomates by high levels of ozone is well documented in the literature (Mansfield and Majernik 1984, Rich and Turner 1972). Recent research at our lab by Taylor *et al* (1988 submitted) indicates that stress ethylene produced by exposure of plants to ozone plays a major role in inducing observed reductions in net photosynthesis and transpiration. The fact that ethylene is relatively more effective in inhibiting transpiration than reducing photosynthesis (Gunderson *et al* 1988 submitted) supports the possible

role of our mid-day exposures in the field causing potentially favorable shifts in water status at the cost of relatively less significant reductions in net photosynthesis.

One concern in evaluating responses of plants grown in charcoal filtered and ozonated air is possible differences in nitrogen nutrition induced by the influence of the ozonator on NO_x levels in the supply air. Some ozonating systems can produce a mixture of nitrogen oxides by induction of reactions between nitrogen in the supply air and the ozone generated (Harris et al, 1982). When ambient air, such as we used, is the supply air, this can be more of a problem than when pure oxygen is used. Ozone-induced nitrogen oxide formation, however, cannot be considered a significant factor in these experiments for two principal reasons. First, the amount generated would be greatly diluted by the mixing required to produce final chamber ozone concentrations. Based on published generation rates (Harris et al, 1982), it can be calculated that only about 1 ppb of NO_x would have been added to ambient levels of about 8 ppb in our area by ozonation. Second, the foliar nutrient levels reported in Section 5 indicate that nitrogen levels were actually lower in the foliage of ozone treated seedlings than in those grown under charcoal filtration.

The predominant effect of simulated acid rain in these studies was on height growth, as was noted with ozone. Height growth was typically stimulated at near ambient pH levels and reduced by an average of 19% at pH 3.5 when seedlings were grown in CF air. Sensitivity to acid rain was not significantly influenced by family origin; however, there was a significant overall interaction between ozone and acid rain for some families, and this influence did vary significantly across families.

The antagonistic interaction between acid rain and ozone noted in this study is interesting because it implies a physiological inhibition of the phytotoxicity of these pollutants when they occur together at high levels. The physiological basis of this interaction cannot be ascertained at this time; however, the addition of nitrogen in acid rain may be beneficial to plants stressed by ozone if reduced transport

of carbon to the root system is one of the significant adverse effects of ozone (McLaughlin and McConathy 1983). The influence of acid rain and ozone on photosynthesis, carbon allocation, and foliar nutrition are discussed in later sections of this document.

The consistent response of families to ambient levels of ozone is one of the most interesting results of this study. This may be relevant to the measured slowdown of radial increment that has been reported for southern pines based on analyses of continuous forest inventory data by the U.S. Forest Service (Sheffield and Cost 1987). Analyses of data from continuous inventory plots across a wide diversity of sites within the region show that diameter growth of southern pines has dropped by 30-50% below expectation across the region. Earlier reports indicated that the radial slowdown was more pronounced in the Piedmont region (Sheffield and Knight 1983) than in coastal areas. However, these more recent analyses indicate a similar overall magnitude of reduction during the last three decades (Sheffield and Cost 1987). More recent analyses of the role of climate and competition in the observed growth responses indicate that a substantial portion ($\geq 10\%$) of the observed decline cannot be explained by these variables (Zahner and Meyers 1987). Such evidence provides inferential support but does not prove the involvement of atmospheric pollution in the observed growth declines.

Reductions in growth of loblolly pine seedlings grown in open-top chambers have been reported for loblolly pine in this region. Shafer et al. (1987) estimated that growth of loblolly pine would be reduced by 10% in ambient air. This estimate was based on ozone-dose-response relations derived from studies with four open-pollinated families that were exposed to ozone in open-top chambers near Raleigh, North Carolina. Shafer et al grew their seedlings directly in the soil, whereas, in the present study, the use of seedling containers placed aboveground in either the greenhouse or field setting raise questions about possible changes in sensitivity that might be induced by increased soil temperatures associated with seedling containers. For this reason, we conducted a second-year study in which nine seedlings

from each of four sensitive families and two resistant families were grown directly in the soil in three charcoal-filtered and three non-filtered chambers. These seedlings, which were from the same initial group planted in a CF greenhouse the previous year, were allowed to grow in ambient air in all plots for 80 days before chambers were installed in mid-July. During the following four months, those seedlings growing in ambient air experienced an approximate 20% reduction in diameter growth and a 10% reduction in height growth relative to the CF controls. These growth reductions indicated a generally greater sensitivity of diameter growth than height growth for these 1-year-old seedlings compared to mean responses (8% diameter and 20% height suppression) observed during the first year with these same families grown as containerized seedlings. These data, which will be discussed more completely in a subsequent report, support the validity of both the experimental protocol and the results of the larger study during the first year.

The primary uses of growth and sensitivity data from this study are twofold: (1) as an indicator of relative sensitivity across loblolly pine families and within loblolly pine as a species and (2) as a measure of expected responses for the larger trees in the field. Our results indicate that there are substantial differences between families in sensitivity to both ozone and acid rain. Comparisons of sensitivity rankings for the nine common families involved in the interlab studies indicate that the sensitivity of seedlings to ozone in the ORNL studies was lower than that observed at either the USDA/North Carolina State or Texas A&M sites (Dick Rheinert and Frank Fong, personal communication). The relative sensitivity between sites was comparable. This leads us to suggest that intersite comparisons may provide useful data for evaluating relative sensitivity among families; however, these comparisons must be evaluated carefully in terms of estimating actual levels of response. The generally good agreement between the growth responses observed for our seedlings between first- and second-year studies and the previous results of Shafer et al. (1987) support the validity of responses we measured in ambient air.

In the evaluation of growth responses of mature trees to ambient ozone levels, differences in carbon allocation strategies between seedling and mature trees would be a major factor in limiting direct extrapolation. However, the seedling growth stage is an important growth stage for all forest trees, and alterations in the rate or characteristics of growth at this stage may have major economic or ecological consequences later in the growth cycle, even if larger trees are relatively resistant to pollution stress.

3.4 SUMMARY AND CONCLUSIONS

These studies have examined growth and physiological responses of 53 families of loblolly pine selected from among families planted in significant quantities across the southeastern United States. As expected, there were large differences in inherent growth rates among these families and in their responses to ozone and acid rain. Analyses of growth and physiological responses of these families to acid rain and ozone have led to the following principal conclusions:

1. Exposure to ambient air, in which ozone is the principal phytotoxic component, reduced average height (-26%), diameter (-5%), and volume (-14%) growth compared to growth of seedlings exposed to a 50% lower dose as a result of charcoal filtering supply air.
2. Increasing ozone levels above those in ambient air resulted in growth responses that were occasionally stimulatory at the lowest level, varied widely between families, and although they became increasingly inhibitory at the highest levels, did not significantly exceed growth reductions found in ambient air.
3. Acid rain caused a general stimulation of height growth and reduction of diameter growth across families at ambient levels (pH 4.5) while both height and diameter growth were reduced at a median pH of 3.3.
4. Significant interactions between rainfall acidity and

ozone were detected principally in responses of height growth. In general, acid rain effects were greatest in CF air and decreased as the level of ozone increased.

5. Generally seedlings were more sensitive to changes in both growth and physiology following ozone exposure in field experiments than when exposed in the laboratory.

Collectively, these studies indicate that adverse growth responses of loblolly pine seedlings to ambient levels of atmospheric ozone are very likely but will be strongly dependent on genetic variations associated with seed source and on associated levels of acid rain. Responses to ambient levels of acid deposition are likely to be much more complex and may involve growth stimulation, particularly in height. Ozone and acid rain interactions appear likely, but in most cases, the influence of combined exposures was antagonistic rather than additive or synergistic.

The more obvious growth responses observed in the field compared to results of laboratory studies argue for increased emphasis on field work in the future. A major difference between the lab and field studies may have been the influence of continuous low-level exposures that occur between ozone additions in the field. This "respite dose" contributed approximately 40% of the total daytime dose received at the highest ozone treatment level in the field (see Table 2.4) and may well have played a major role in responses observed. The influence of respite dose (Section 1) should be examined much more closely in future studies.

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4. NET CARBON DIOXIDE EXCHANGE CHARACTERISTICS OF PINUS TAEDA L. SHOOTS

P. J. Hanson and S. B. McLaughlin

4.1 INTRODUCTION

Evidence of regional changes in growth and vigor of some species of forest trees in both Europe and the eastern United States has accumulated in the past 5 years (McLaughlin 1985). In the United States unexpected changes in radial growth rate and mortality have been noted in red spruce (Picea rubens Sarg.) growing at high elevations in the Appalachian mountains (Johnson and Siccama 1983; McLaughlin et al. 1987). At lower elevations, recent reductions in radial increment have been observed in shortleaf pine (Pinus echinata Mill.) in East Tennessee (Baes and McLaughlin 1984), which coincide in time with shifts to slower growth by red spruce at high elevations. In addition, analysis of forest inventory data from permanent survey plots by the U.S. Forest Service has revealed an unexplained decline (approximately 25% during the past decade) in softwood growth in the piedmont regions of South Carolina (Tansey 1984) and Georgia (Sheffield and Knight 1984). Sheffield and Cost (1987) analyzed the decline evidence for natural pine forests in the southeastern United States but were unable to attribute the decline to any single causal factor.

The recent reductions in growth rate have been suggested to result from a combination of stress factors (i.e., drought, pathogens, anthropogenic pollutants) or from reductions in site fertility resulting from years of intensive management. Atmospheric pollutants have been implicated as causative agents for growth reductions in coniferous forests in the United States and Europe (McLaughlin 1985). Because ozone is known to reduce growth and productivity of many plant species (Reich and Amundson 1985; Heck et al. 1984) and is present on a regional scale (Pinkerton and Lefohn 1987; Taylor and Norby 1984), it is thought to be a contributing cause of the declining growth rates of pines in the southern United States (McLaughlin 1985). Anthropogenically altered precipitation ("acid rain") is also found on

a regional scale and thought to have the potential to affect plant growth (Johnson and Siccama 1983).

Air pollutants have been shown to directly affect the CO₂ exchange rate [CER (in $\mu\text{mol g}^{-1} \text{s}^{-1}$)] of many forest tree species (Reich and Amundson 1985), and altered CER (i.e., photosynthetic and respiratory processes) can be expected to affect dry matter accumulation of plants. Because CERs represent both primary carbon fixing and respiratory processes in plants, CER should be a sensitive indicator of changing plant health. The objective of the current study was to investigate the effects of ozone and rain chemistry on the photosynthetic and respiratory processes of loblolly pine (Pinus taeda L.) seedlings from two half-sib families.

4.2 MATERIALS AND METHODS

4.2.1 Plant Material and Exposure Conditions

Loblolly pine seedlings were obtained from a parent study conducted during the late summer and fall of 1986 (Sect. 2). The seedlings were grown from seed in a charcoal-filtered greenhouse under well-watered and fertilized conditions for approximately 6 months prior to beginning the experimental treatments. In the parent study, various treatment combinations of ozone (O₃) and rain chemistries were applied to loblolly pine seedlings from 51 different seed sources under field conditions (field study) and to seedlings from 8 of the 51 families in the laboratory (lab study). We used seedlings from two families: "family 8" from Gates County, North Carolina (Weyerhaeuser Company 8-80), and "family 9" from Beaufort County, North Carolina (Weyerhaeuser Company 8-130).

All field exposures were made in open-top chambers outfitted with sliding covers for the exclusion of ambient rain (Johnston et al. 1986), and laboratory exposures were executed in continuously stirred

tank reactors (CSTRs) (Taylor et al. 1983). Laboratory-treated seedlings were grown in a charcoal-filtered greenhouse during all respite periods between exposures in the CSTRs.

Detailed information on the application of ozone and rain treatments is given in Sect. 2. Field application of elevated ozone treatments varied from week to week (e.g., no additions during natural rain events), but, during a typical week, exposures were provided 4 d/week from 0930 to 1530. Laboratory ozone exposures were also conducted 4 d/week at the same time. Rain chemistries having a sulfate:nitrate ratio of approximately 2:1 were applied weekly in 1-cm events.

Field seedlings from combinations of ozone (charcoal filtered = 14 ppb ozone, or ozonated = 167 ppb ozone) and rain pH treatments (pHs of 3.3, 4.5, and 5.2) and lab seedlings exposed to pH 4.3 rain and three levels of ozone (0, 160, and 320 ppb) were measured after 6 and 12 weeks of exposure. The 167 ppb field ozone treatment was 3 to 4 times mean ambient air concentrations of ozone at the field site; and rain pHs of 3.3, 4.5 and 5.2 approximated rain from polluted, ambient, and pristine environments, respectively. Actual ozone concentrations applied, mean ozone concentrations during exposures, total dose data (ppm.h), and information on exposure durations for treatments used in our study are summarized in Table 4.1.

4.2.2 Measures of Carbon Dioxide Exchange Rates

Following 6 or 12 weeks of exposure to their respective treatments, whole-shoot CER, were measured as a function of photosynthetic photon flux densities (PPFDs) on seedlings from the field and lab studies. Measurements of CER-PPFD relationships were made during a 1-week period following the last ozone treatment. One seedling from each treatment was measured daily in a random order (3 or 4 seedlings per day), until all seedlings had been utilized (3 or 4 seedlings per family-treatment combination). Whole-shoot CERs were

Table 4.1. Ozone exposure data by substudy and treatment. Dose levels at 6 weeks were approximately half of those listed below.

Treatment	Experiment duration (d)	Number of exposure (d)	Total daytime exposure (h)	Daytime respite duration (h)	Mean O ₃ exposure level ppb ^a	Mean O ₃ respite level ppb ^a	Total O ₃ dose ppm.h
<u>Field study^b</u>							
CF ^b	96	49	328	833	14 ± 9	14 ± 9	19
Ozonated	96	49	328	833	167 ± 41	26 ± 17	79
<u>Lab study^d</u>							
0 (nLl ⁻¹ O ₃)	86	49	294	720	14 ± 9	14 ± 9 ^e	14
160 (nLl ⁻¹ O ₃)	86	49	294	720	160 ± 15	14 ± 9	57
320 (nLl ⁻¹ O ₃)	86	49	294	720	320 ± 30	14 ± 9	104

^aExposure O₃ levels are listed as the mean ± standard deviation.

^bField exposures were normally applied for 6 h (0900 to 1500) on weekdays when no precipitation was taking place.

^cCF = charcoal-filtered chamber.

^dLab exposures were made 6 h/d 4 times per week, independent of external weather conditions.

^eAssumes charcoal filtration in the laboratory/greenhouse environment is equally efficient as in the field chamber system.

measured in an open infrared gas analysis system (Koch et al. 1968) at six descending levels of PPF_D (1600, 800, 410, 60, 33, and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Four high-pressure sodium vapor lamps (General Electric, Lucalox - LU400/BU) were used to illuminate the plants during measurements, and combinations of neutral density filters (Lee Filters, Andover England, #209, 210 and 211) were used to produce the range of PPF_Ds. Shoots were sealed in the cuvette of the gas analysis system between 0830 and 0930 hours eastern daylight time and allowed to acclimate under a PPF_D of 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ before starting subsequent CER measurements. Calculations of CER in units of $\mu\text{mol CO}_2 \text{ g}^{-1} \text{s}^{-1}$ were made as described by Long and Hallgren (1985) using total needle mass to normalize data between seedlings. Cuvette temperatures were $25 \pm 2^\circ\text{C}$ for all measurements. Needle temperatures, measured with a hypodermic thermocouple inserted into the needle, were within $2 \pm 1^\circ\text{C}$ of cuvette temperatures. CO_2 concentrations of air exiting the cuvette (i.e., the effective ambient CO_2 concentration) were between 330 and 350 ppm. Dew points of the air entering and exiting the cuvette were monitored (EG&G Model 660 dew point hygrometer), and the data were used to calculate leaf diffusive resistance to water vapor (Long and Hallgren 1985). Air flow through the cuvette was maintained at approximately 10 L/min. Approximately 2 h was required to generate a single CER-PPF_D curve for each shoot. Dry weight of needles was obtained after drying the needles for 2 d at 65°C .

Estimates of light-saturated CER [P_{max} (in $\mu\text{mol CO}_2 \text{ g}^{-1} \text{s}^{-1}$)], light compensation point [LCP in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$], and CER at zero PPF_D [dark respiration (R_d) in $\mu\text{mol CO}_2 \text{ g}^{-1} \text{s}^{-1}$] were obtained from the following equation [modified from the original (Hanson et al. 1987)] and nonlinear regression techniques:

$$\text{CER} = P_{\text{max}} \left[1 - \frac{(1 - R_d/P_{\text{max}})^{xx}}{(1 - \text{PPFD}/\text{LCP})^{xx}} \right] \quad (1)$$

The parameter "xx" in the equation is a constant that allows a better fit to the "whole-shoot" data. The regressions were run on the pooled data from seedlings of an individual treatment.

4.2.3 Statistical Analysis

CER-PPFD relationships of seedlings between treatments were compared using an F test that is approximate for nonlinear situations (Hanson et al. 1988). The comparison tests for differences in shape of the CER-PPFD response surface. Dry weight data were analyzed with a one-way analysis of variance.

4.3 RESULTS

4.3.1 Field Study

Compared with plants exposed to charcoal-filtered air, ozone-treated family 8 seedlings exhibited mean reductions in CER at saturating PPFD ($2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) of 12.5 and 25% for plants measured at 6 and 13 weeks, respectively (Fig. 4.1). At 6 weeks, the CER-PPFD relationships were not significantly influenced by the rain chemistry treatments (Table 4.2). However, after 12 weeks, pH 3.3 and 4.5 rain treatments enhanced mean CER at saturating PPFD by an average of 52% over that observed for seedlings exposed to the pH 5.2 treatment (Fig. 4.2). It should also be noted that CER decreased by up to 50% in the field and 40% in laboratory studies between the 6 and 12 week measurements. No differences in Rd or LCP were detected between any treatments (Figs. 4.1 and 4.2; Table 4.3). Water vapor exchange observations taken during measurement of CER-PPFD relationships showed no statistically significant treatment-related changes in stomatal conductance (data not shown).

4.3.2 Lab Study

Family 8 seedlings exposed to 320 ppb ozone showed a 12% reduction in CER at saturating light after 6 weeks but not after 13 weeks, and family 9 seedlings showed the opposite response -- no reductions at 6 weeks but a 14% ozone induced reduction in CER at 12 weeks (Tables 4.2 and 4.3). No differences in Rd or LCP were detected between any treatments (Table 4.3).

Whereas the lab seedlings exhibited few significant responses to ozone, family CER-PPFD characteristics did vary with time. No

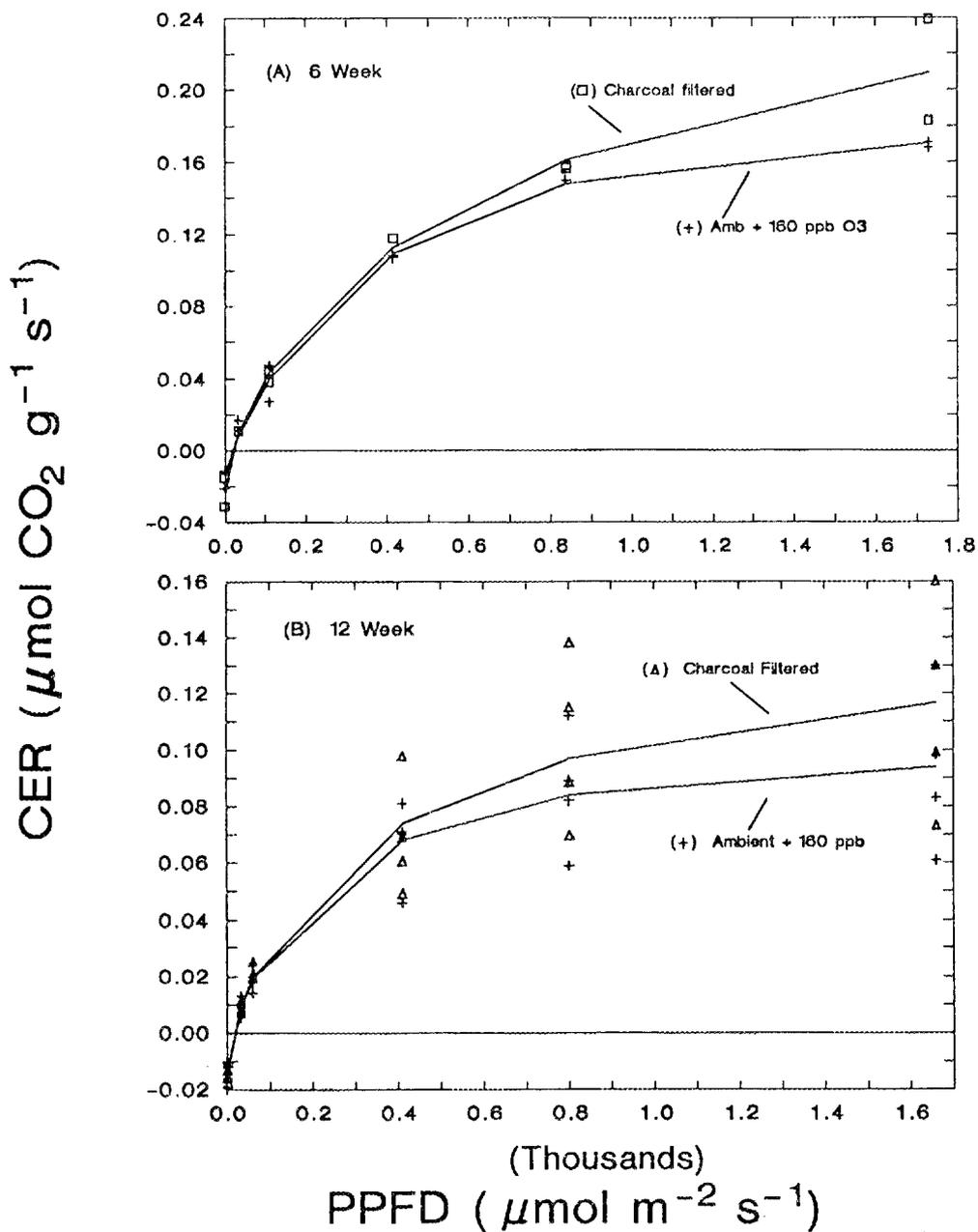


Fig. 4.1. Mean CER-PPFD relationships of control and ozone-treated "field" seedlings after 6 or 12 weeks of exposure. Each fitted curve represents the mean of 2 or 4 seedlings from family 8.

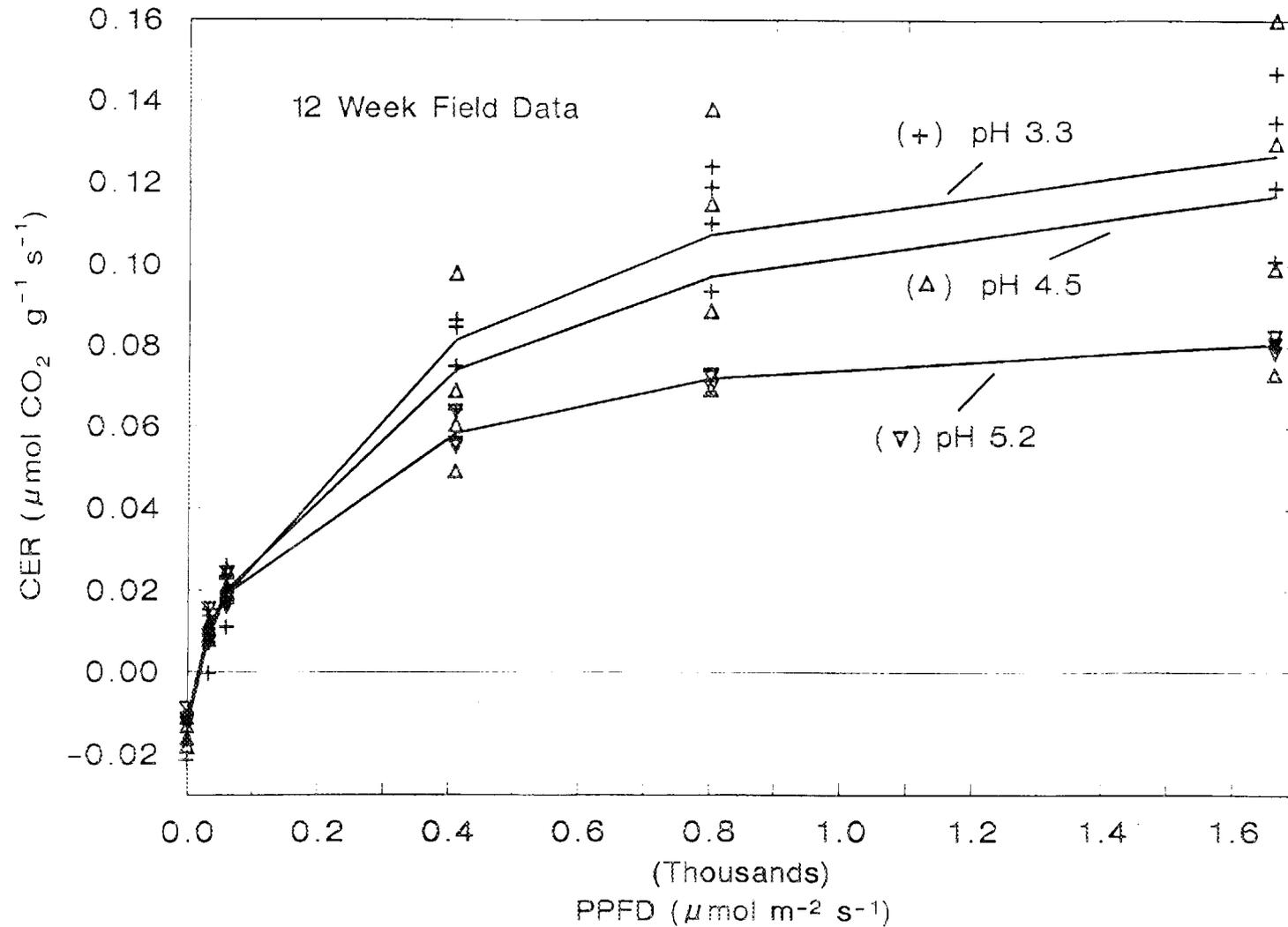


Fig. 4.2. Mean CER-PPFD relationships of "field" seedlings exposed for 12 weeks to charcoal-filtered air and rain chemistries of pH 3.3, 4.5, or 5.2. Each curve represents the mean of 3 or 4 seedlings from family 8. Curves for pH 3.3 and 4.5 are each differ from the curve for the pH 5.2 treatment (Table 4.2).

Table 4.2. Statistical significance of paired comparisons between the CER-PPFD response surfaces of seedlings exposed to the indicated O₃ and/or rain pH treatments.

Treatments compared	F-statistic df(x,x) = F	P-value	Percent change in Pmax ^a
<u>Field study (Family 8)</u>			
6 weeks			
CF ^b vs Ozonated			
pH 3.3	(4,16)=3.21	0.041	-16
pH 5.2	(4,16)=2.74	0.065	- 9
5.2 vs 3.3 pH			
CF	(4,16)=1.64	0.213	+12 (ns?)
Ozonated	(4,16)=0.48	0.750	ns
12 weeks:			
CF vs Ozonated	(4,64)=3.24	0.017	-25
4.5 vs 3.3 pH	(4,40)=0.45	0.772	ns
5.2 vs 3.3 pH	(4,34)=21.47	0.000	+46
5.2 vs 4.5 pH	(4,34)=3.80	0.012	+57
<u>Lab study (Families 8 and 9)</u>			
6 weeks:			
Family 8			
0 vs 160 ^c	(4,28)=1.02	0.414	ns
0 vs 320	(4,28)=6.84	0.001	-12
160 vs 320	(4,28)=6.30	0.001	- 8
Family 9			
0 vs 160	(4,28)=0.70	0.600	ns
0 vs 320	(4,28)=0.15	0.960	ns
160 vs 320	(4,28)=1.01	0.420	ns
Family 8 vs 9			
0	(4,28)=0.33	0.855	ns
12 weeks:			
Family 8			
0 vs 160	(4,28)=0.06	0.990	ns
0 vs 320	(4,28)=0.16	0.957	ns
160 vs 320	(4,28)=0.08	0.988	ns
Family 9			
0 vs 160	(4,28)=1.83	0.151	-14 (ns?)
0 vs 320	(4,28)=1.74	0.169	-14 (ns?)
160 vs 320	(4,28)=0.08	0.988	ns
Family 8 vs 9			
0	(4,28)=12.28	0.000	+51

^aThe percentage of change of CER under light saturation (Pmax; PPFD = 2000 μmol m⁻² s⁻¹) is provided to indicate the direction of the treatment effect.

^bCF = charcoal-filtered

^c0, 160, 320 = XXXppb O₃

Table 4.3. Predicted-light saturated CER (P_{max}), light compensation point (LCP), and dark respiration (Rd) obtained from nonlinear regressions of 3 to 4 plants per treatment.

Treatment Family No.	P_{max}		LCP		Rd	
	$\mu\text{mol g}^{-1} \text{s}^{-1}$		$\mu\text{mol m}^{-2} \text{s}^{-1}$		$\mu\text{mol g}^{-1} \text{s}^{-1}$	
	8	9	8	9	8	9
<u>Field study</u>						
6 weeks ^a						
CF - pH 3.3	0.266		19		-0.023	
CF - pH 5.2	0.188		15		-0.012	
160 O ₃ - pH 3.3	0.177		19		-0.013	
160 O ₃ - pH 5.2	0.173 (0.059) ^b		20 (16)		-0.020 (0.015)	
12 weeks						
CF - pH 3.3	0.136	na	17	na	-0.013	na
CF - pH 4.5	0.132	na	14	na	-0.014	na
CF - pH 5.2	0.084	na	10	na	-0.011	na
160 O ₃ - pH 4.5	0.097 (0.033)	na (na)	16 (15)	na (na)	-0.014 (0.013)	na (na)
<u>Lab study</u>						
6 weeks						
0	0.187	0.192	9	10	-0.013	-0.013
160	0.177	0.170	9	10	-0.013	-0.010
320	0.174 (0.019)	0.180 (0.030)	3 (5)	9 (11)	-0.008 (0.008)	-0.013 (0.016)
12 weeks						
0	0.107	0.163	16	19	-0.015	-0.021
160	0.115	0.141	17	17	-0.018	-0.017
320	0.116 (0.034)	0.142 (0.027)	14 (24)	17 (16)	-0.016 (0.026)	-0.022 (0.020)

^aField data at 6 weeks represent the mean of families 8 and 9.

^bThe value in parentheses subtending a column of numbers is the mean one-sided 95% confidence interval for those treatments.

differences between families were evident after 6 weeks of exposure, but by 12 weeks family 9 seedlings had higher CER over a range of PPFDs (Tables 4.2 and 4.3; Fig. 4.3).

4.3.3 Final Seedling Dry Weights

The dry weight data showed no statistically significant consistent trends with respect to pH or ozone treatments in either the field or lab studies (Table 4.4), but there were inherent differences between families. Family 9 seedlings had consistently less needle, stem, and root dry matter than family 8 in the field study. As with the dry weight data, root:shoot ratios (Table 4.4) and height and diameter growth (Table 4.5) showed differences due to seed source but not to the pH or O₃ treatments.

4.4 DISCUSSION

Our field data indicate that ozone levels approximating peak field concentrations (i.e., 167 versus ambient peaks of approximately 100 to 120 ppb ozone) reduced the photosynthetic capacity of loblolly pine shoot systems without changing mitochondrial (dark) respiration of the same tissue. The reductions in CER at saturating PPFd (approximately 20%) correspond to previous reports of reduced photosynthesis for other tree species (Carlson 1979; Coyne and Bingham 1982; Kress et al. 1982; Reich and Amundson 1985). Reich (1987) summarized a large number of studies reporting effects of ozone on conifer photosynthesis and showed up to 30% reductions in CER over a range of total dose (0 to 160 ppb•h).

Previous studies of white pine [Pinus strobus L. (McLaughlin et al. 1982)], Scots pine [Pinus sylvestris L. - (Skärby et al. 1987)], and hybrid poplar (Reich 1983) provided evidence of increased respiration rates in response to ozone exposures. In contrast, our data on shoot CER and those of Reich et al. (1986a) for soybean leaves showed no change in mitochondrial respiration with ozone exposure. The increased respiration in Reich's hybrid poplar leaves was present in the younger leaves but not in leaves that were 40 d old. The majority

Table 4.4. Final mean dry weight (DW) data of selected treatments for field and laboratory-treated seedlings. Average standard deviations about the mean dry weights in the field study were 0.59 for needles, 0.31 for stems, and 0.41 for roots and 0.63, 0.29, and 0.32, respectively, for the laboratory experiment.

Treatment Family No.	Needle DW (g)		Stem DW (g)		Root DW (g)		Root/shoot ratio	
	8	9	8	9	8	9	8	9
<u>Field study (n= 7-11)</u>								
Charcoal filtered								
pH 3.3	2.82	1.93	1.40	0.76	1.77	1.33	0.42	0.49
pH 4.5	3.51	2.24	1.78	0.66	2.09	1.56	0.40	0.54
pH 5.2	3.27	1.95	1.70	0.83	2.09	1.24	0.42	0.45
Ambient + 160 ppb								
pH 3.3	3.47	2.02	1.67	0.90	1.91	1.29	0.37	0.44
pH 4.5	3.02	1.75	1.45	0.76	1.85	1.09	0.42	0.43
pH 5.2	3.58	2.26	1.64	0.98	2.01	1.16	0.39	0.36
<u>Lab study (n=8)</u>								
0-pH 4.3	2.70	3.08	1.13	1.23	1.03	1.02	0.27	0.24
160-pH 4.3	2.56	3.22	1.26	1.27	0.97	1.14	0.25	0.25
320-pH 4.3	2.73	3.00	1.22	1.11	1.02	0.97	0.26	0.24

Table 4.5. Height and diameter growth of selected treatments for field and laboratory-treated seedlings. Although trends in the data are evident, only effects of family can be declared statistically significant.

Treatment Family No.	Height growth (mm)		Diameter growth (mm)	
	8	9	8	9
<u>Field study (n = 8-16)</u>				
Charcoal filtered				
pH 3.3	29.7	40.2	2.83	2.41
pH 4.5	40.9	58.8	2.99	2.51
pH 5.2	36.7	59.3	2.81	2.54
Ambient + 160 ppb				
pH 3.3	38.2	68.4	2.86	2.60
pH 4.5	37.7	73.9	2.53	2.41
pH 5.2	48.1	44.7	2.53	2.32
<u>Lab study (n = 8)</u>				
0 - pH 4.3	23.5	80.5	1.34	1.96
160 - pH 4.3	35.1	72.3	1.72	1.54
320 - pH 4.3	37.5	59.9	1.50	1.37

of the needles on our loblolly pine shoots were fully expanded and, therefore, would have exhibited little growth respiration. Perhaps our shoot CER measurements did not detect altered respiration rates because of a limited amount of developing tissue present in our shoots. However, the data for white pine, an "ozone-sensitive" species (McLaughlin et al. 1982), and Scots pine (Skärby et al. 1987) did find increased respiration for fully expanded needles as an apparent response to atmospheric ozone. Additional research will be needed to explain why ozone increases respiration rates in some studies but not in others.

In contrast to the ozone-induced reductions in shoot CER, rain chemistries of pH 3.3 and 4.5 resulted in enhanced shoot CER over pH 5.2-exposed plants that was of a similar magnitude to the ozone-induced CER reductions. This observation suggests that there is a potential for low pH chemistries (i.e., nitrogen or sulfur fertilization) to counteract the deleterious effects of ozone under combined exposure conditions. Previous studies have shown no effect, reductions, or enhancements of CER as a result of exposing plant materials to low pH rain. Studies of oak and maple [Quercus and Acer (Reich et al. 1986b and Jensen 1987, respectively)], ash [Fraxinus (Elliott et al. 1987)], and tulip poplar [Liriodendron (Jensen 1986)] indicated no change in growth or photosynthesis as a function of acid rain treatments. Chappelka and Chevone (1986) observed reduced growth of ash seedlings in response to pH 4.3 and 3.0 rain chemistries. Studies of red spruce (Taylor et al. 1986), loblolly pine (Seiler and Paganelli 1987), and white pine [Pinus strobus (Reich et al. 1987)] have either indicated a trend toward or documented enhanced photosynthesis and/or growth in response to rain pH chemistries below 4.0. In the studies showing enhanced photosynthesis, the increasing CER has been attributed to nitrogen fertilization (nitrate ions from dissolved nitric acid). Wood and Bormann (1977) also attributed increases in productivity of white pine to fertilization with nitrate ions from a low-pH rain chemistry treatment. Reich et al. (1987) demonstrated that white pine seedlings grown on nitrogen-deficient

soils exhibited a greater enhancement of photosynthesis in response to acidic pH rain than did similar seedlings grown on soils of high nutrient content. Nutrient analyses on similar plants from the parent study showed no distinct trend in needle nitrogen concentration with respect to the pH treatments (Sect. 5, Table 5.6). Fertilization effects due to added nitrogen and sulfur in acidified rain and/or delayed senescence of plants treated with higher rainfall acidities are possible explanations for the CER enhancements.

Ozone-induced reductions in CER of family 8 shoots were observed in the "field" study, but reductions in CER were not as apparent for families 8 and 9 when they were exposed to similar ozone dosages under laboratory conditions (Tables 4.1 and 4.2). It is unclear why similar dosages (Table 4.1) induced a different response in loblolly shoot CER under laboratory conditions. However, because laboratory-reared and -treated seedlings were exposed to charcoal-filtered conditions between ozone exposures, they may have had a more favorable environment for repairing ozone damage during their respite periods. Furthermore, because laboratory seedlings received liquid instead of "slow-release" fertilizer (Appendix E) they may have had a better nutrient regime allowing them to withstand the ozone exposures (i.e., repair themselves). Our contrasting field and laboratory results suggest the need for caution when attempting to extrapolate laboratory data to field situations.

Even though laboratory ozone exposures did not produce consistent alterations in CER-PPFD characteristics, family differences in CER at saturating PPFD were observed. The difference was not apparent at 6 weeks, but by 12 weeks, family 9 seedlings had higher CER (Fig. 4.3). Dark respiration rates were not different between the two families (Fig. 4.3). Kress et al. (1982) documented differences in susceptibility of Pinus taeda families to ozone, and Weir (1977) showed that seedlings from family 9 exhibited a lower percentage of visible injury resulting from ozone exposures than did family 8 seedlings. Our data showed little change in CER or growth with respect to ozone treatments for families 8 and 9 in the laboratory exposures. However,

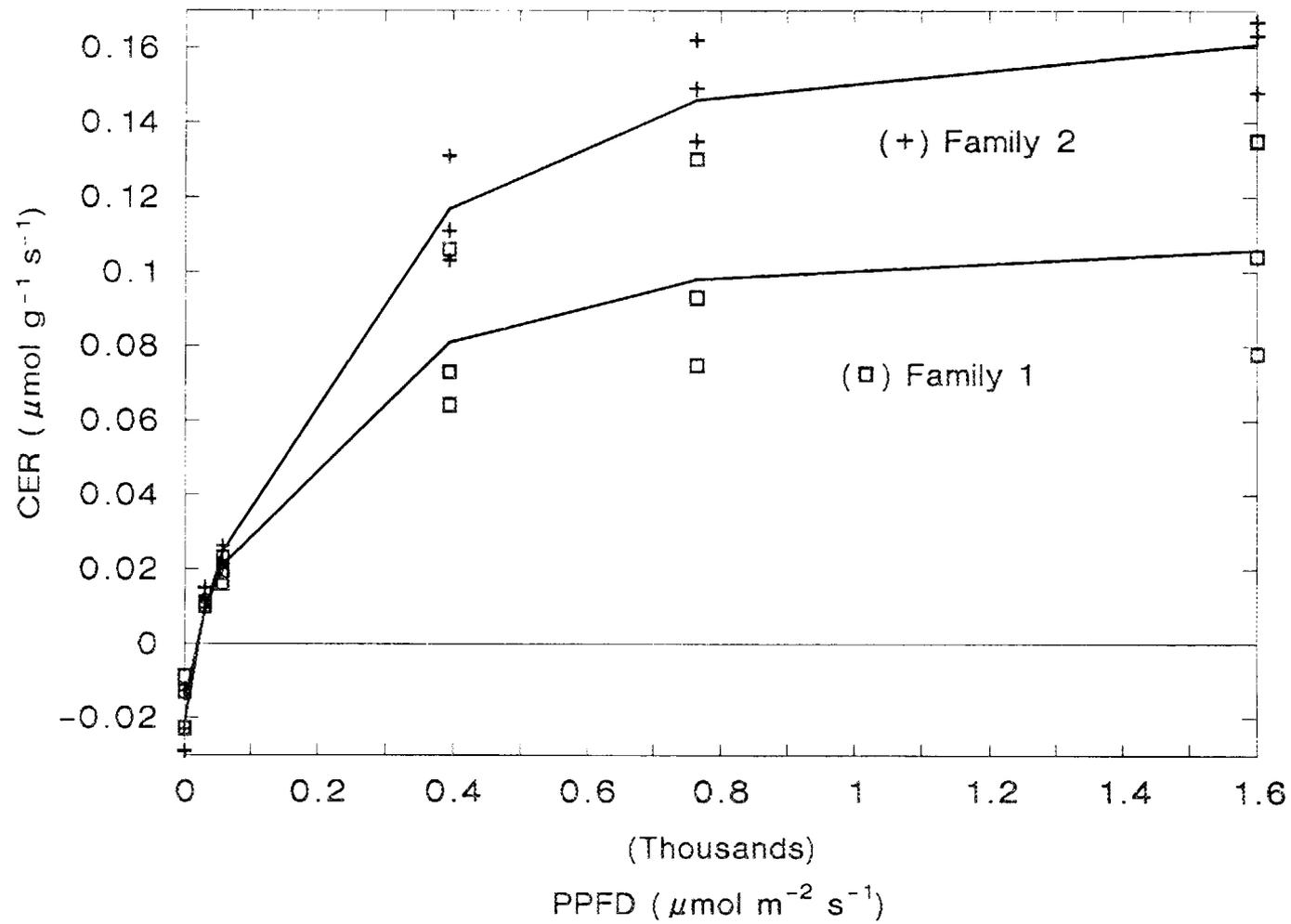


Fig. 4.3. Mean CER-PPFD relationships of "lab" seedlings from families 8 and 9 grown for 12 weeks in charcoal-filtered air (0 ppb O₃). Each curve represents the mean of 3 seedlings. The two curves are different (Table 4.2).

the higher rates of photosynthesis that we have observed for family 9 seedlings may have allowed them to avoid permanent tissue damage from ozone by providing additional carbon compounds for repair and could explain why Weir (1977) found them to be less susceptible to ozone under their experimental conditions.

Altered shoot CER characteristics (e.g., reduced CER because of ozone) were not translated into significant differences in final seedling dry weights over the 12-week experiment.

However, because these studies could logistically include only a very limited number of seedlings, the statistical powers of detection were rather low. For this reason we have examined the consistency of photosynthetic responses and various indices of growth in Table 4.6. The observed responses include both stimulation and inhibition of growth and photosynthesis associated with various family + treatment combinations. In looking across these trends, there is an apparent positive correlation between changes in CER and growth responses for ozone, but not for acid rain. Of the 15 possible comparisons of responses of seedling weight, height, or diameter to measured responses in CER for family x treatment combinations, photosynthetic responses were in the same direction as growth responses on 13 occasions where effects of ozone were being evaluated. On the other hand, of the 15 comparisons, photosynthetic responses and differences in plant weight between treatments agreed in 3 of 5 comparisons. In all 10 comparisons involving changes in height or diameter, the photosynthetic and growth responses agreed in direction. Growth responses to acid rain opposite measured responses in CER in two of three cases.

Previous studies of ozone and rain pH effects on forest tree seedlings have shown reduced growth (Kress 1982; Chappelka and Chevone 1986; Percy 1986). However, these studies were done on very young seedlings grown from seed (from 0 to 5 weeks old). Our seedlings, which were 15 to 20 cm tall and 3 months old prior to exposures, may have had a better capacity for repairing cellular damage caused by ozone (i.e., greater starch reserves, greater photosynthetic surface area). Our short-duration experiment may not have allowed us to

Table 4.6. Comparison of relative responses of net photosynthesis and growth to ozone and acid rain exposure after 12 weeks exposure.

Family	Setting	Treatment comparison ^b	Measured response % ^a			
			Ps	Total weight	Height growth	Diameter growth
8	Field	CF 3.3 vs. CF 5.2	+39	-16	-20	+1
	Field	CF 4.5 vs. A160 4.5	-39	-15	-8	-16
8	Lab	0 vs. 160	+7	-2	+49	+28
		0 vs. 320	+8	+2	+60	+12
9	Lab	0 vs. 160	-14	+6	-10	-21
		0 vs. 320	-14	-5	-26	-31

^aResponses are expressed as % change from appropriate control treatments.

^bComparisons are indicated by both the pH of rain treatment and the ozone treatment designation (160 is ambient + 160 ppb). Lab treatments are in ppb and were all exposed to pH 4.3 rainfall.

adequately document small changes in dry weight accumulation that longer-term experiments would have the ability to resolve. Reich (1987) discussed length of ozone exposure for crop, broadleaf tree, and coniferous tree species and demonstrated that conifers require longer exposures before changes in dry weight can be measured. Furthermore, McLaughlin (1987) emphasized that not only photosynthetic data but also data on maintenance respiration, metabolite translocation, and growth are all required to characterize a plant's response to pollutant stress; therefore, lack of correlation between shoot CER and final dry weight does not necessarily indicate the absence of a relationship between CER and dry matter.

The ozone treated seedlings in our study received peak ozone concentrations on approximately 50% of the days throughout the experiment (Table 4.1). This is a higher percentage than would be expected under current ambient conditions where plants might expect to receive peak ozone exposures (> 80 ppb) on only 30% of the days (Taylor and Norby 1984; Adams and Taylor 1987). Because the number of ozone events in our study exceeded current ambient expectations (i.e., 50 vs 30% of the days) and the exposures still produced only small alterations in CER-PPFD responses and small and/or variable changes in final dry weight, one might conclude that loblolly pine seedlings should be considered resistant to ozone under current atmospheric conditions. However, results of the parent study (Sect. 3) have shown a wide range of family-dependent responses to ambient or elevated ozone levels and acid rain exposures, indicating that species-wide conclusions concerning the susceptibility of loblolly to ozone and/or low-pH rain chemistries should consider potentially significant variations in sensitivity associated with genetic background.

4.5 SUMMARY OF EXPERIMENTAL OBSERVATIONS

Carbon dioxide exchange rate (CER) was measured as a function of light levels (PPFD) for seedlings of two loblolly pine families following 6- or 12-week exposures to two ozone (charcoal-filtered or ambient air + ozone) and three acid rain treatments (pH 3.3, 4.5, and

5.2). Treatment effects were not consistent between field and laboratory-exposed seedlings. Ozone-treated "field" seedlings exhibited statistically significant reductions in light-saturated CER of 12.5 and 25% for plants measured at 6- and 12-weeks, respectively. Lab seedlings exhibited mixed responses, with one family showing reduced CER only after 6 weeks and the other only after 12 weeks of ozone exposure. After 12 weeks of exposure, pH 3.3 and 4.5 rain treatments enhanced light-saturated CER by an average of 52% over that observed for seedlings exposed to the pH 5.2 treatment. No differences in dark respiration were detected between treatments. The enhanced CERs due to acid rain were of the same magnitude (3 to 5 $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) as ozone-induced CER reductions, suggesting the potential for an interaction between treatments.

Although ozone and acid rain treatments altered seedling CER, the effects on plant weight and height and diameter growth were less consistent. There were subtle growth trends in responses to some applied treatments under both laboratory and field conditions. In general measured CER responses agreed well qualitatively with those trends, particularly diameter response to ozone. Responses in CER did not agree with growth responses to acid rain. Future research will be needed to resolve interactions between the effects of ozone and acid rain on seedling CER and growth and should carefully consider the potential for differences in exposure patterns and associated toxicity of the applied dose between field and laboratory studies.

4.6 DEVELOPMENT OF AN IN SITU CANOPY PHOTOSYNTHESIS SYSTEM

The interpretation of photosynthesis data obtained from point measurements taken during the course of the growing season is highly dependent on the frequency of those measurements relative to the frequency of principal stress events. Measurements discussed in the preceding section have demonstrated that differences in photosynthetic capacity associated with ozone treatment could be detected over the three days after seedlings were removed from the chambers in which they were exposed in the field. However, additional information on the

seasonal and diurnal kinetics of the photosynthetic response and recovery could not be obtained with that system for logistical reasons.

Because patterns of photosynthetic response and recovery are very important in evaluating both the causes (ie. chronic vs acute exposures, concentration thresholds, etc.) and quantitative significance of pollution - induced changes in the carbon economy of canopies of forest trees, we have been developing a gas exchange system that can be placed in the canopy for continuous in situ measurement of gas exchange processes. The system we developed is based on a prototype originally designed for use in open top chamber studies with soybeans (McConathy and McLaughlin, 1987). The original system was developed to examine the diurnal and seasonal patterns of gas exchange of soybean canopies. That system was used successfully to detect differences in photosynthesis as they developed over the growing season in response to both ozone and acidic deposition (McConathy and McLaughlin, 1987). It was based on measurements of changes in CO₂, H₂O, and O₃ in the boundary layer immediately below 10 individual leaflets using 10 3 mm. (OD) teflon tubes through which air was drawn to a single mixing manifold for each canopy sample.

During the 1986 growing season, exploratory studies were initiated to adapt the system for use on field grown trees. The test specimens were 5 m tall open-grown loblolly pine trees growing at our field research site (see the location of these trees in Figure 1.1). Modifications made to the system to utilize pine foliage included the use of small glass cuvettes (2.0 cm in diameter by 4.0 cm long) designed to create a slightly wind-buffered air space around sets of 6-9 individual needles. The individual cuvettes were self ventilating, produced only small temperature increase around the partially enclosed needles and were flushed approximately 8 times per minute by the approximately 100 cc per minute flow through each individual unit. They are light weight and can be mounted on the branches under study. Ten of these units were attached to a single sampling manifold and were used to obtain an average measurement for a particular canopy location.

Results from the exploratory tests with this system were very encouraging. The system was able to measure the diurnal pattern of photosynthetic as a well defined, radiation dependent signal that stood out clearly from background noise induced by within canopy fluctuations in CO₂ concentrations (see Figure 4.4). The basic design considerations, test data for both the original broadleaf system and the loblolly prototype are discussed in a manuscript (McLaughlin, 1988) included in its entirety in Appendix D. In this manuscript the developmental concepts, scientific rationale, and applications of branch level measurements of carbon assimilation are discussed in relation to interpretation of pollution induced changes in carbon economy of trees.

While the original broadleaf system was designed to provide qualitative measures of relative changes in the diurnal and seasonal cycles of gas exchange to characterize and contrast treatment effects, results to date indicate that measurements obtained using the pine cuvette have good quantitative potential for estimating actual photosynthetic rates as well as characterizing differences in photosynthetic patterns. Additional experiments are currently under way to explore this potential further.

The development of this system offers many possibilities for evaluating essential features of both pollutant dose and plant response to ambient or altered concentrations of pollutants. Of particular relevance are the lightweight cuvettes and the fact that they can be used to integrate measurements from different portions of the canopy or for different age classes. Such capabilities will be of great importance as investigative efforts move increasingly to the field and towards the larger size classes of trees that are of immediate concern.

CANOPY PHOTOSYNTHESIS

4M LOBLOLLY PINE -3 DAYS IN OCTOBER

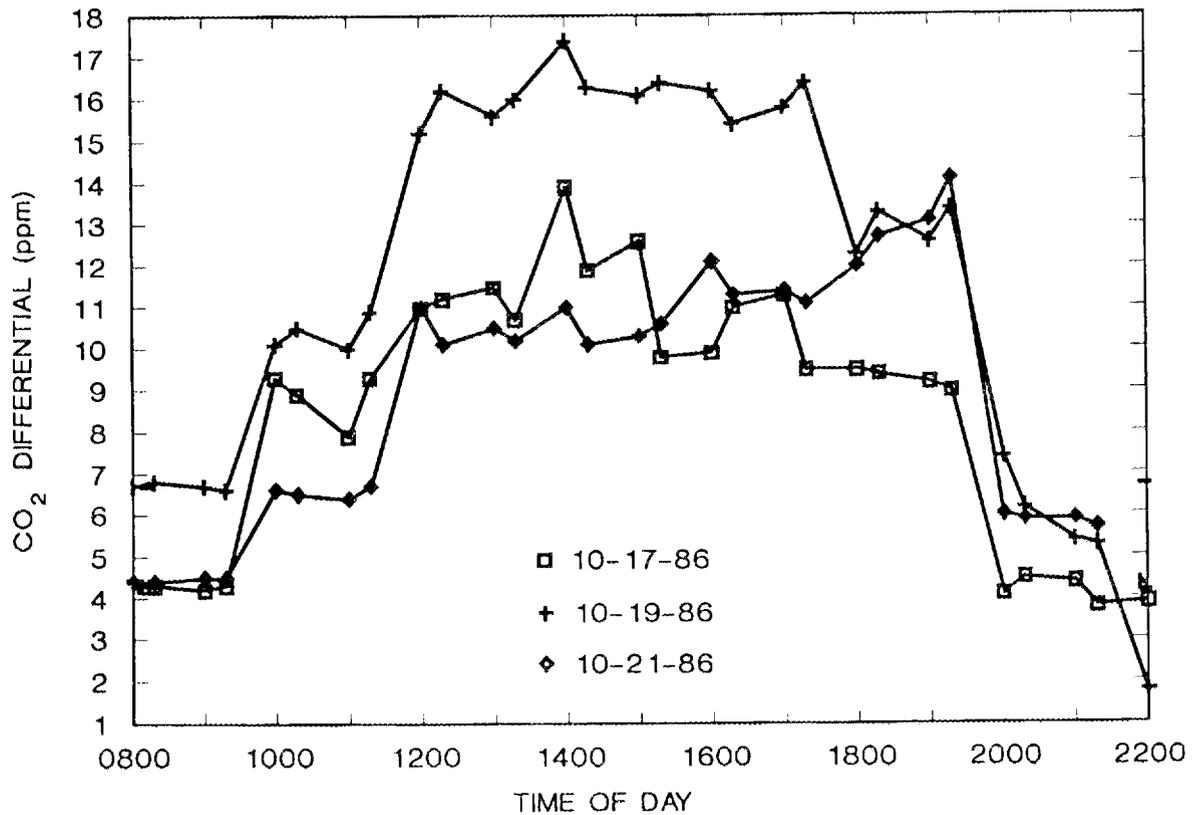


Fig. 4.4. Diurnal pattern of CO₂ exchange of loblolly pine canopy determined for three successive days in October, 1986. Data are in ppm CO₂ differential for a flow of approximately 1.5 l min⁻¹ across approximately 360 cm of loblolly needle length.

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5. CARBON ALLOCATION AND NUTRITION

M. B. Adams

5.1 INTRODUCTION

In recent years, highly visible symptoms of decline of some forest trees have led to increased investigation of the role of air pollutants in forest health. Researchers quickly became aware of the need for an understanding of the effects of atmospheric pollutants on tree physiology and functioning as a basis for evaluating the mechanistic basis for stand-level effects. One area of physiological research of particular relevance that has to date attracted relatively little research activity is that of carbon allocation. The study of carbon economy as affected by air pollutants may identify short-term mechanistic responses as well as longer-term responses that integrate seasonal or multiyear effects. Carbon allocation patterns affect, directly or indirectly, other physiological processes (e.g., nutrient uptake, reproduction) as well as tree growth and vigor (Kramer and Kozlowski 1979, Webb 1981, McLaughlin and Shriner 1980).

Elevated ozone levels may alter patterns of carbohydrate allocation within a plant either through changes in the various carbohydrate fractions (Constantinidou and Kozlowski 1979, Jensen 1981) or in the spatial allocation of photosynthetically fixed carbon within the plant (McLaughlin et al. 1982, Wilkinson and Barnes 1973). Changes in allocation of carbon to the various carbohydrate fractions may have significant implications if less carbon is allocated to reserve carbohydrates or is diverted to compounds used in repair of damaged tissue at the expense of growth or other functions. Spatial shifts in allocation may also result in altered growth patterns or decreased tree vigor. Recent research provides somewhat inconsistent and contradictory support for the hypothesis that altered carbon allocation is a major result of ozone fumigation (Reich et al. 1986, Wang et al. 1986, respectively). The interaction of ozone dose with rain chemistry further confounds the question.

The main objectives of this study were (1) to examine individual and interactive effects of ozone and rain chemistry on whole-plant carbon allocation patterns of loblolly pine seedlings and (2) to determine whether carbohydrate reserves, in particular starch, are significantly affected by elevated ozone levels, acidic rain, or an interaction of the two. To achieve these objectives, and to allow examination of possible interactions among family, ozone level and rain chemistry, seedlings of several families were used. Both field-grown and continuously stirred tank reactor- (CSTR-) grown seedlings were used to allow comparison between field and lab studies. (See Sect. 2 for complete treatment descriptions.) Table 5.1 displays the family and treatment combinations used in this study. To explore the effects of ozone fumigation and its interaction with rain chemistry on carbon allocation, we examined patterns of allocation of photosynthetically fixed ^{14}C within individual seedlings. Starch is the major reserve carbohydrate in woody plants and is predominantly stored in the roots (Ebell 1969). Therefore, to assess the effects of ozone and acidic rain on carbon reserves, starch concentrations in the roots were examined. Because tree nutrition may be affected by changes in carbon allocation as well as by the ionic content of acidic deposition, foliar nutrient concentrations of two representative families were also examined.

5.2 METHODS

5.2.1 $^{14}\text{CO}_2$ Allocation

Four-month-old loblolly pine seedlings were exposed to ^{14}C -enriched CO_2 after 13 weeks of treatment. A 90 X 60 X 72 cm wood and clear Teflon chamber was used to expose the plants to ^{14}C -enriched CO_2 (360 ppm CO_2 , $19.9 \mu\text{Ci l}^{-1}$). High-intensity-discharge sodium vapor lamps (400 W) provided illumination at light-saturation conditions (500 to $600 \mu\text{mol m}^{-2} \text{s}^{-1}$). The $^{14}\text{CO}_2$ gas was delivered into the chamber at a flow rate of $6 \text{ l}^{-1} \text{ min}$ (0.10 L s^{-1}) for 30 s. A small fan within the chamber ensured circulation of the gas. After the initial 30 s, ^{14}C

Table 5.1 . Families utilized in the study of carbon allocation^a.

Ozone treatment	Rain Treatment (pH)		
	5.2	4.5	3.3
<u>Field study</u>			
Charcoal-filtered	8,9 (2,5)	8,9,40,49 (2,5)	8,9 (2,5)
Ambient chambered		40,49	
Ambient + 80 ppb O ₃	8,9	8,9	8,9
Ambient + 160 ppb O ₃	8,9 (2,5)	8,9 (2,5)	8,9 (2,5)
<u>CSTR study</u>			
		4.3	
0 ppb O ₃		8,9,10 (2,5)	
160 ppb O ₃		8,9,10 (2,5)	
320 ppb O ₃		8,9,10 (2,5)	

^aSee Methods section for complete treatment and genotype descriptions. Numbers in parentheses represent families used for foliar nutrient analysis.

injection was halted and the air was circulated an additional 90 s. Then the chamber was vented and the air was pumped out of the chamber. The plants were then removed from the chamber and a representative sample of foliage was collected (approximately 0.02 g dry weight) from each seedling. (This is referred to as the day 0 sample.) The foliage sample was frozen immediately with liquid nitrogen, then stored frozen until dried to a constant weight in a forced-draft oven at 70°C. This day 0 sample was used to determine initial ^{14}C uptake. Subsamples of foliage were again collected on the day after tagging (day 1) and at one week (day 7). On day 7, the seedlings were removed from the pots, separated into shoots and roots, and frozen. Later, prior to drying, these were further separated into foliage, stem, and fine and coarse root (< 1.0 mm and \geq 1.0 mm, respectively) components. The plant components were then dried and weighed. Fine and coarse roots and stems were ground to pass a 40-mesh Wiley mill screen for further analysis. Samples were oxidized using a Packard Model 306 Tri-Carb sample oxidizer. Released CO_2 was trapped in scintillation cocktail and counted in a Packard Tri-Carb 460C automatic liquid scintillation counting system. Carbon allocation, expressed as the percentage of the original (day 0) ^{14}C uptake of the individual seedling, and as the percentage of activity remaining in the plant after 7 d, was examined; and comparisons were made among plant components, across treatments and families.

5.2.2 Starch Assays

Starch concentrations in the roots of the seedlings tagged with ^{14}C were assayed to determine sensitivity of carbon storage to ozone fumigation and rain chemistry. Starch concentrations were determined for the fine and coarse roots separately using an enzymatic hydrolysis method similar to that described by Haissig and Dickson (1979). Briefly, reducing sugars and pigments were extracted from 20-mg subsamples with a mixture of methanol:chloroform:water (12:5:3, v:v:v) and the residue dried (50°C) overnight. After rewetting the sample with ethanol, 4 mL of distilled water were added and samples were boiled for 10 min to gelatinize starch. Starch was then hydrolyzed to

glucose by a mixture of two enzymes, an alpha-amylase and an amyloglucosidase, during a 24-h incubation at 50°C. Glucose concentrations were then measured colorimetrically by means of a glucose-oxidase peroxidase reagent (Sigma Chemical Company 1983).

Starch standards were prepared identically to tissue samples to determine that starch recovery was complete. Replicates of "standard" samples were run with each batch of samples to assess variability of the method, and approximately 20% duplication of samples was also used.

Data were analyzed using analysis of variance techniques for a split-plot design (SAS Institute 1985). Mean comparisons were conducted at the $p \leq 0.05$ level of significance unless otherwise indicated. Because of selection of differing treatment levels, families 8 and 9 were analyzed separately from families 40 and 49 (field study).

5.2.3 Nutrient Analyses

Two representative families (2 and 5) were selected from the larger study for nutrient analyses. Two seedlings per ozone-rain combination were selected from each block. Treatments included in the analyses were: from the field study, charcoal-filtered, ambient air + 160 ppb ozone, and pH 5.2, 4.5, and 3.3 rain; for the CSTR study, 0, 160, and 320 ppb additional ozone, rain of pH 4.3 (Table 5.1).

Foliage from these seedlings was dried to a constant weight at 70°C then ground to pass a Wiley mill 40-mesh screen. Nutrient analyses were conducted by personnel of the University of Georgia Soil Testing and Plant Analysis Laboratory in Athens, Georgia. Total nitrogen was determined using a macro-Kjehldahl procedure. Concentrations of other nutrients (P, K, Ca, Mg, Mn, Fe, Al, B, Cu, Zn) were determined using a direct reading emissions spectrograph (Jarrell-Ash, Inc., Walton, Mass.).

Data were analyzed using analysis of variance procedures for a factorial design (SAS Institute 1985). All mean comparisons were conducted at the $p \leq 0.05$ level of significance unless otherwise stated.

5.3 RESULTS

Only 13-week harvest data will be reported here for families 8 and 9 (both the field and CSTR studies), 10 (CSTR only), and 40 and 49 (field study only). Nutrient data for families 2 and 5 will also be presented.

5.3.1 Field Study

5.3.1.1 Biomass

There were no significant effects of varying ozone concentration or rain pH on any of the biomass components of the families analyzed. Total plant biomass (Table 5.2) was also not affected. Root:shoot ratios declined slightly with increasing O₃ level, but differences were not statistically significant. (See Sect. 3 for a discussion of the growth of these families relative to all others used in the larger screening study.) Significant family differences were detected, however. Seedlings of family 8 were consistently the largest, both aboveground and belowground. There were also significant differences in root:shoot ratios among the families, indicating different carbon allocation patterns.

5.3.1.2 ¹⁴C Allocation

During the first week after tagging, approximately 50% of the ¹⁴C taken up by the seedlings was lost from the foliage, either through translocation elsewhere in the plant, or through respiration (Fig. 5.1). Much of this reduction occurred within the first 24 h after tagging. Losses from the plant 1 week after tagging (largely due to respiration, with perhaps slight losses due to root exudation) varied significantly with family, averaging 10.7% for family 8, 27.7% for family 9, 29.3% for family 40, and 33.3% for family 49 (Table 5.3). For families 8 and 9, ¹⁴C losses increased with ozone concentration, though this was not statistically significant (Figure 5.2). An increase in ¹⁴C retention by foliage of the ozone-exposed trees was significant for families 40 and 49. No other significant ozone or rain

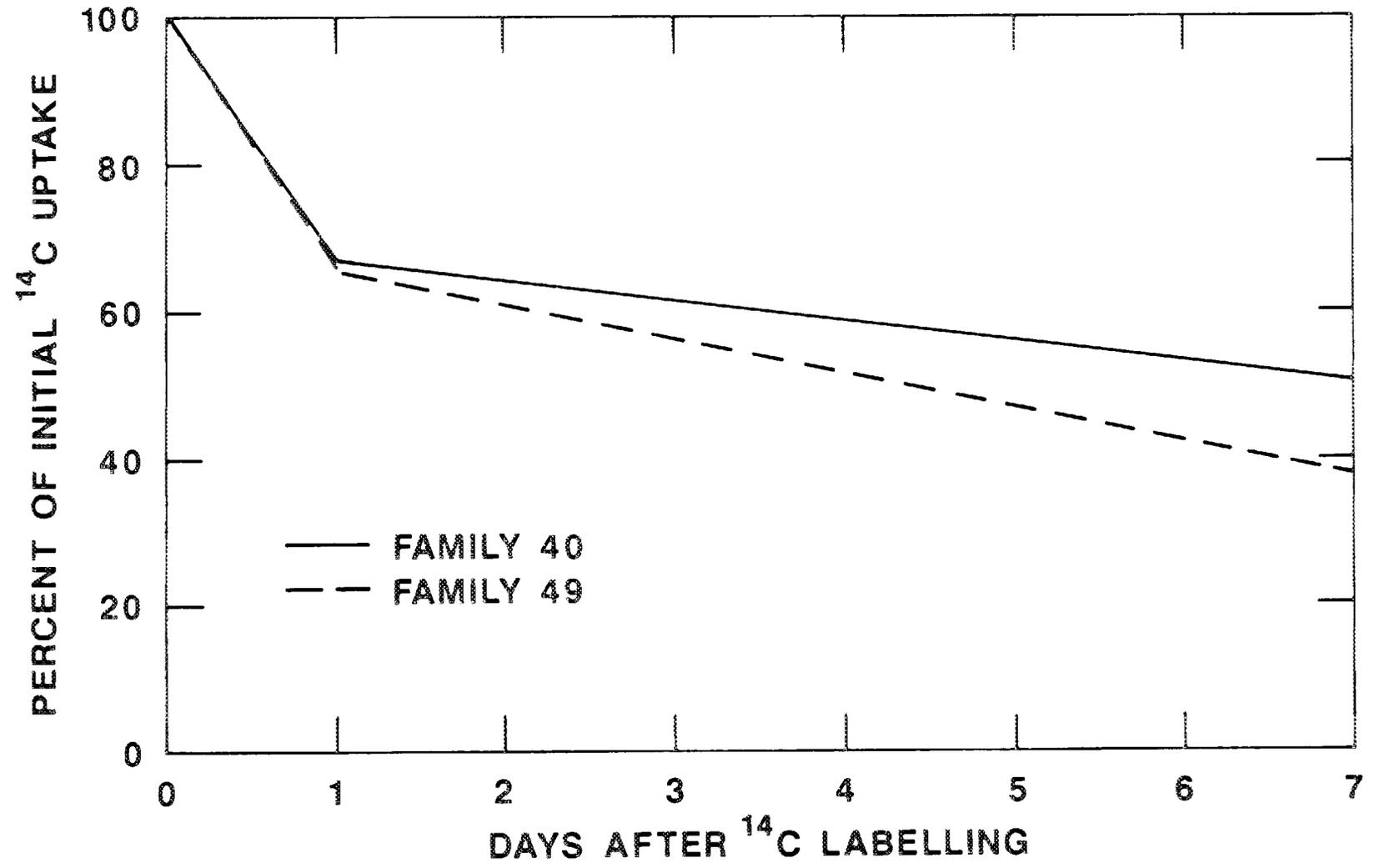


Fig. 5.1. Comparisons between loblolly pine families 40 and 49 in retention of ^{14}C by foliage with time after labeling with $^{14}\text{CO}_2$ (field study).

^{14}C Allocation (% of total uptake)

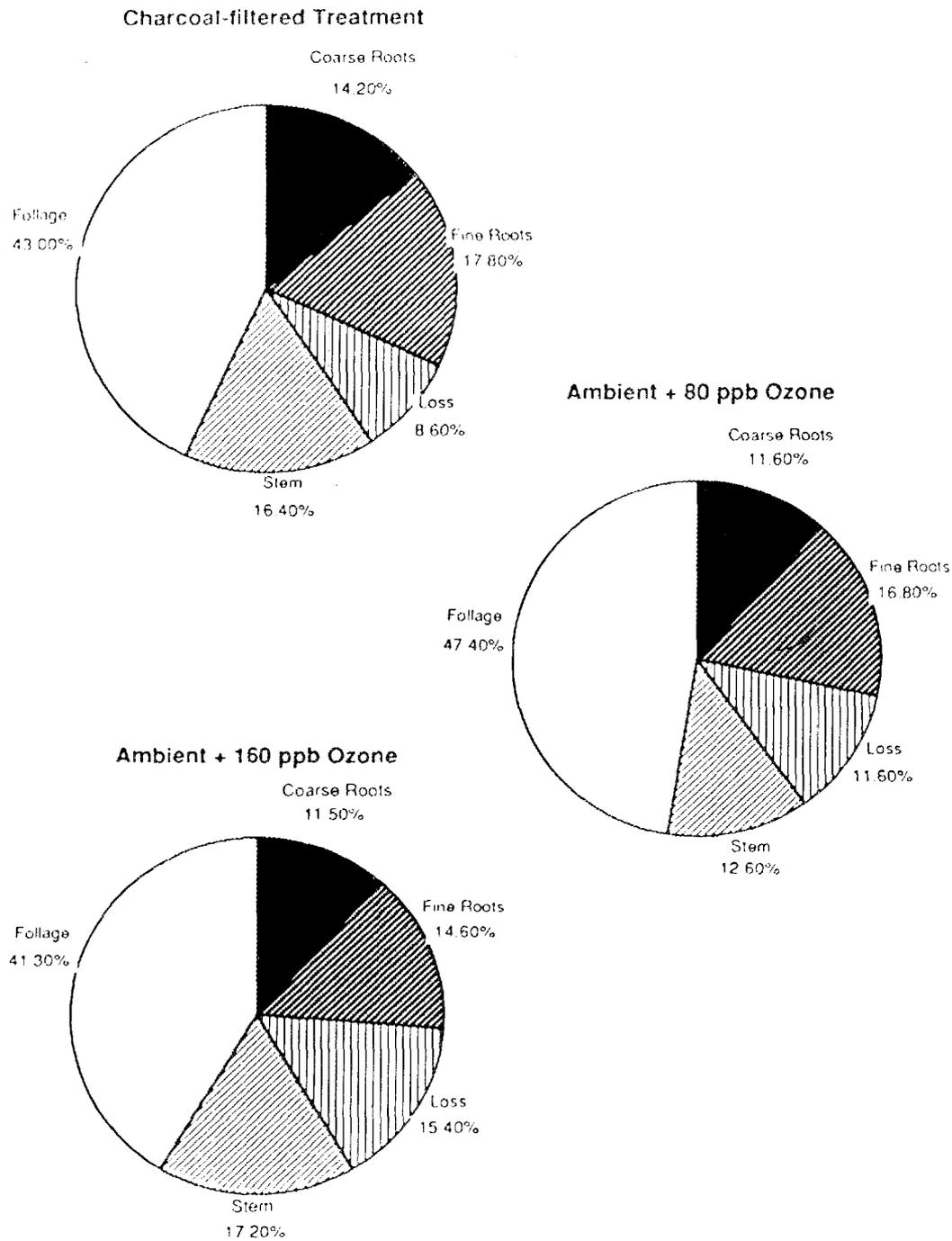


Fig. 5.2. Whole-plant allocation of ^{14}C -photosynthate by loblolly pine seedlings as affected by ozone treatment (field study). Note decreasing allocation to roots and increasing total plant losses (an estimate of maintenance costs) with increasing ozone concentration.

Table 5.2. Biomass components of loblolly pine seedlings by family after 13 weeks of treatment (field study)

	Foliage	Stem	Aboveground biomass	Fine roots	Coarse roots	Total roots	R:S
Family	<u>Means across all treatments (g) n = 104 (8,9), 26 (40, 49)</u>						
8	3.54A ^a	1.62A	5.15A	1.13A	0.95A	2.08A	0.397A
9	2.23B	0.87B	3.07A	0.82B	0.49B	1.31B	0.426B
40	2.75a	0.764a	3.509a	0.93a	0.44a	1.37a	0.392a
49	2.150b	0.796a	2.890b	0.84a	0.53a	1.38a	0.474a
Ozone	<u>Means of families 8 & 9 across all rain pH levels (g) n=35 per level</u>						
Charcoal- filtered	2.80A	1.20A	4.00A	0.98A	0.75A	1.73A	0.433A
Ambient + 80 ppb	2.94A	1.20A	4.14A	1.07A	0.75A	1.82A	0.428A
Ambient + 160 ppb	2.91A	1.24A	4.15A	0.95A	0.72A	1.67A	0.410A
Rain pH	<u>Means of families 8 & 9 across all O₃ levels (g) n = 35 per level</u>						
5.2	2.94A	1.28A	4.22A	1.01A	0.77A	1.78A	0.420A
4.5	2.82A	1.19A	4.01A	1.00A	0.75A	1.75A	0.438A
3.3	2.88A	1.18A	4.06A	0.98A	0.70A	1.68A	0.415A

^aMeans within the same column are not significantly different at the p=0.05 level if they are followed by the same letter. Capital letters are used to indicate differences for families 8 and 9; lower case letters are used to indicate differences for families 40 and 49.

Table 5.3. Allocation of ^{14}C label in loblolly pine seedlings 1 week after labeling, expressed as mean percentage of initial uptake (field study)

	Fine roots	Coarse roots	Total roots	Foliage	Stem	Loss from plant
Family	<u>Means across all treatments (%)</u> n = 104 (8,9), 26 (40,49)					
8	17.6A ^a	14.4A	32.0A	50.5A	15.5A	10.7A
9	15.2A	10.5B	25.7B	37.1B	15.1A	27.7B
40	19.5a	7.2a	26.7a	62.8a	10.5a	29.3a
49	21.9a	11.8b	33.7b	55.0a	11.3a	33.3a
Ozone by Family	n = 18 (8,9) or 13 per treatment (40,49)					
8 & 9						
CF	17.8A	14.2A	32.0A	43.0A	16.4A	8.6A
Ambient + 80	16.8A	11.6A	28.4A	47.4A	12.6A	11.6A
Ambient + 160	14.6A	11.5A	26.1A	41.3A	17.2A	15.4A
40 & 49						
Ambient	13.4a	6.2a	19.6a	43.1a	7.4a	30.5a
CF	13.7a	5.9a	19.3a	41.0b	7.2a	32.4a

^aMeans within the same column are not significantly different at the $p = 0.05$ level of significance if followed by the same letter. Capital letters are used to indicate differences for families 8 and 9; lower case letters are used to indicate differences for families 40 and 49.

pH effects on the proportion of ^{14}C initially taken up that was allocated to individual plant parts were detected for these two families, and differences are small. However, for both families 8 and 9, the percentage allocated to coarse roots varied significantly ($p \leq 0.05$) with rain pH, and was highest at the ambient (pH 4.5) rain treatment (Fig. 5.3). Genetic differences in allocation of the labeled photosynthate to the different plant components were also significant, with family 8 allocating significantly more carbon to foliage and roots (coarse and total roots) than family 9, despite significantly higher root:shoot ratios for family 9. Families 40 and 49 differed only in allocation to coarse roots and total roots, with family 49 allocating a larger percentage of the ^{14}C to roots relative to family 40 (Table 5.3).

Of the activity remaining in the seedlings after 7 d, approximately 50% was in the foliage, with the remainder divided approximately equally among the fine and coarse roots and the stem, though proportions varied among the families (Table 5.4). Family 40 allocated more carbon to foliage at the expense of the root system, while family 9 allocated a larger proportion to stems. Biomass allocation followed approximately the same pattern as carbon allocation: 45% in foliage, 30% in roots, 20% in the stem. No statistically significant ozone or rain pH effects on carbon allocation on day 7 were detected.

5.3.1.3 Starch

Fine-root starch concentration and content and coarse-root starch content were found to vary widely among families (Table 5.5). Starch concentrations and content were highest in family 8, the fastest growing family. Differences between families 40 and 49 were not statistically significant, nor were differences resulting from ozone levels detected for families 40 and 49. For families 8 and 9, starch concentration of the coarse roots and total root system starch concentration were found to vary significantly among ozone treatments at the $p \leq 0.10$ level (Fig. 5.4). Concentrations were significantly higher (by approximately 20%) in roots of seedlings grown under the

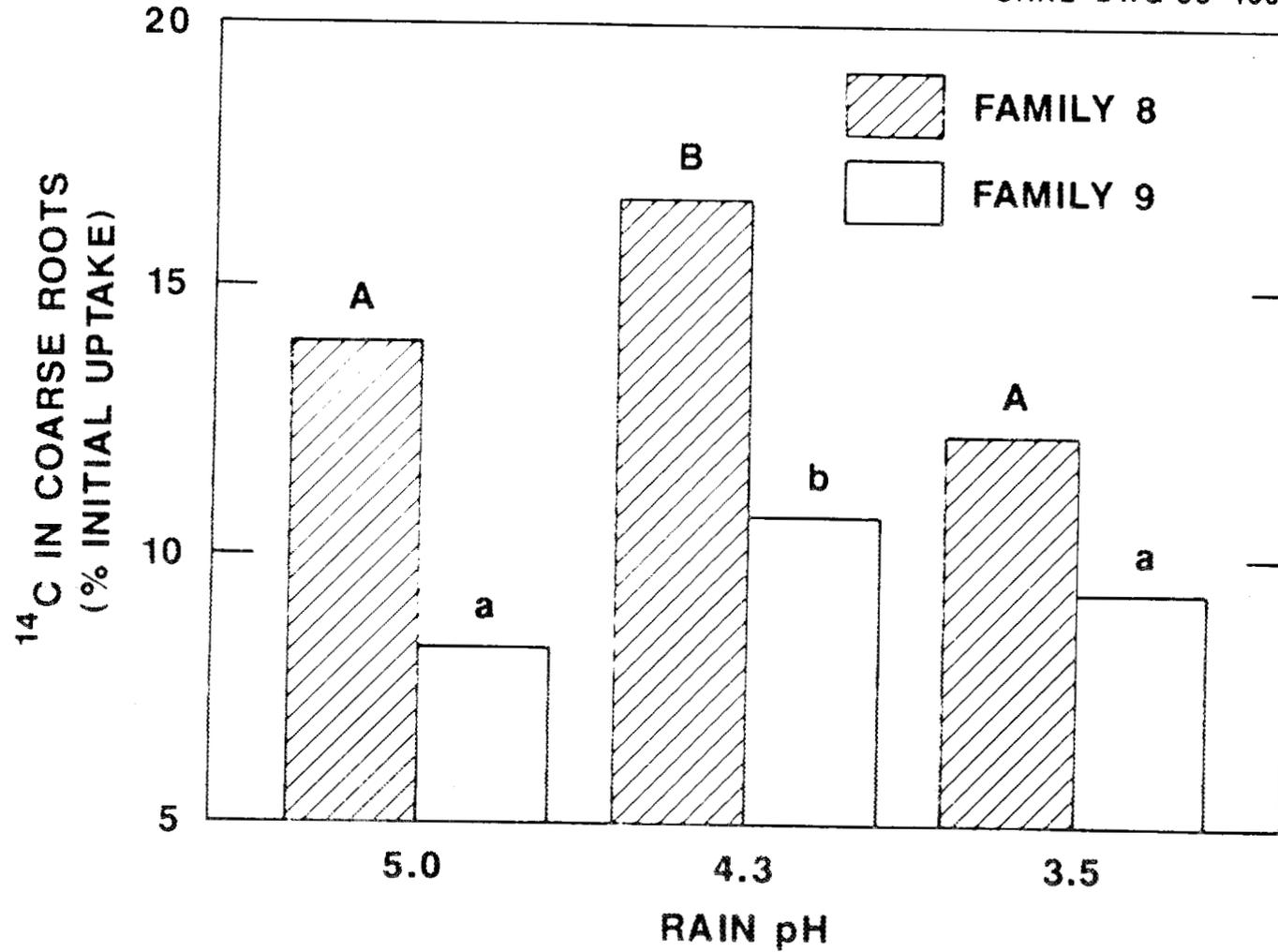


Fig. 5.3. Allocation of ^{14}C to coarse roots of loblolly pine seedlings from families 8 and 9, as affected by rain pH (field study). Letters above the bars are used to indicate statistically significant differences among rain pH levels. Capital letters are used to indicate treatment differences within family 8; lower-case letters are used to indicate differences among treatments within family 9.

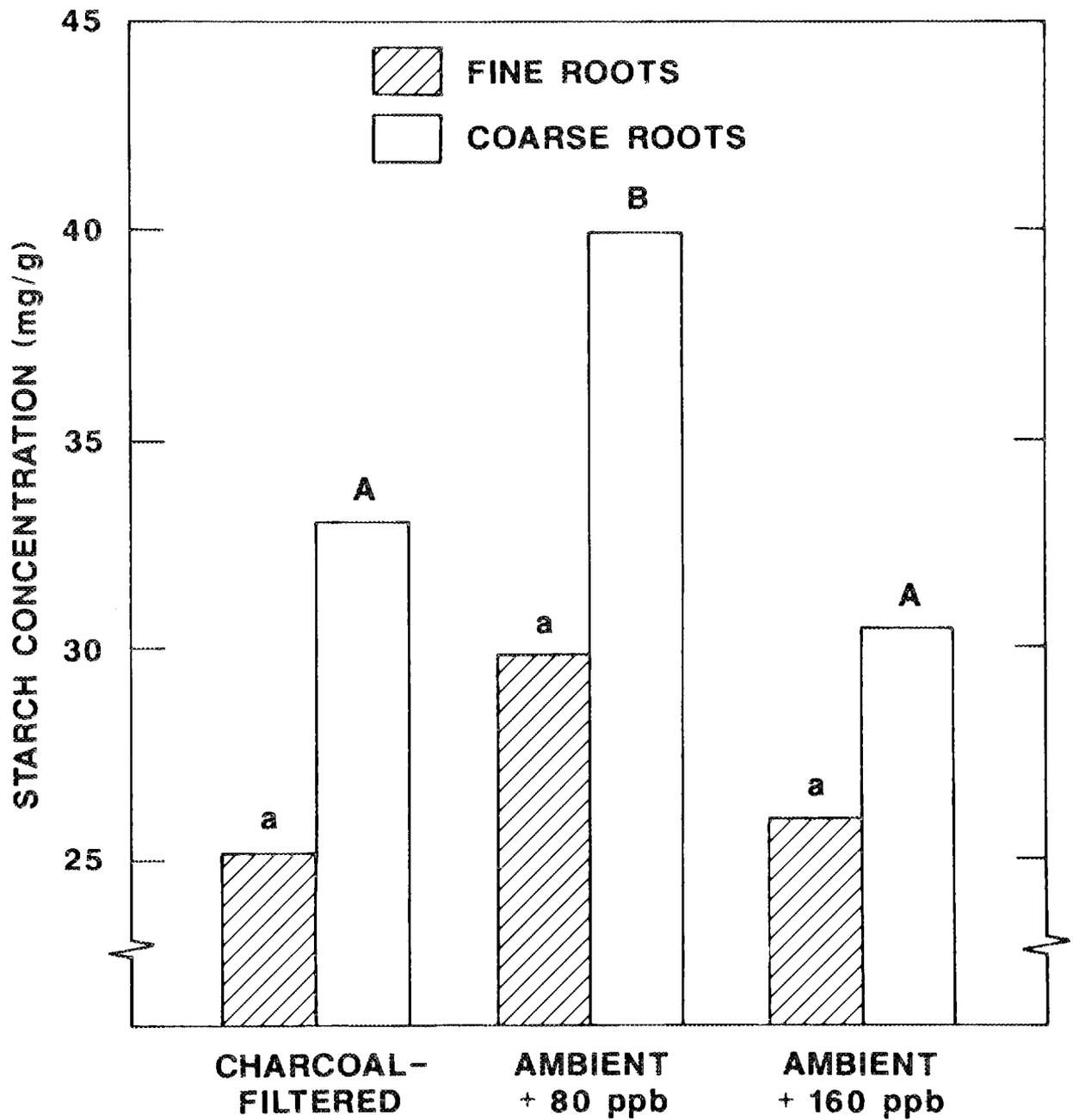


Fig. 5.4. Root starch concentration as affected by ozone treatment, families 8 and 9 combined (field study). Letters above the bars are used to indicate statistically significant differences among O_3 treatments.

Table 5.4. Allocation of ^{14}C label in loblolly pine seedlings 1 week after labeling, expressed as mean percentage of total activity remaining in the whole plant (field study)

	Fine roots	Coarse roots	Total roots	Foliage	Stem
Family	<u>means across all treatments (%)</u> n = 52 (8,9) or 13 (40,49)				
8	18.0A ^a	14.6A	32.6A	51.8A	15.6A
9	21.2A	13.6A	34.8A	45.2A	20.0B
40	19.5a	7.2a	26.7a	62.8a	10.5a
49	21.9a	11.8b	33.7a	55.0a	11.3a
Ozone by Family n = 14 (8,9) or 6-7 (40,49) per level					
8 & 9					
Charcoal- filtered	19.4A	13.8A	33.2A	49.7A	17.2A
Ambient + 80 ppb O ₃	20.7A	14.6A	35.3A	48.4A	16.3A
Ambient + 160 ppb O ₃	18.0A	14.1A	32.1A	48.3A	19.6A
40 & 49					
Charcoal- filtered	20.9a	9.6a	30.5a	58.5a	11.0a
Ambient	20.3a	9.0a	29.3a	60.0a	10.7a

^aMeans within the same column are not significantly different at the p = 0.05 level of significance if followed by the same letter. Capital letters are used to indicate differences for Families 8 and 9; lowercase letters are used to indicate differences for families 40 and 49.

Table 5.5. Starch concentration and content of loblolly pine roots after 13 weeks of treatment (field study)

Family	Fine roots		Coarse roots		Total roots	
	Concentration (mg g ⁻¹)	Content (mg)	Concentration (mg g ⁻¹)	Content (mg)	Concentration (mg g ⁻¹)	Content (mg)
	<u>Means across all treatments n = 54 (8,9) or 13 (40,49)</u>					
8	29.58A ^a	35.37A	36.85A	35.90A	32.83A	71.27A
9	22.93B	20.00B	31.24B	16.13B	26.21B	36.13B
40	23.05a	22.31a	26.59a	12.27a	24.30a	34.58a
49	21.19a	18.53a	29.60a	14.45a	24.48a	34.98a
	<u>Rain pH (families 8 & 9) n = 18 per level</u>					
5.2	25.39A	27.56A	34.07A	28.06A	29.56A	55.62A
4.5	28.11A	29.20A	33.44A	26.09A	29.92A	51.78A
3.3	25.59A	26.77A	34.73A	25.01A	29.35A	55.29A

^aMeans within the same column are not significantly different at the p = 0.05 level if followed by the same letter. Capital letters compare families 8 and 9, lower case letters compare families 40 and 49.

ambient + 80 ppb ozone treatment than in seedlings grown under either charcoal-filtered or high (+ 160 ppb) ozone conditions. This increase in starch storage at low ozone concentrations occurred simultaneously with a slight increase in total root biomass relative to roots of control plants. Mean fine-root starch concentrations followed a similar bimodal response pattern, but the differences were not statistically significant. Rain pH did not significantly affect root starch concentrations or content, though total root starch content declined by 7% at the pH 4.5 treatment (Table 5.5).

5.3.1.4 Nutrient Concentrations

No nutrient deficiencies were detected in any of the applied treatments, based on foliar nutrient analyses (South and Davey 1983). With the exception of iron and manganese, mean nutrient concentrations were lower in the foliage of seedlings receiving the ambient + 160 ppb ozone treatment (Table 5.6). This difference was statistically significant in only a few cases, however. Significant differences in foliar nitrogen, potassium and aluminum concentrations were detected between ozone treatments, with lower concentrations of each of these elements in foliage of plants grown under the ambient + 160 ppb ozone regime. Manganese concentrations were significantly higher in foliage of trees receiving additional ozone.

Only foliar aluminum and manganese concentrations were found to vary significantly with pH of the rain simulant (Table 5.6). The lowest foliar aluminum levels were found in seedlings receiving pH 5.2 rain, while the lowest manganese levels were detected in seedlings receiving rain of pH 3.3.

A significant interaction of ozone and rain chemistry was detected for P, Fe, Al, and Mn (Figure 5.5). Phosphorus concentrations were significantly lower in seedlings grown with 160 ppb ozone relative to those grown with charcoal-filtered air at a rain pH of 4.5 ("ambient"). No significant differences were detected at other pH levels. At the low pH treatment, iron and aluminum levels were lower in plants grown under the 160 ppb ozone regime than in seedlings grown with

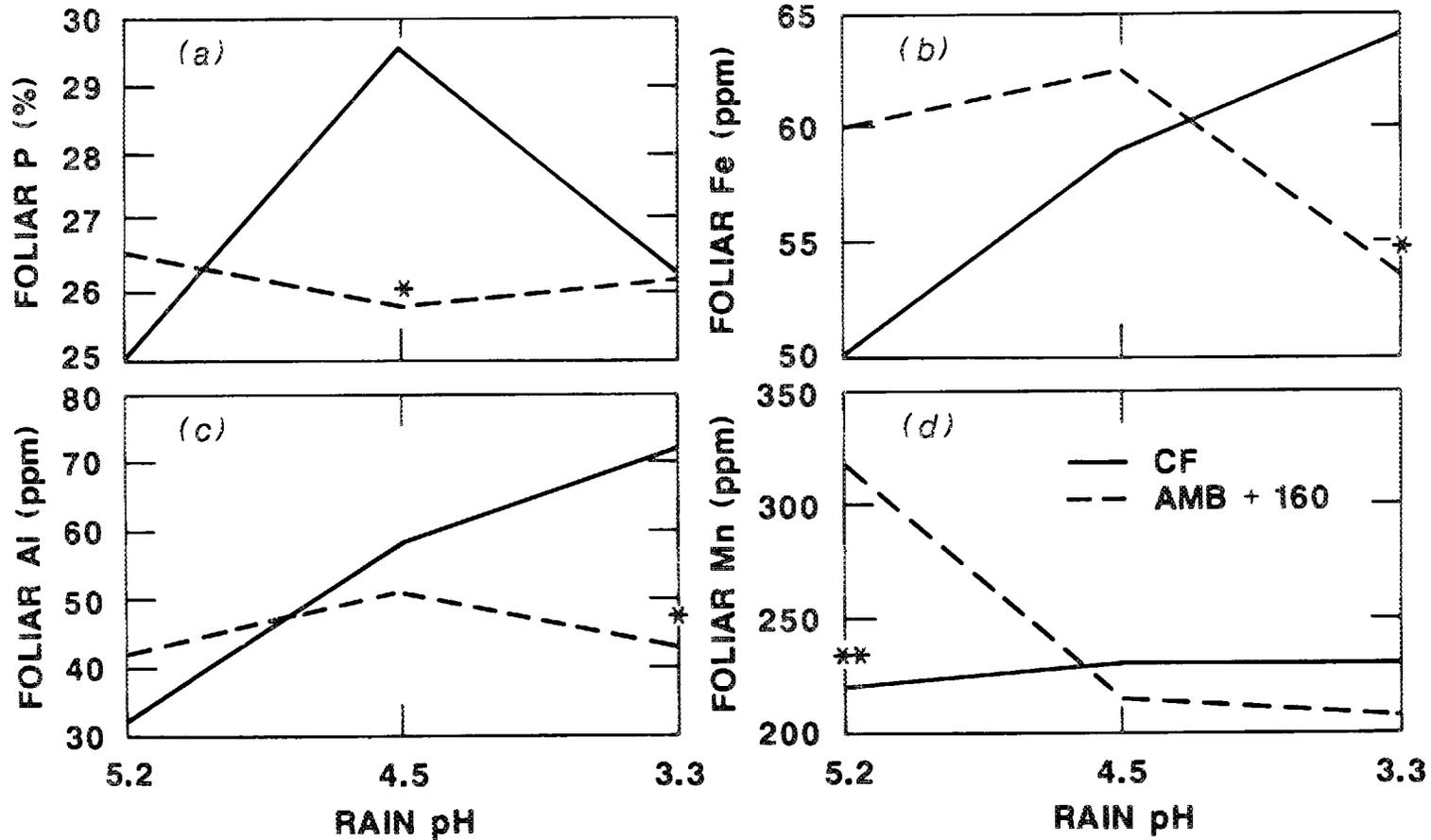


Fig. 5.5. Significant ozone-rain pH treatment interactions on foliar nutrient concentrations (field study). (a) phosphorus concentrations (b) iron concentrations (c) aluminum concentrations (d) Manganese concentrations. Asterisks indicate significant differences at $p \leq 0.05$ (*) and $p < 0.10$ (**) level.

Table 5.6. Foliar nutrient concentrations of loblolly pine seedlings by treatment (field study)

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Al (ppm)
<u>Ozone</u> n = 36 per treatment							
Charcoal-filtered	3.34a ^a	0.270a	1.415a	0.184a	0.160a	57.5a	54.6a
Ambient + 160 ppb O ₃	2.96b	0.262a	1.310b	0.174a	0.158a	58.6a	46.2b
<u>Rain pH</u> n = 24 per treatment							
5.2	3.11a	0.258a	1.363a	0.169a	0.157a	54.8a	37.8a
4.5	3.22a	0.276a	1.334a	0.186a	0.160a	60.6a	55.1b
3.3	3.11a	0.265a	1.391a	0.181a	0.160a	58.8a	58.2b
	B (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)			
<u>Ozone</u>							
Charcoal-filtered	126.2a	13.4a	227.2a	36.7a			
Ambient + 160 ppb O ₃	116.0a	8.0a	249.0b	35.8a			
<u>Rain pH</u>							
5.2	120.3a	8.8a	269.8a	36.3a			
4.5	121.9a	8.7a	224.8ab	34.1a			
3.3	121.2a	14.6a	219.7b	38.3a			

^aMeans within the same column and main treatment (ozone, rain) are not significantly different at the p = 0.05 level if followed by the same letter.

charcoal-filtered air. Manganese concentrations varied only at the pH 5.2 treatment.

Significant differences between families were also detected for foliar P, K, Fe, B, Cu and Mn concentrations.

5.3.2 CSTR Study

5.3.2.1 Biomass

Biomass of foliage and stems varied significantly among the three families examined (Table 5.7). Unlike the seedlings in the field study, however, family 9 allocated more biomass aboveground than family 8, and the root-to-shoot ratios of these two families were reversed in magnitude from the field study. Unlike family 8, family 9 grew faster in the CSTR study than in the field study. In addition root-to-shoot ratios were generally lower in the CSTR study than in the field study. Family 10 was consistently the smallest of the three families used in the CSTR study.

Of the biomass components examined only the coarse-root biomass was found to vary significantly with ozone level ($p \leq 0.10$) (Fig. 5.6). Coarse-root biomass of seedlings receiving 320 ppb O_3 was significantly less (-17%) than coarse-root biomass of the control seedlings; biomass of the ambient + 160 ppb ozone was intermediate (-6% compared to control). In general, R:S ratios were more consistent across ozone treatments in the lab study relative to the field study (See Sect. 3, Fig. 3.16). A similar pattern of decreasing foliar biomass was observed with increasing ozone level, but no significant differences among treatment levels were detected (Table 5.7).

A significant family-ozone interaction effect upon foliar biomass was found and is graphed in Fig. 5.7. Significant differences in the response by foliar biomass to varying ozone levels were detected primarily due to a relatively greater reduction of foliar weight at the 320 ppb treatment for family 9 than for families 8 or 10. Family 9 exhibited little response to the 160 ppb ozone treatment.

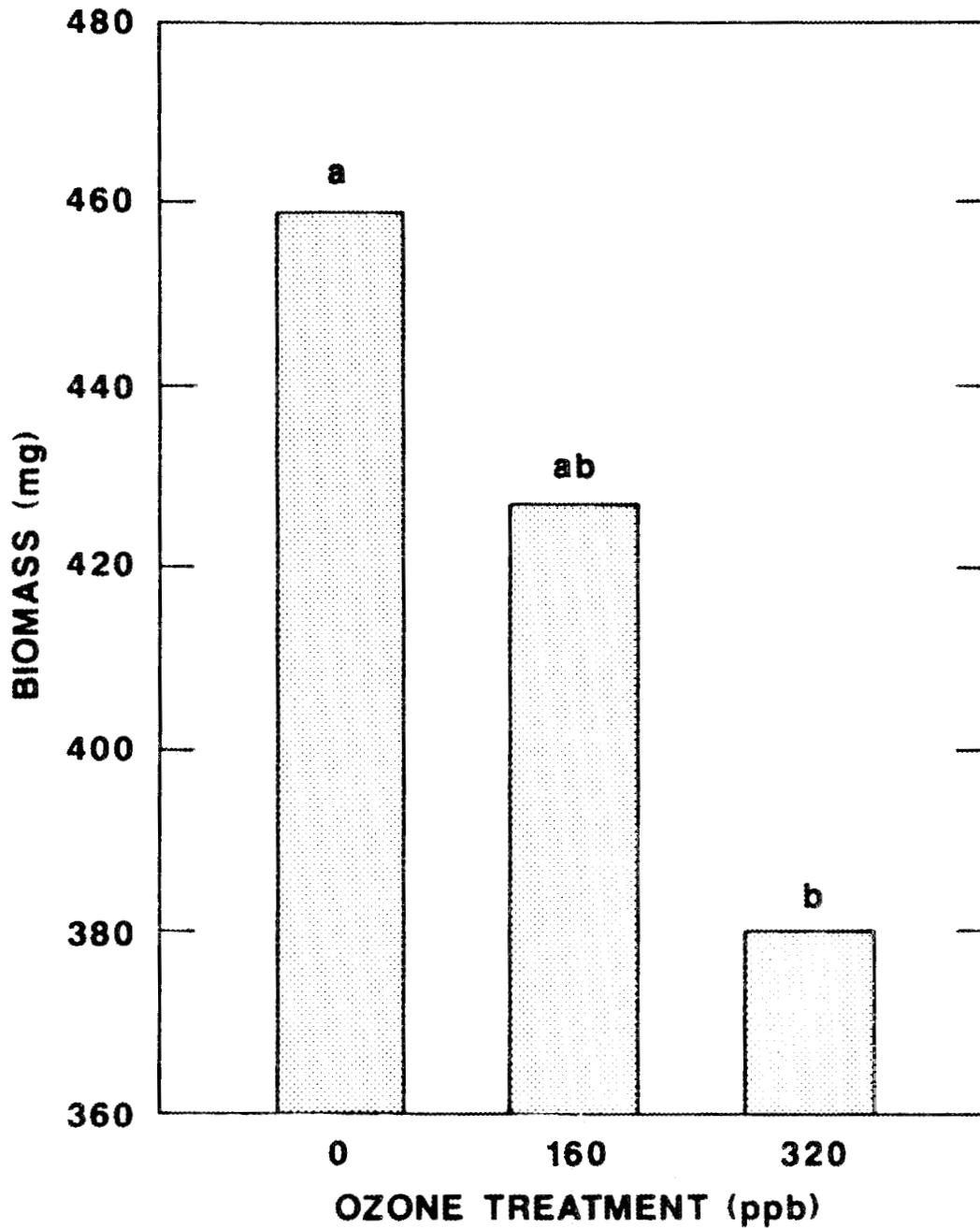


Fig. 5.6. Coarse root biomass by ozone treatment, families 8, 9 and 10 (CSTR study). Letters indicate significant differences at the $p < 0.10$ level of significance.

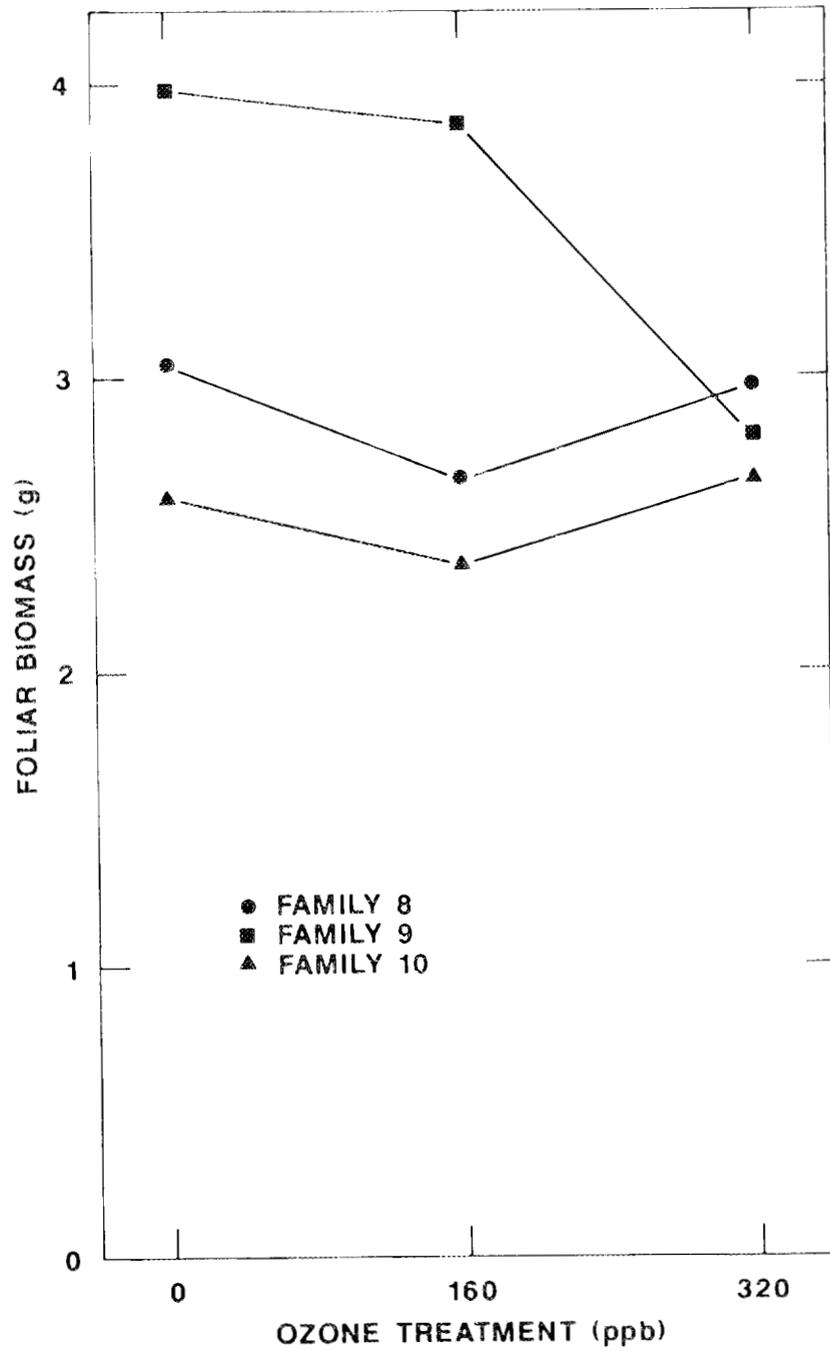


Fig. 5.7. Interaction of family and ozone level on foliar biomass (CSTR study).

Table 5.7. Biomass components of loblolly pine seedlings after 12 weeks of treatment (CSTR study)

	Foliage (g)	Stem (g)	Aboveground biomass (g)	Fine roots (g)	Coarse roots (g)	Total roots (g)	R:S
Family n = 15 (8,9,10)							
8	2.88a ^a	1.21a	4.09a	0.68a	0.41a	1.09a	0.268a
9	3.54b	1.20a	4.74a	0.59a	0.48a	1.07a	0.232b
10	2.54a	0.92b	3.46b	0.60a	0.37a	0.97a	0.280a
Ozone n = 15 per treatment							
0 ppb O ₃	3.23a	1.08a	4.32a	0.61a	0.46a ^b	1.06a	0.253a
160 ppb O ₃	2.96a	1.18a	4.14a	0.65a	0.43ab	1.08a	0.265a
320 ppb O ₃	2.81	1.07a	3.88a	0.61a	0.38b	0.99a	0.260a

aMeans within the same column are not significantly different at the p = 0.05 level if followed by the same letter.

bMeans within the same column are not significantly different at the p = 0.10 level if followed by the same letter.

5.3.2.2 ^{14}C Allocation

The pattern of loss/removal of the initial ^{14}C from the foliage during the week following tagging was similar to that observed for the field study trees (Fig. 5.8). A large decrease in foliar ^{14}C activity (25-35%) during the first 24 h was followed by a more gradual decline over the remainder of the week. However, retention by the foliage of the CSTR trees was higher than that of the field study trees at day 7, averaging 62% compared with 44% for the field study. Losses from the plant (mostly respiratory losses) varied among the ozone treatments at the $p \leq 0.10$ significance level (Table 5.8). A bimodal response was observed, with the largest relative losses (22%) from the seedlings receiving 160 ppb ozone. No differences in relative ^{14}C loss between the control plants and those receiving 320 ppb ozone were detected. This pattern was consistent across families, though the magnitude varied, with family 9 showing the largest response (Fig. 5.9). Families 9 and 10 had significantly higher respiration losses than did family 8.

The proportions of labeled photosynthate allocated to coarse roots and foliage varied significantly with ozone concentration ($p \leq 0.10$). For the coarse roots, the largest percentage (6.75%) was allocated to the coarse roots of control plants, and the percentage declined with increasing ozone concentration level (Table 5.8). This followed the same pattern as coarse-root biomass. The retention of ^{14}C by the foliage 1 week after tagging also showed a rather consistent pattern across ozone concentrations. Allocation of photosynthate to the stem varied significantly with family ($p \leq 0.05$), as did allocation to fine roots ($p \leq 0.10$), reflecting the differences in respiration losses.

The relative distribution of ^{14}C within the plant components on day 7 was somewhat different from that observed for field-grown seedlings (Table 5.9). Allocation to the roots was much lower (by 7 to 12% total activity) and foliar ^{14}C retention was higher (by 15 to 25%) in the CSTR-grown seedlings relative to field study seedlings. Lower light levels and less water stress in the CSTRs may have

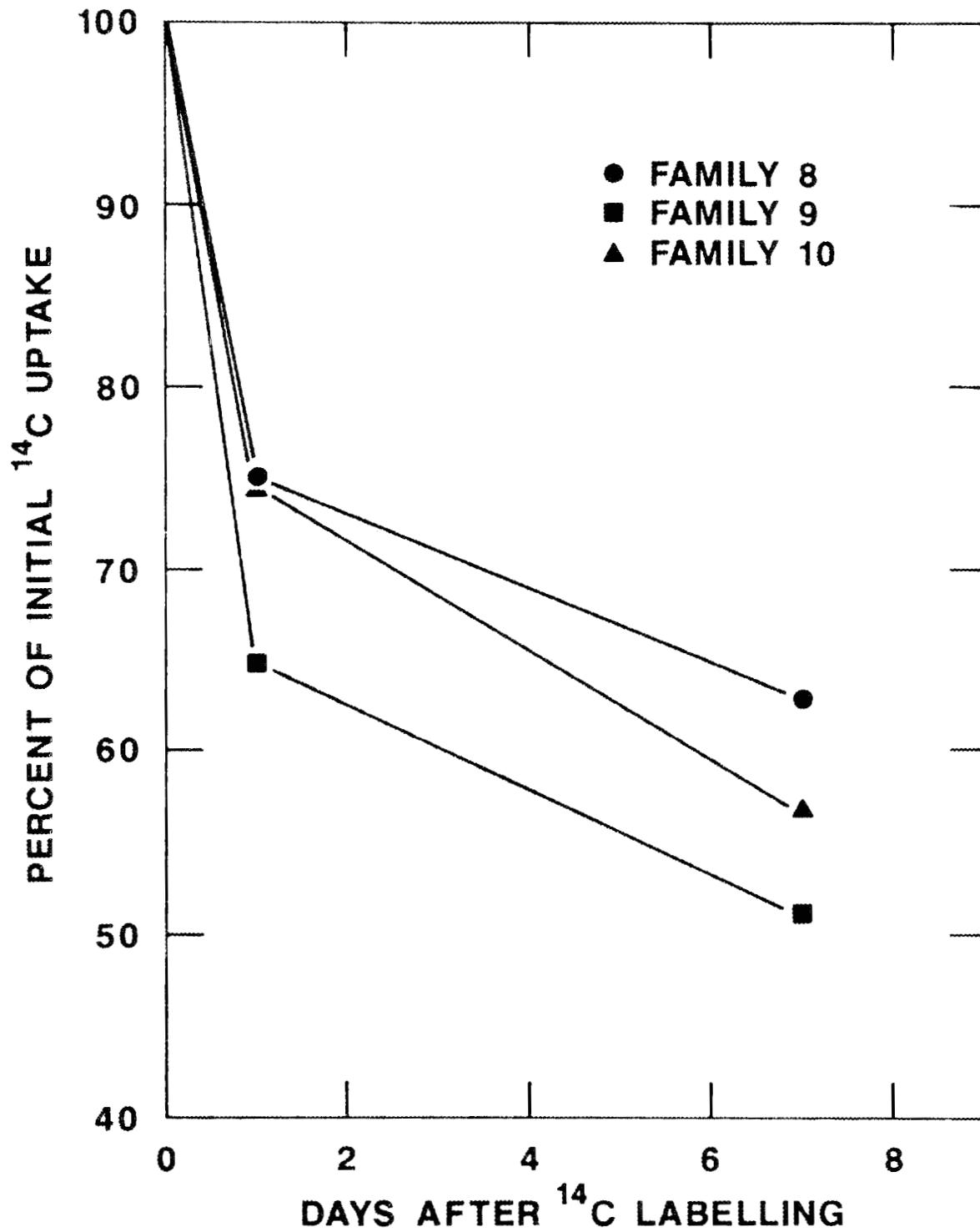


Fig. 5.8. Comparisons among loblolly pine families in retention of ^{14}C by foliage with time after labeling with $^{14}\text{CO}_2$ (CSTR study).

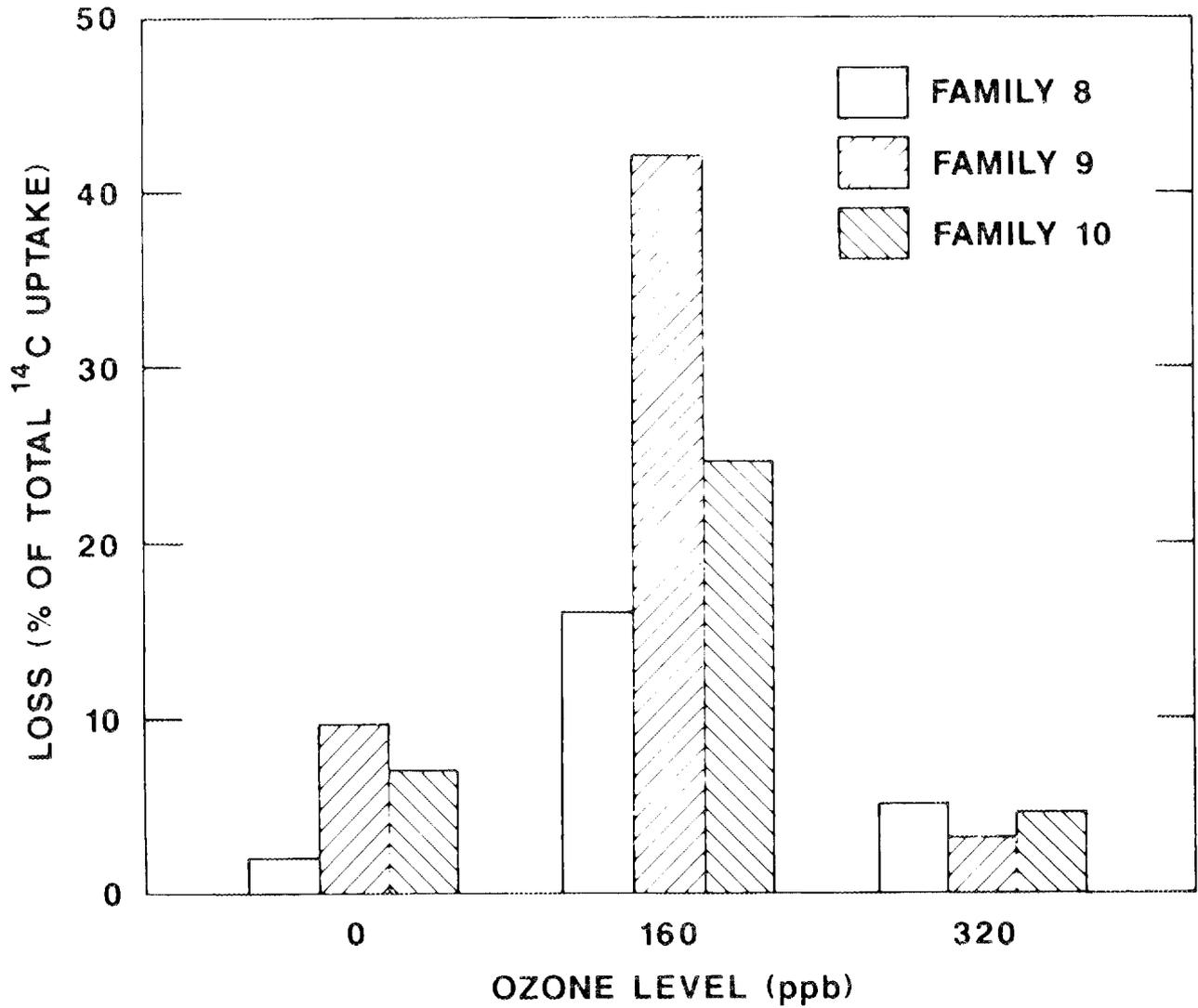


Fig. 5.9. Labeled (^{14}C) carbon losses from loblolly pine seedlings by family and ozone level (CSTR study).

Table 5.8. Allocation of ^{14}C label by loblolly pine seedlings 1 week after labeling, expressed as mean percentage of initial uptake (CSTR study)

	Fine roots	Coarse roots	Total roots	Foliage	Stem	Loss from plant
Family	<u>Means across all treatment (%) n = 46 (15 per family)</u>					
8	5.98ab ^a	5.81a	11.79a	63.90a	17.40a ^b	7.30a
9	5.22b	6.08a	11.30a	60.91a	14.77b	11.68b
10	6.74a	6.24a	12.97a	62.43a	13.49b	11.03b
Ozone	<u>Means across all families (%) n = 15 per treatment</u>					
0 ppb O ₃	5.92a	6.75a ^a	12.67a	64.98a ^a	14.65a	5.72 ^a
160 ppb O ₃	6.26a	5.98ab	12.24a	51.43b	15.77a	22.48b
320 ppb O ₃	5.68a	5.40b	11.08a	69.38a	15.22a	4.24a

^aMeans within the same column and main effect are not significantly different at the $p = 0.05$ level if followed by the same letter.

^bMeans within the same column and main effect are not significantly different at the $p = 0.10$ level if followed by the same letter.

Table 5.9. Allocation of ^{14}C in loblolly pine seedlings 1 week after labeling, expressed as percentage of total activity remaining in whole plant (CSTR study)

	Fine roots	Coarse roots	Total roots	Foliage	Stem
Family	<u>Means across all treatments (%) n = 46 (15 per family)</u>				
8	6.63ab ^a	6.75a	13.38a	67.16a	19.46a ^b
9	5.58b	6.19a	11.77a	72.38a	15.85b
10	7.76a	7.27a	14.75a	69.57a	15.40b
Ozone	<u>Means across all families (%) n = 15 per treatment</u>				
0 ppb O ₃	6.20a	7.05a	11.68a	71.46a	15.29a
160 ppb O ₃	7.65a	7.33a	14.98a	65.05a	19.96a
320 ppb O ₃	6.00a	5.73a	13.25a	72.71a	15.56a

^aMeans within the same column and main effect are not significantly different at the $p = 0.05$ level if followed by the same letter.

^bMeans within the same column and main effect are not significantly different at the $p = 0.10$ level if followed by the same letter.

contributed to this difference. No significant differences in ^{14}C allocation among treatments 1 week after labeling were detected, though mean allocation to the roots was highest at 160 ppb ozone and allocation to foliage lowest at the same concentration. Family differences in allocation to fine roots and to the stem were significant ($p \leq 0.05$ and 0.10 , respectively), with family 9 allocating the least to the fine roots.

5.3.2.3 Starch

Root starch concentrations were considerably lower than those in the field study and generally declined with increasing ozone additions (Table 5.10). This pattern reflects the lowering of R:S with treatment. These decreases were statistically significant only for total root starch concentration, however, which was reduced 19% by the 320 ppb ozone treatment relative to the 0 ppb ozone treatment. Total starch content also differed among treatments, with the lowest levels found in roots of seedlings grown under the highest ozone level. Coarse-root starch content was decreased by 25% in roots at 320 ppb ozone ($p \leq 0.10$) and coarse roots appeared more affected than fine roots. Differences among families were not statistically significant, though family 8, which contained the most starch in the field study, had the lowest average starch content in the CSTR study.

5.3.2.4 Nutrients

There were no indications of nutrient deficiencies or severe imbalances in these seedlings (Table 5.11). Micronutrient concentrations were generally lower in seedlings grown under 160 ppb ozone, though ozone effects were significant only for the micronutrients Fe, Cu, Mn, and Zn. For each of these micronutrients, the lowest concentrations were found in foliage of trees receiving 320 ppb ozone. Unlike the field study, no significant differences in foliar N were detected among ozone treatments. Significant differences in concentrations of N, P, K, Fe, B, Cu, and Mn were again detected between the two families (2 and 5).

Table 5.10. Starch concentration and content of loblolly pine roots after 13 weeks of treatment (CSTR study)

	Fine roots		Coarse roots		Total roots	
	Concentration (mg g ⁻¹)	Content (mg)	Concentration (mg g ⁻¹)	Content (mg)	Concentration (mg g ⁻¹)	Content (mg)
<u>Family</u> n = 15 per family						
8	10.32a ^a	6.84a	18.85a	7.63a	13.35a	14.47a
9	9.29a	5.70a	19.16a	9.73a	13.79a	15.43a
10	11.88a	7.23a	21.96a	8.57a	15.81a	15.80a
<u>Ozone</u> n = 15 per treatment						
0 ppb O ₃	11.82a	7.12a	20.62a	9.83a ^b	15.33a	16.95a
160 ppb O ₃	10.92a	7.19a	21.92a	9.41a	15.27a	16.60a
320 ppb O ₃	8.76a	5.46a	17.43a	6.69b	12.35b	12.16b

^aMeans within the same column and family or treatment are not significantly different at the p = 0.05 level of significance if followed by the same letter.

^bMeans within the same column are not significantly different at the p = 0.10 level of significance if followed by the same letter.

Table 5.11. Foliar nutrient concentrations of loblolly pine seedlings (CSTR study)^a

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Al (ppm)
<u>Ozone</u> n = 12 per treatment							
0 ppb O ₃	2.23a	0.242a	1.097a	0.189a	0.142a	45.40a	72.07a
160 ppb O ₃	2.16a	0.231a	1.045a	0.185a	0.139a	48.32a	70.30a
320 ppb O ₃	2.24a	0.250a	1.115a	0.195a	0.151a	36.70a	59.18a
<u>Family</u> n = 18 per family							
2	2.22a	0.241a	1.086a	0.189a	0.144a	43.47a	67.18a
5	2.34b	0.264b	0.996b	0.178a	0.137a	47.47b	59.18a
	B (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)			
<u>Ozone</u>							
0 ppb O ₃	24.1a	7.7a	303.2a	36.1a			
160 ppb O ₃	21.1a	8.0a	251.8a	38.3a			
320 ppb O ₃	21.2a	6.6b	164.7b	32.1a			
<u>Family</u>							
2	24.6a	7.1a	257.8a	36.7a			
5	19.7b	7.8b	222.1b	34.2a			

^aMeans within the same column and treatment are not significantly different at the p = 0.05 level of significance if followed by the same letter.

5.4 DISCUSSION

The most obvious differences in carbon allocation found in these experiments were associated with the different families. Loblolly pine has a broad genetic base, thus the differences in carbon partitioning and nutrient utilization among families is perhaps not surprising. Sensitivity to ozone has been found to vary among loblolly pine genotypes (Adams et al. 1988, Kress et al. 1982, Wier 1977). From these studies, a significant family-ozone interaction on carbon allocation might be expected. (A significant interaction might suggest that the families varied in their mechanism of response to ozone). While a statistically significant an effect was detected only for foliar biomass in the CSTR study, there were several allocation patterns that were of interest in evaluating likely mechanisms of ozone impact at the whole plant level. However, a relatively small number of families was used in this study as were an equally small number of ozone treatments. The families examined also were less sensitive to ozone than the average of all families in the study.(see Section 3). More combinations would have been desirable to more clearly characterize such allocation responses.

Of the $^{14}\text{CO}_2$ initially taken up by the seedlings, the majority of it moved very quickly (within 24 h) from the foliage to the stems and roots in both studies. Carbon allocation patterns of seedlings in the CSTR study varied somewhat from those observed in the field study, with higher retention by foliage at the expense of the roots in the CSTR study. This is probably a result of different environmental conditions inherent to the CSTR and field studies. One hypothesis is that lower light levels in the CSTR study contributed to decreased carbon allocation to the roots. Van den Dreissche (1987) found new root growth to be proportional to light intensity. The much lower root starch concentrations and contents in the CSTR study relative to the field study support this hypothesis. Lower root starch concentrations could also have resulted if a faster rate of growth had been observed, as a consequence of diminished photosynthesis and carbohydrate production, or from altered carbon allocation patterns. With the

exception of family 9, which grew faster in the CSTR study than in the field study, seedlings consistently grew more slowly under laboratory conditions. Thus it is unlikely that lower root starch concentrations were the result of lower growth rates under these conditions. Although water stress was believed to be lower in the CSTR study, diminished carbohydrate production or altered carbon partitioning (R:S ratios were substantially lower than in the field study) in response to lower light levels are more likely explanations for the observed differences between the two studies. Ozone has been shown to reduce photosynthesis in a number of tree species (Reich and Amundson 1985), which could lead to decreased carbon allocation to roots (Jensen 1981). McLaughlin and coauthors (1982) suggest that in addition to decreased photosynthate production, increased utilization of carbon for repair of damaged foliar tissue may occur in response to chronic air pollutant stress. Though elevated ozone significantly reduced coarse-root biomass in the CSTR study, root:shoot biomass ratios were not significantly affected by the elevated ozone treatments, suggesting a decrease of photosynthate production rather than a change in carbon partitioning. However, net photosynthesis did not vary appreciably between the lab and the field (Sect. 4). Increased respiratory losses in response to ozone fumigation have been documented (Barnes 1972) and could also divert carbon resources from the root systems. While no changes in foliar respiration were noted during gas exchange measurements (see Section 4.3.1), increased respiratory losses resulting from elevated ozone were observed in both of the current studies and were found to be statistically significant in the CSTR study at a concentration of 160 ppb ozone but not at 320 ppb. These higher losses were accompanied by small, weakly significant or nonsignificant reductions in ^{14}C allocation to the root systems of these trees (reductions of 11.2 and 3.4%, field and CSTR, respectively). Changes in respiratory losses could foreshadow further, more significant shifts in carbon allocation patterns. Such shifts in carbon allocation could have significant implications for water stress tolerance (Cannell 1986), nutrient

uptake, mycorrhizal associations (Adams et al. in press) and other important plant processes.

In the field study, the highest root starch concentrations were found in the coarse roots of trees receiving the ambient + 80 ppb ozone treatment, while concentrations in roots of ambient + 160 ppb ozone trees were not significantly different from those in roots of control plants. This probably reflects a slight stimulation in carbon allocation and biomass production at the intermediate level of ozone. This stimulatory effect on growth was seen in most of the families (see Sect. 3), but the cause as yet is unidentified. A similar unexplained stimulatory effect was observed for red spruce (Taylor et al. 1986).

In the CSTR study, no statistically significant differences in root starch concentration or content were detected until the 320 ppb ozone level. At this treatment level, relative carbon allocation to the root system decreased by only 12.5% (not statistically significant), while the amount stored as starch decreased by 28%. Thus, storage and utilization of reserve carbohydrates was more affected than was allocation of current photosynthate. This is likely to be a chronic accumulation effect. Cooley and Manning (1987) found that plants appear to use accumulated starch to maintain a steady growth rate regardless of light or darkness in the diurnal cycle. Thus, if photosynthesis is adversely affected by ozone, plant levels of sucrose and other soluble sugars may increase as starch reserves are mobilized to accommodate decreased photosynthate production. If photosynthetic production is impacted over a long period of time, the resulting diminished carbohydrate reserves could prove insufficient to meet the needs of trees that rely on stored carbohydrates for dark respiration or spring growth, for example. Such trees may become less able competitors if reserves are limited because of ozone stress. McLaughlin et al. (1982) hypothesized that the decline of ozone-sensitive field-grown white pine was a result of both increased respiratory activity and altered carbon allocation patterns.

In both field and laboratory studies, concentrations of micronutrients were lowest at the 160 ppb ozone treatment level. Lower

foliar nutrient levels in ozone-stressed trees might suggest decreased nutrient uptake, because of decreased root biomass, or perhaps increased leaching because of damaged foliar tissue. However, nutrient levels in these plants did not approach published deficiency levels (South and Davey 1983, Stone 1985), and such effects are not indicated by the magnitude of the changes in nutrient levels. However, long-term, significant decreases in nutrient levels could contribute to declining photosynthesis (Reid et al. 1983, Linder and Axelsson 1982), altered carbon allocation (Linder and Rook 1984), and impacts on tree health and vigor.

Rain pH was a significant treatment effect only for root biomass, with the largest root biomass at the ambient (pH 4.5) treatment. Micronutrient concentrations were significantly altered by rain pH, but no patterns were evident.

No interactions of ozone and rain pH were detected for any of the biomass components, carbon allocation, or starch concentrations in either the field or CSTR study. Such interactions have often been hypothesized (Chappelka and Chevone 1986, Reich et al. 1986, Taylor et al. 1986, Elliott et al. 1987) but seldom documented. Interactions of ozone and rain pH on growth for the larger study were found to be antagonistic (see Sect. 3). The relatively small number of plants and the inherent high variability may have precluded detection of an interaction in the two studies discussed in this chapter. Interactions were detected for several micronutrients, but the patterns were variable and firm conclusions cannot be made at this time.

Analysis of patterns of carbon partitioning and starch accumulation revealed a significant relationship between tissue starch levels and biomass partitioning. These two parameters reflected the lowering of R:S ratios with ozone treatment and appeared to be more sensitive indicators of ozone stress than traditional biomass measures.

One of the primary objectives of these studies of carbon allocation was to evaluate various indicators of physiological response in terms of their usefulness for indicating the extent of pollutant - induced stresses. In this study we have evaluated three potentially

useful indicators - biomass, distribution of C-14 photosynthate, and root starch concentration. The preceding discussion has indicated that in general the root systems appear to be a primary site of ozone induced stress. A comparison of ozone-induced effects on these three response parameters is presented in Table 5.12. It can be concluded from these comparisons that, in general, changes in allocation of C-14 photosynthate to roots are a useful indicator of changes in both biomass allocation and starch at the highest ozone treatment levels. At intermediate ozone levels reduced allocation of C-14 to roots was less consistently related to the direction of changes in biomass or starch levels. The lack of a consistent relationship and the fact that changes in biomass or starch were sometimes opposite in direction from changes induced in C-14 allocation may in fact indicate that reduced allocation to root systems was a response that developed only after longer duration exposure at these lower ozone levels. Longer term studies involving sequential sampling of these indicators would be needed to evaluate whether trends in C-14 partitioning noted in these studies are early warning signs of physiological disfunction. The responses observed at the higher ozone levels suggest that in fact this may be the case.

5.5 SUMMARY

Strong genetic differences in growth, carbon allocation, and foliar nutrient concentrations of loblolly pine were observed in both the field study and the CSTR study. Although a statistically significant genotype - ozone interaction was detected only for foliar biomass in the CSTR study, the families did differ in sensitivity to ozone. Whole-plant carbon allocation patterns among families within each study were consistent, proportions allocated to roots and foliage as well as growth varied between the field and CSTR study. This is hypothesized to be primarily a result of lower light levels in the CSTR study. The primary effects of elevated ozone were increased respiratory losses of carbon and decreased carbon allocation and starch concentrations in the roots. Coarse-root biomass also decreased with

Table 5.12. Comparative responses of transport of ^{14}C photosynthate to roots, root biomass, and starch concentration of roots in responses to ozone treatments of loblolly pine seedlings in laboratory and field environments. (see Tables 5.2, 5.3, 5.8 and 5.10 and Figure 5.4 for date on which those responses are based).

Conditions	Response	Tissue Type	a Response (%)	
			A80	A160
Field	Allocation of ^{14}C	Fine roots	-6	-18
		Coarse roots	-18	-18
	Allocation of biomass	Fr	+9	-3
		Cr	0	-4
	Starch concentration	Fr	+20	+8
		Cr	+21	-7
Laboratory	Allocation of ^{14}C	Fr	<u>160</u> -6	<u>B20</u> -4
		Cr	-11	-20
	Allocation of biomass	Fr	+6	0
		Cr	-6	-17
	Starch concentration	Fr	-8	-26
		Cr	+6	-16

^aResponses are expressed as a % of controls values.

ozone fumigation. Rain pH had little effect on carbon allocation or root starch concentrations, nor were any significant ozone - rain pH interactions detected. Ozone fumigation in the field resulted in slightly decreased micronutrient (N and K) and Aluminum concentrations, while acidic rain significantly increased foliar levels of Aluminum (+52%) and reduced foliar Mn levels.

The families selected for allocation studies were not among the most sensitive examined in the larger study (see Section 3). However, the trends in allocation of biomass and the fate of ^{14}C photosynthate from support the labelling studies support our original hypothesis that the root system may be an initial site of pollution-induced stress. In general the reduction in allocation of carbon belowground determined from the radiochemical experiments was supported by observed differences in the root starch levels and trends in root biomass. Thus the allocation techniques used in these studies may provide a sensitive early warning signal for incipient changes in allocation of dry matter to the belowground system. Experiments with more pollution-sensitive families or longer term experiments where growth patterns are more completely developed in response to applied treatments would be useful in more definitively evaluating these relationships.

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6. MYCORRHIZAL RESPONSES TO OZONE AND ACID DEPOSITION

E. G. O'Neill

6.1 INTRODUCTION

Although a considerable amount of research is being conducted on direct, aboveground impacts of both ozone and acid precipitation on forest tree species, very little attention has been given to potential indirect impacts on belowground processes. Interactions of rhizosphere organisms and root symbionts (particularly mycorrhizae) with tree species are frequently ignored in speculation about forest responses. This is a serious oversight, especially since reduced nutrient acquisition and increased root pathogen invasion are often invoked as causes or consequences of pollutant exposure. Both of these phenomena could result from a reduction in the effectiveness of mycorrhizal symbiosis.

Stresses on the mycorrhizal system could result from pollutant exposure via effects on the host tree, or from direct toxic effects of trace element mobilization due to acid precipitation. The results of studies examining the effects of ozone on mycorrhizae are conflicting. Ozone exposure could reduce photosynthate translocation, thus limiting mycorrhizal infection, since the fungal partner is dependent on host-derived carbon. However, Reich and coworkers (1986) reported that mycorrhizal infection of northern red oak seedlings increased following exposure to ozone, and Mahoney et al. (1985) detected no effect of ozone on loblolly pine mycorrhizae. McCool and Menge (1983) observed dramatic (up to 63%) reductions in the infection of endomycorrhizal tomatoes. In spite of these reductions in mycorrhizal status, dry weights of mycorrhizal tomatoes exposed to 300 ppb ozone were less than those in nonmycorrhizal plants similarly exposed. The authors proposed that ozone exposure changed the nature of the mycorrhizal association from symbiotic to "pathogenic", because of competition for carbon resources between the host and the fungal symbiont. It is important at this point to note that McCool and Menge were working with endotrophic

mycorrhizae. Ectotrophic mycorrhizae, such as those associated with pine species, although likewise dependent on the host plant for their carbohydrate supply, belong to a completely different taxonomic group than do the endomycorrhizas. Consequently, the morphology, physiology, and the nature of interaction with their host can be very different from those found in endosymbiotic relationships.

Complication increases when one considers the potential for interactions of ozone with acidic precipitation (Reich et al. 1986). In this case, mycorrhizae are subject not only to indirect effects through host response, but they are also vulnerable to changes in soil pH, soil nutrient status, and heavy metal solubilization. Visser et al. (1987), in a report for the Acid Deposition Research Program on the potential effects of acid precipitation on soil microbial populations and processes, stated that effects of acid rain on ectomycorrhizae are likely to be negligible. Basidiomycetous fungi (the group to which most of the ectotrophs belong) are naturally acid adapted and might not be expected to show a response to changes in soil pH that might realistically result from acid precipitation. This assumption conflicts in some aspects and agrees in others with Shafer et al. (1985), who found a quadratic relationship of loblolly pine mycorrhizal colonization with rain acidity, where moderate pH (4.0 and 3.2) inhibited infection and increased acidity (pH = 2.4) enhanced infection. Stroo and Alexander (1985) also reported changes in mycorrhization with Pisolithus tinctorius on white pine; however, these changes were linked this to soil type and anion specie as well as rain pH.

The objective of this preliminary experimental work was to quantify mycorrhizal infection responses to ozone and simulated acid precipitation and to look for differences in response in two families of commercially grown loblolly pine.

6.2 METHODS

6.2.1 Field

Mycorrhizal assessment was performed on two of the common families (8 and 9), that had been subjected to other intense physiological measurements. Seedlings of these two families from selected ozone/acid rain combinations were harvested after 0, 6, and 12 weeks' exposure in the chambers (Table 6.1). At harvest in 6 weeks, all rain pH levels in the control-filtered and ambient air plus 160-ppb treatments were included; however, in the ambient air plus 80-ppb treatment, only seedlings exposed to a rain pH of 4.3 were assessed. Eight replicate seedlings (maximum) were examined for each family/ozone/pH combination at harvest in 6 weeks. At harvest in 12 weeks, seedlings from all pH levels were assessed for each ozone level included. Twelve replicate seedlings (maximum) were assessed for each family/ozone/pH combination at this harvest. Time zero mycorrhization was determined as the mean of seven seedlings from each family just prior to initiation of exposure.

The percent infection of short roots by mycorrhizae was assessed using visual estimation (Grand and Harvey 1982). At each harvest, seedlings were "read" in groups of 15 to 20 after being nonsystematically assigned code numbers to minimize subjectivity in measurements. Each seedling was examined, and the estimated percent of mycorrhizal short roots was determined to the nearest 5%. Groups of seedlings were examined twice in random order, with the individual performing the assessment on the second trial unaware of results from the first. When the difference between the first and second assessment exceeded 15%, the seedling was examined a third time. The percent mycorrhizal infection for each seedling was recorded as the mean of two or three trials. In this manner, standard errors of measurement were maintained at less than 5% of the mean. Data for this and the Continuously Stirred Tank Reactor (CSTR) study were arc-sine transformed before analysis by ANOVA.

Table 6.1. Levels of ozone and simulated acid rain used in screening studies of 53 loblolly pine families.

Rain ph	Ozone treatment ^a					
	AO	AC	CF	A40	A80	A160
3.5			X		X	X
4.3	X	X	X	X	X	X
5.0			X		X	X

^aAO = ambient air, no chamber; AC = ambient air, chamber; CF = charcoal-filtered air; AXX = ambient air plus XX parts per billion ozone.

^bBoxed X's indicate those treatments in which seedlings were assessed for mycorrhizal colonization

6.2.2 Laboratory

Seedlings from the same two families used in the field study were assessed in laboratory exposures. In this case, simulated rain pH was held constant at 4.3, and the seedlings were exposed to charcoal-filtered air plus 0-, 160-, or 320-ppb ozone. Mycorrhizal assessment was performed in the same manner as in the field exposures.

6.3 RESULTS

Percent colonization by Pisolithus in all seedlings was lower at the conclusion of both studies than expected, given the extent of infection at the initiation of exposure. After 24 weeks' growth (including the 12-week treatment period), a mean infection level of 70% or greater in the controls would not have been unusual. Actual colonization ranged from 20 to 55% in the control treatments. The percent mycorrhizal colonization (PMC) changed very little over the treatment period regardless of family, treatment, or study. Seedlings were relatively well fertilized (see Section 1.4), which could account for some degree of inhibition since high plant nutrient status has been linked to mycorrhizal depression. In the CSTR study, low light levels might also have contributed towards a general reduction in mycorrhizal infection percentages.

6.3.1 Field

The overriding main effect for all parameters measured was family. Root infection by mycorrhizae was greater in family 8 at all harvests ($p < 0.0001$ at the 6- and 12-week harvests), although prior to the beginning of the exposure period, there was no significant difference in mycorrhization of the two families. The initial PMC was 40.6% in family 8 and 32.6% in family 9.

There were no interactions of family, ozone, or rain pH in the percentage of roots colonized for either harvest, nor was PMC affected by rain pH as a main effect (Table 6.2). Ozone effects were consistent across both families and were significant at 6 and 12 weeks at $p \leq 0.08$ and $p \leq 0.10$, respectively (Fig. 6.1). At 6 weeks, mycorrhization in

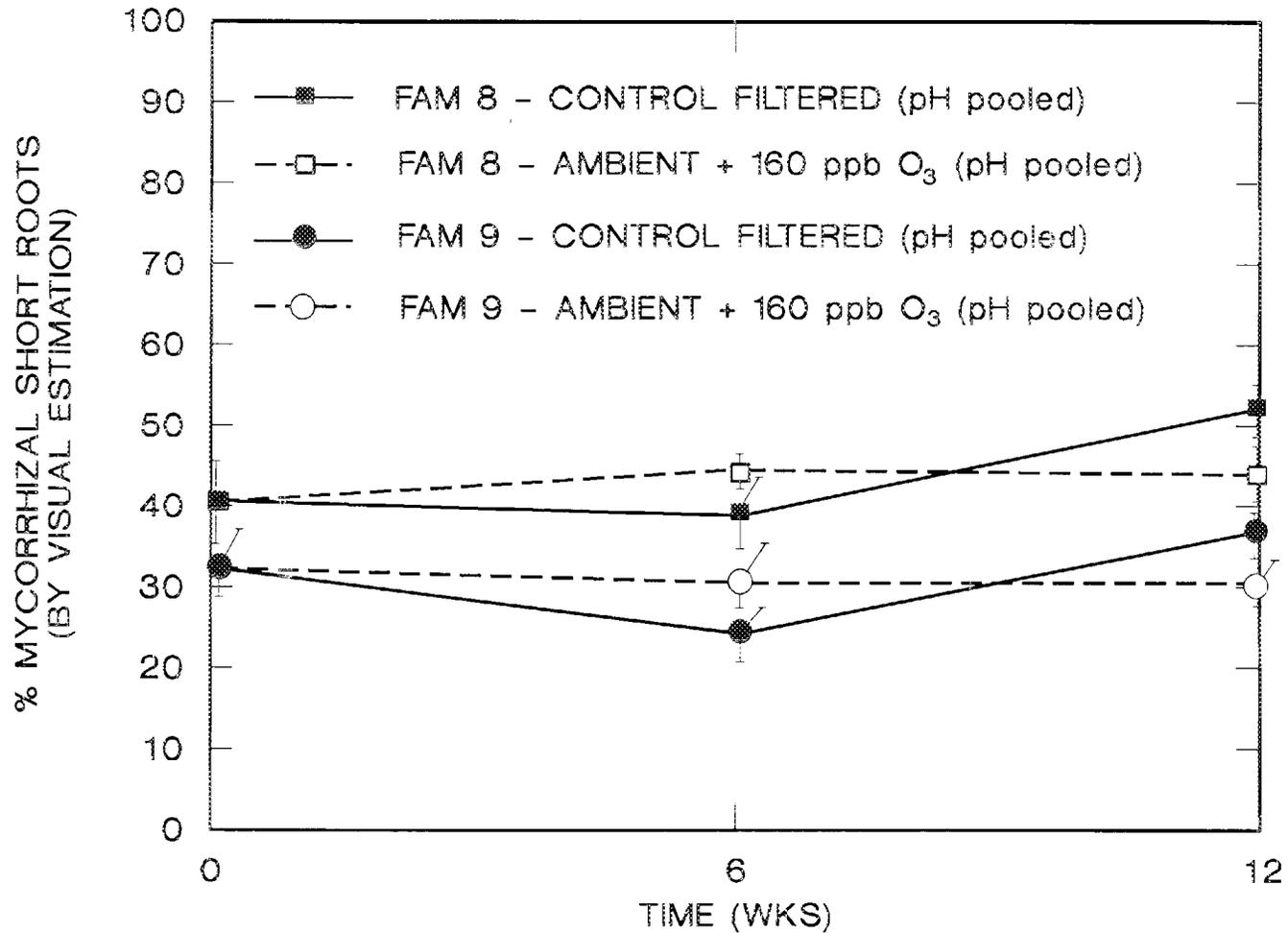


Fig. 6.1. Percent short roots mycorrhizal with *Pisolithus tinctorius* in two families of loblolly pine exposed to charcoal-filtered air or ambient air plus 160 ppb ozone. For purposes of illustration, pH treatments were pooled within families and within ozone and filtered-air treatments. Data points represent means of 6 to 8 seedlings (0, 6 weeks) or 8 to 12 seedlings (12 weeks) \pm 1 S.E.

Table 6.2. Percent mycorrhizal colonization of loblolly pines in the field study. Data represents the mean \pm standard deviation.

Time	pH	CF	Ozone treatment	
			Amb + 80	Amb + 160
Family 8 -- 40.6% mycorrhizal short roots at time zero				
6 weeks	3.5	32.8 \pm 16		43.8 \pm 11
	4.3	44.5 \pm 18	32.9 \pm 9.5	45.0 \pm 9
	5.0	39.3 \pm 10		40.4 \pm 9
12 weeks	3.5	54.8 \pm 15	43.9 \pm 9	45.2 \pm 17
	4.3	54.0 \pm 15	50.4 \pm 12	44.7 \pm 20
	5.0	44.6 \pm 11	49.5 \pm 20	45.0 \pm 14
Family 9 -- 32.6% mycorrhizal short roots at Time Zero				
6 weeks	3.5	20.8 \pm 12		29.0 \pm 16
	4.3	26.0 \pm 10	29.0 \pm 18	34.2 \pm 16
	5.0	26.7 \pm 11		34.0 \pm 16
12 weeks	3.5	35.0 \pm 17	34.1 \pm 26	25.8 \pm 11
	4.3	36.8 \pm 10	28.1 \pm 16	37.8 \pm 11
	5.0	36.6 \pm 12	24.3 \pm 14	26.4 \pm 5

aCF = Control-filtered; Amb + XX = Ambient air plus XX ppb ozone.

the seedlings grown in charcoal-filtered air was less than that measured at time zero and less than that on seedlings exposed to ozone, lending some support to the idea that very low levels of ozone (in this case interns of duration of exposure) have a stimulatory effect as compared to a charcoal-filtered air environment. The slight depression that occurred in the control-filtered treatments for both families did not occur in the seedlings exposed to ambient air plus 160 ppb ozone.

At the 12-week harvest, however, seedlings exposed to ozone were significantly less mycorrhizal than controls, and equally or less mycorrhizal than the ozone-treated seedlings from the 6-week harvest. Several seedlings in the high ozone treatment, for family 9, had visibly abnormal mycorrhizae with reduced fine roots and dark-colored mycorrhizal tips, although no attempt was made in these assessments to differentiate between viable and nonviable mycorrhizae.

6.3.2 Laboratory

Family differences in PMC were again observed in the CSTR exposures, with mycorrhization of family 8 at all harvests greater than family 9 (Table 6.3). A third family (No. 10), was included in this set of assessments and responded similarly to family 8.

Ozone had no effect on PMC in the CSTR study. In fact, PMC changed very little from the initiation of exposure until 12 weeks later when the exposures were concluded. Root systems in general appeared healthier (by subjective criteria) in the CSTR study than in the field study.

6.4 CONCLUSIONS

The extent of infection by mycorrhizae was clearly related to family origin in these seedlings. It cannot be determined by these screening studies whether different degrees of mycorrhization are responsible for, or a consequence of, differences in biomass and reaction to ozone. It is possible that what is considered to be "resistance" or "susceptibility" to any stress in different loblolly

Table 6.3. Percent mycorrhizal colonization of loblolly pines in the GSTR study. Data represents the mean \pm standard deviation.

Time	pH	CF	Ozone treatment	
			CF + 160	CF + 320
Family 8 -- 40.6% mycorrhizal short roots at time zero				
6 weeks	4.3	44.5 \pm 11	37.5 \pm 12	38.2 \pm 12
12 weeks	4.3	44.3 \pm 19	43.2 \pm 13	37.1 \pm 11
Family 9 -- 32.6% mycorrhizal short roots at time zero				
6 weeks	4.3	25.8 \pm 5	27.1 \pm 12	29.4 \pm 16
12 weeks	4.3	20.0 \pm 11	27.5 \pm 9	32.1 \pm 22
Family 10 -- 35.8% mycorrhizal short roots at time zero.				
6 weeks	4.3	37.1 \pm 12	25.8 \pm 6	33.3 \pm 15
12 weeks	4.3	35.0 \pm 10	36.7 \pm 14	40.0 \pm 19

^aCF = Control-filtered; CF + XX = Control-filtered air plus XX ppb ozone.

pine families is tied to infection patterns of their symbiont, even though in this series of exposures, family did not interact with ozone.

Mycorrhization in both families examined in the field study was reduced by 12 weeks' exposure to ozone. Fine root starch concentrations in both ozone treatments were increased relative to controls (Table 6.4). Slankis (1973) presented evidence from several sources that mycorrhizal auxins hydrolyse starch to soluble sugars. A reduction in mycorrhizal infection would be reflected in higher fine root starch concentrations. Results from biomass measurements and carbon allocation patterns (see Section 5) support the generally held presumption that reduction in translocation to the belowground system will negatively affect mycorrhizal numbers and possibly mycorrhizal function. In this study, unlike that of McCool and Menge (1983), there was no suggestion of a shift towards preferential allocation of photosynthate to mycorrhizal tissue at the expense of the host. In fact, relative reductions in PMC were larger than reductions in fine root biomass (Table 6.4), suggesting just the opposite. Reduced PMC may be a precursor to losses of fine root vigor.

As predicted by Visser and coworkers (1987), acid deposition within the range examined here had no effect on quantifiable mycorrhizal infection. However, no measure of mycorrhizal "effectiveness" was employed in this study. Mycorrhizal benefits to host species are not always simply correlated with numbers of infected root tips; consequently, failure to find changes in PMC because of acid deposition does not rule out the possibility of a change in mycorrhizal effectiveness. Further study should examine some aspect of mycorrhizal function under both ozone and acid deposition exposures, to more completely clarify the mycorrhizal contribution to tree response to pollutants.

Table 6.4. Comparison of mycorrhizal infection at 12 weeks transport of ^{14}C photosynthate to fine roots, fine root biomass, and fine root starch concentrations in response to ozone exposures of loblolly pine seedlings in the field study (see Tables 5.12 and 6.2 for data on which these responses are based)

<u>Condition</u>	<u>Parameter</u>	<u>Response (%)</u> ¹	
		<u>A80</u>	<u>A160</u>
Field	% Mycorrhizal infection	-13	-14
	Allocation of ^{14}C	-6	-18
	Allocation of biomass	+9	-3
	Starch concentrations	+20	+8

¹Per Cent increase or decrease relative to control-filtered.

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7. PROJECT SYNTHESIS AND CONCLUSIONS

S. B. McLaughlin

This project was initiated in April of 1986 as one of three first phase studies within the Southern Commercial Forest Research Cooperative. Overall project objectives were to provide a quantitative and mechanistic basis for evaluating the potential effects of atmospheric pollutants on physiology and growth of southern commercial forests. During the first year specific objectives were to (1) quantify differences in growth responses of seedlings representing 53 families of loblolly pine to the individual and interactive effects of simulated acid rain and O₃ in the field (2) characterize the physiological basis of observed responses in field and laboratory studies; (3) compare and contrast results obtained with similar experimental protocols in field and laboratory approaches.

7.1 EXPERIMENTAL DESIGN

During the first year field exposures of 9950 containerized 12-week-old seedlings were conducted in a 36-plot field research facility comprising of 33 open-top chambers and three open plots in which six ozone levels [ambient open plots (AO), ambient chambered (AC), charcoal-filtered (CF), A + 40 ppb, A + 80 ppb, and A + 160 ppb] were applied for 6 hr/d and 4 d/week. Simulated rain at pH 4.5 was applied across all six ozone treatments. For CF, A80, and A160, three levels of simulated acid rain (pH 3.3, 4.5, and 5.2) were used in a 3 x 3 factorial design. Laboratory studies of 384 similar seedlings representing 8 common denominator families involved application of O₃ at three levels (0, 160 ppb, and 320 ppb) for 6 h/d, 4 d/per week in continuously stirred tank reactor (CSTR) chambers with a background of pH 4.3 rain (1.1 cm twice each week). These plants were placed in a charcoal-filtered greenhouse air during the alternate 3 d.

Growth and physiological measurements were conducted on subsets of seedlings of 2 to 5 families after 6 and 12 weeks of exposure.

7.2 SUMMARY OF PRINCIPAL CONCLUSIONS

The following conclusions can be drawn from analyses of growth and physiological responses of loblolly pine to ozone and acid rain in these experiments.

7.2.1 Growth and Yield

As expected, there were large differences in inherent growth rates among these families and in their responses to ozone and acid rain. Mean volume growth during the 12 weeks of these experiments as indicated by the parameter diameter² x height (D²H) ranged from approximately 1000 mm³ to 5000 mm³ per across the 54 families examined in the field study. Seedlings grew approximately 30% faster under field conditions than in the greenhouse/CSTR environment. Analyses of growth and physiological responses of these families to acid rain and ozone have led to the following principal conclusions:

1. Exposure to ambient air, in which ozone was the principal known phytotoxic component, reduced average height (-26%), diameter (-5%), and volume (-14%) growth compared to growth of seedlings exposed to a 50% lower dose as a result of charcoal filtering supply air
2. When ozone levels were increased approximately 20% above those in ambient air, growth was generally stimulated. Responses to ozone varied widely between families, and, while they became increasingly negative at the highest ozone levels, they did not significantly exceed growth reductions found in ambient air.
3. Acid rain caused a general stimulation of height growth across families at near ambient levels (pH 4.5), while height growth was reduced at a median pH of 3.3.
4. Significant interactions between rainfall acidity and ozone were detected for some families, principally in responses of height growth. In general, acid rain effects were greatest in charcoal-filtered air and decreased as the level of ozone increased. Similarly, ozone effects were greatest in at high rainfall pH and diminished as the acidity of rainfall increased.

5. Growth rates of seedlings under field conditions were more rapid than for those grown under controlled laboratory conditions, and differences in responses to ozone were first apparent during the interval between 3 and 6 weeks after exposures were initiated.

6. Seedlings were generally more sensitive to changes in both growth and physiology following ozone exposure in field experiments than when exposed in CSTR chambers in the laboratory. The pattern of differences in sensitivity to ozone among families was generally similar in field and laboratory settings.

7.2.2 Carbon Assimilation

Carbon dioxide exchange rates were measured as a function of photosynthetic photon flux densities on seedlings of two loblolly pine families (8 and 9) after 6 or 12 weeks of exposure. Treatments compared for these families were charcoal filtered (CF) vs the highest ozone doses in the field (A160) or laboratory (320 ppb). Treatment effects of three acid rain levels (pH 3.5, 4.3, and 5.0) were also compared at each ozone level for field grown seedlings. Results may be summarized as follows:

1. Field-grown seedlings were more sensitive to ozone-induced reductions in net photosynthesis (P_n) than those grown under laboratory conditions. Field-grown seedlings exhibited statistically significant reductions in light-saturated P_n of 13% and 25% after 6 and 12 weeks of exposure respectively. Reductions in P_n of laboratory-grown seedlings were observed but were less pronounced and inconsistent in timing, occurring only after 6 weeks in one family and only after 12 weeks in the other.

2. The shape of the light response surface was not affected by any of the treatments; however, differences were generally more significant statistically at saturating radiation levels.

3. Stimulation of P_n was observed in response to increasing acidity of precipitation after 12 weeks of exposure, and average P_n rates were 52% higher for pH 4.5 and 3.3 treatments compared to controls.

4. Dark respiration of foliage was not affected significantly by any of the treatments.

5. Measurements of carbon assimilation capacity provided generally useful index trends in biomass increment for ozone exposures but not for studies with acid rain. For ozone-treated seedlings, induced reductions in Ps capacity were accompanied by reduced seedling biomass, and where differences in Ps were absent, differences in seedling biomass were minimal. Acid rain-induced stimulation of Ps was not accompanied by increasing seedling weight, indicating that the Ps response had either developed only recently or was offset by other unfavorable physiological changes.

6. Continuous measurements of photosynthesis in canopies of large trees appear to be feasible using an open-flow gas exchange system and may provide a basis for in situ measurements of tree responses to ozone under ambient exposure regimes (see Appendix D).

7.2.3 Carbon Allocation

The main objectives of the carbon allocation studies were (1) to examine individual and interactive effects of ozone and rain chemistry on whole-plant carbon allocation patterns and (2) to determine whether carbohydrate reserves, particularly starch, are significantly affected by elevated ozone levels, acidic rain, or an interaction of the two. To achieve these objectives, and to allow examination of possible interactions among genotype, ozone level, and rain chemistry, seedlings of five families representing both field-grown and CSTR-grown sources were used to allow comparison between field and lab studies. Principal findings were as follows:

1. Strong genetic differences in growth, carbon allocation and foliar nutrient concentrations of loblolly pine were observed in both the field study and the CSTR study. Although a statistically significant family-ozone interaction was detected only for relative allocation to foliar biomass in the CSTR study, the families did differ in sensitivity to ozone, and these differences were reflected in differing patterns of carbon allocation.

2. Whole-plant carbon allocation patterns were generally similar between the field and CSTR study but proportions allocated to roots and foliage and total growth differed. The lower rate of growth and the lower root:shoot ratios observed in the CSTR chambers are hypothesized to be due to lower light levels and possibly lower levels of moisture stress under CSTR/greenhouse conditions.

3. Changes in levels of root starch and altered patterns of allocation of ^{14}C labeled photosynthate provided generally useful indicators of changes in biomass distribution of ozone stressed seedlings. Elevated ozone increased apparent whole plant respiratory losses of carbon and decreased carbon allocation and starch concentrations in the roots. Coarse-root biomass also decreased with ozone fumigation.

4. Rain pH had little effect on carbon allocation or root starch concentrations, nor were any significant O_3 x rain pH interactions detected.

5. Ozone effects on carbon allocation to roots in the CSTR study were not noted until the highest O_3 treatment level (320 ppb). The greater effect on root starch levels (-28%) than on allocation of current photosynthate (-13%) suggested an accumulative effect that was supported by the trend toward lower root:shoot ratios observed with increasing ozone in these studies.

6. Observed reductions in foliar content of the macronutrients nitrogen and potassium of ozone-treated seedlings were observed in the field but not in CSTR studies and were associated with decreased allocation of carbon to roots. The extent to which reduced nutrient uptake or increased foliar leaching may have been involved in observed foliar nutrient levels cannot be determined from the present data. Foliar Manganese levels, on the other hand, were slightly higher (10%) in ozone-treated seedlings. Although ozone fumigation resulted in slightly decreased macronutrient concentrations, acidic rain primarily affected micronutrient concentrations, including increasing foliar aluminum concentrations.

7.2.4 Effects on Mycorrhizae

Assessment of percent mycorrhizal infection of two families (8 and 9) used extensively in other physiological studies was performed after 0, 6, and 12 weeks of exposure to determine whether disruption of root function had been induced by ozone or acid rain-induced alteration of carbon allocation patterns. Principal findings were as follows:

1. The major effect noted in these experiments was the influence of family on the percent mycorrhizal colonization (PMC). The range of PMC across treatments for family 8 was approximately 40 to 55% and for family 9, 25 to 35%.

2. Ozone effects on PMC of roots were significant (15 to 20% reduction) for both families examined in the field study but not under laboratory conditions.

3. Acid rain had no significant effect on PMC over the pH 3.3 to 5.2 range examined.

4. The relative response of PMC to ozone exposure was bimodal over time, a consequence of an initial reduction in PMC of seedlings growing in charcoal-filtered air during the interval 0 to 6 weeks. This pattern was reversed by 12 weeks because of a greater relative increase of mycorrhizae in the CF treatment.

5. Reductions in PMC induced by ozone exposure were larger than changes in root biomass and were generally accompanied by decreasing root shoot ratios, indicating that PMC may be a useful indicator of decreased root vigor.

7.3 DISCUSSION AND CONCLUSIONS

Collectively, these studies indicate that adverse growth responses of loblolly pine seedlings to ambient levels of atmospheric ozone are likely but will be strongly dependent on genetic variation associated with family origins. Responses to ambient levels of acid deposition are likely to be much more complex and may involve growth stimulation, particularly in height. Ozone-acid rain interactions at very low or very high pH levels appear likely, but in most cases the influence of

combined exposures at the highest levels tested was antagonistic rather than additive or synergistic. Because ambient levels of acid deposition did influence growth of test seedlings relative to growth observed at near pristine pH, it is apparent that more work is needed to ascertain the significance of possible acid rain - ozone interactions at ambient levels of both pollutants.

The more obvious growth responses observed in the field compared to results of laboratory studies argue for increased emphasis on field work in the future and for better understanding of the physiology of pollutant uptake and effects. In these studies, seedlings grew faster in the field even though the slow-release fertilizer used produced slightly slower growth when tested against the liquid application regime of the greenhouse/CSTR studies. A major difference between the studies was the relatively lower radiation levels of the CSTR/greenhouse system and the apparent influence of continuous low level exposures between ozone additions in the field. The influence of this "respite dose" should be examined much more closely in future studies. The basis of this recommendation is the potential significance of chronic stress induced by continuous exposure of plants to ozone under actual field conditions.

Charcoal filtration of the growing environment between exposures allows one to isolate the effects of specific applied doses, but it may also reduce the impact of that dose because of the operation of inherent recovery and repair processes.

In the future, major emphasis should be placed on evaluating responses of loblolly pine to ambient and near-ambient exposure doses. The observed -26% mean height growth response to ambient air in these studies was the quantitatively most significant response detected. The fact that follow-up studies carried out with 1 year-old seedlings planted in the soil during the second year produced similar results further substantiates the results of these studies with containerized seedlings. The significance of ambient air responses is further reinforced by results from other past (Shafer et al. 1987) and ongoing (Adams et al., 1988 and L. Allen-SCFRC/Duke, personal communication)

studies. Our results show that addition of ozone to ambient air may stimulate growth initially when ozone levels are low. Observed responses at higher levels are likely to be an integrative function of stomatal regulation, actual internal dose received by the plant, and the balance of alteration of both carbon and water relations.

The results we have obtained do not indicate that acid rain at ambient levels will cause significant adverse effects on growth of loblolly pine seedlings; however, these results must be interpreted with caution. Aluminum was significantly more available to seedlings at near ambient and higher acidity levels even in the more organic potting medium used in these experiments. More research is needed to determine whether mobilization of aluminum in poorly buffered, more-acid soils is a significant factor to consider in evaluating the response of loblolly pine to acid deposition. Ambient levels of rainfall acidity did have an effect on height growth. While that effect was positive, it does demonstrate the capacity of ambient levels of acidity to influence growth processes. Such changes may be beneficial under some conditions and a disadvantage under others where another resource such as water is limiting.

The physiological measurements made during the course of these studies generally support the utility of physiological indicators as early warning signs of dysfunction of the growth process. Results obtained with measurements of carbon assimilation, carbon allocation, and mycorrhizal infection support the observed differences in dry matter production and distribution. The measurements we have made suggest that carbon assimilation and the distribution of that assimilate particularly to the root system are both adversely affected by ozone. The fact that these responses were observable during the course of a 12-week experiment suggests that they may be even more useful in evaluating the longer-term effects of chronic exposures in the field. It should also be noted that the families chosen for physiological measurements were not particularly sensitive to ozone compared to others examined within the study. Results of continuous in situ measurements of canopy gas exchange suggest that examination of

the kinetics of CO₂ exchange of both saplings and larger trees is possible and would be valuable to pursue.

The significance of the responses we have observed lies primarily in providing inferential evidence that ambient and higher levels of ozone can produce significant effects on physiology of loblolly pine and that responses can be expected to vary widely across families. Although it would be a mistake to assume that responses of equal magnitude would be produced for mature trees, these results can not be dismissed as irrelevant for more mature forests. The rate of maturation of both natural and managed forests very obviously depends on the rate and success of initiating those forest stands from seedlings. Growth impacts at the seedling stage can have far-reaching consequences on the mature forests that follow based on the changes in the competitive status and vigor of the individuals and species of which those forests will be composed.

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APPENDIX A.
MEAN HEIGHT AND DIAMETER GROWTH DATA
BY TREATMENT AT FINAL HARVEST

Table A. 1. Average height growth (mm) by loblolly pine family for seedlings grown for 12 weeks in charcoal filtered air and exposed to three rain pH levels.

Family	Rain pH		
	3.3	4.5	5.2
2	17	30	20
3	36	42	41
4	55	69	58
5	14	30	16
6	27	52	46
7	33	53	59
8	29	40	33
9	40	58	59
10	34	54	59
11	35	46	37
12	35	43	61
13	30	38	40
14	17	30	22
15	23	38	27
16	14	33	23
17	34	37	42
18	21	22	21
19	38	57	40
20	14	40	23
21	34	43	39
23	37	59	50
24	43	63	53
25	20	23	19
26	44	56	60
27	48	87	51
28	24	38	31
29	52	80	50
30	53	74	55
31	50	53	53
32	62	84	69
33	23	46	30
34	19	30	27
35	29	36	37
36	27	33	32
37	13	42	26
38	13	34	27
39	43	54	34
40	33	47	36

Table A.1. (continued)

Family	Rain pH		
	3.3	4.4	5.2
41	22	38	20
42	50	53	44
43	24	35	30
44	17	34	27
45	32	48	54
46	13	29	26
47	23	33	38
48	22	28	30
49	19	34	32
50	21	24	23
51	31	48	43
52	48	87	55
53	24	53	39
54	26	31	27
55	25	28	25

Table A. 2. Average height growth (mm) by loblolly pine family for seedlings grown for 12 weeks in ambient + 80 ppb ozone air and exposed to three rain pH levels.

Family	Rain pH		
	3.3	4.5	5.2
2	14	27	29
3	37	44	45
4	55	63	56
5	12	31	25
6	35	54	34
7	53	53	54
8	34	52	49
9	50	64	73
10	48	73	49
11	41	53	41
12	34	49	42
13	37	38	49
14	13	24	24
15	23	32	42
16	25	24	31
17	46	39	45
18	17	20	18
19	50	63	52
20	32	33	36
21	29	45	51
23	56	49	65
24	56	52	59
25	20	20	41
26	46	60	66
27	43	49	62
28	34	33	35
29	61	51	62
30	58	51	73
31	50	50	53
32	72	64	66
33	38	22	37
34	34	23	37
35	26	36	47
36	29	30	38
37	26	30	29
38	32	36	39
39	34	47	50
40	20	39	39

Table A.2. (continued)

Family	Rain pH		
	3.3	4.5	5.2
41	18	19	27
42	50	55	53
43	20	32	33
44	18	27	35
45	34	52	56
46	33	34	46
47	25	39	43
48	22	35	31
49	24	29	28
50	26	27	37
51	30	54	59
52	49	67	69
53	23	36	43
54	29	39	39
55	21	50	40

Table A.3. Average height growth (mm) by loblolly pine family for seedlings grown for 12 weeks in ambient + 160 ppb ozone air and exposed to three rain pH levels.

Family	Rain pH		
	3.3	4.5	5.2
2	27	29	23
3	54	45	46
4	63	64	68
5	25	24	20
6	42	27	46
7	54	51	46
8	38	37	48
9	68	73	44
10	47	43	49
11	49	52	41
12	42	49	53
13	29	36	34
14	24	26	18
15	37	38	25
16	23	32	27
17	45	43	49
18	30	15	20
19	49	35	47
20	29	32	25
21	40	34	41
23	50	73	54
24	43	48	46
25	34	25	32
26	42	52	53
27	55	52	42
28	36	25	35
29	60	47	44
30	57	46	53
31	54	51	43
32	68	62	53
33	30	26	24
34	34	25	29
35	38	31	34
36	41	23	31
37	38	20	27
38	39	35	32
39	48	35	37
40	45	29	32

Table A.3. (continued)

Family	Rain pH		
	3.3	4.5	5.2
41	23	21	16
42	61	54	40
43	29	26	29
44	25	18	33
45	40	49	28
46	28	19	19
47	39	19	30
48	29	27	26
49	32	24	33
50	36	25	17
51	51	41	45
52	72	59	69
53	46	40	41
54	39	36	36
55	27	21	38

Table A.4. Average height growth (mm) by loblolly pine family for seedlings grown for 12 weeks in three ozone levels and irrigated with artificial rain at pH 4.5.

Family	Charcoal- filtered	Ambient + 80 ppb	Ambient + 160 ppb
2	30	24	29
3	42	44	45
4	69	63	64
5	30	31	24
6	52	54	27
7	53	53	51
8	40	52	37
9	58	64	73
10	54	73	43
11	46	53	52
12	43	49	49
13	38	38	36
14	30	24	26
15	38	32	38
16	33	24	32
17	37	39	43
18	22	20	15
19	57	63	35
20	40	33	32
21	43	45	33
23	59	49	73
24	63	52	48
25	23	20	25
26	56	60	52
27	87	49	52
28	38	33	25
29	80	51	47
30	74	51	46
31	53	50	51
32	84	64	62
33	46	22	26
34	30	23	25
35	36	36	31
36	33	30	23
37	42	30	20
38	34	36	35
39	54	47	35
40	47	39	29

Table A.4. (continued)

Family	Charcoal- filtered	Ambient + 80 ppb	Ambient + 160 ppb
41	38	19	21
42	53	55	54
43	35	32	26
44	34	27	18
45	48	52	49
46	29	34	19
47	33	39	19
48	28	35	27
49	34	29	24
50	24	27	25
51	48	54	41
52	87	64	59
53	53	36	40
54	31	39	36
55	28	50	21

Table A.5. Average height growth (mm) by loblolly pine family for seedlings grown for 12 weeks in three ozone levels and irrigated with artificial rain at pH 3.3.

Family	Charcoal-filtered	Ambient + 80 ppb	Ambient + 160 ppb
2	17	14	27
3	36	37	54
4	55	55	63
5	14	12	25
6	27	35	42
7	33	53	54
8	29	34	38
9	40	50	68
10	34	48	47
11	35	41	49
12	35	34	42
13	30	37	29
14	17	13	24
15	23	23	37
16	14	25	23
17	34	46	45
18	21	17	30
19	38	50	49
20	14	32	29
21	34	29	40
23	37	56	50
24	43	56	43
25	20	20	34
26	44	46	42
27	48	43	55
28	24	34	36
29	52	61	60
30	53	58	57
31	50	50	54
32	62	72	68
33	23	38	30
34	19	34	34
35	29	26	38
36	27	29	41
37	13	26	38
38	13	32	39
39	43	34	48
40	33	20	45

Table A.5. (continued)

Family	Charcoal- filtered	Ambient + 80 ppb	Ambient + 160 ppb
41	22	18	23
42	50	50	61
43	24	20	29
44	17	18	25
45	32	34	40
46	13	33	28
47	23	25	39
48	22	22	29
49	19	24	32
50	21	26	36
51	31	30	51
52	48	49	72
53	24	23	46
54	26	29	39
55	25	21	27

Table A.6. Average height growth (mm) by loblolly pine family for seedlings grown for 12 weeks in three ozone levels and irrigated with artificial rain at pH 5.2.

Family	Charcoal-filtered	Ambient + 80 ppb	Ambient + 160 ppb
2	20	29	23
3	41	45	46
4	58	56	68
5	16	25	20
6	46	34	46
7	59	54	46
8	33	49	48
9	59	43	44
10	59	49	49
11	37	41	41
12	61	42	53
13	40	49	34
14	22	24	18
15	27	42	25
16	23	31	27
17	42	45	49
18	21	18	20
19	40	52	47
20	23	36	25
21	39	51	41
23	50	65	54
24	53	59	46
25	19	41	32
26	60	66	53
27	51	62	42
28	31	35	35
29	50	62	44
30	55	73	53
31	53	53	43
32	69	66	53
33	30	37	24
34	27	37	29
35	37	47	37
36	32	38	31
37	26	29	27
38	27	39	32
39	34	50	37
40	36	39	32

Table A.6. (continued)

Family	Charcoal- filtered	Ambient + 80 ppb	Ambient + 160 ppb
41	20	27	16
42	44	53	40
43	30	33	29
44	27	35	33
45	54	56	28
46	26	46	19
47	38	43	30
48	30	31	26
49	32	28	33
50	23	37	17
51	43	59	45
52	55	69	69
53	39	43	41
54	27	39	36
55	25	40	38

Table A.7. Average height growth (mm) for 53 loblolly pine families for seedlings grown for 12 weeks in five ozone levels and irrigated with artificial rain at pH 4.5.

Family	Charcoal- filtered	Ambient	Ambient + 40 ppb	Ambient + 80 ppb	Ambient + 160
2	30			24	29
3	42			44	45
4	69			63	64
5	30			31	24
6	52			54	27
7	53			53	51
8	40			52	37
9	58			64	73
10	54			73	43
11	46	34	50	53	52
12	43	35	47	49	49
13	38	36	43	38	36
14	30	20	32	24	26
15	38	23	40	32	38
16	33	26	35	24	32
17	37	33	47	39	43
18	22	20	34	20	15
19	54	38	47	63	35
20	40	21	48	33	32
21	43	33	54	45	33
23	59	44	62	49	76
24	63	46	68	52	48
25	23	31	40	20	25
26	56	45	70	60	52
27	87	48	75	49	52
28	38	34	39	33	25
29	80	53	77	51	47
30	74	52	70	51	46
31	35	59	60	50	51
32	84	65	71	64	62
33	46	23	42	22	26
34	30	19	33	23	25
35	36	32	37	36	31
36	33	25	40	30	23
37	42	21	39	30	20
38	34	29	42	36	35
39	54	32	54	47	35
40	47	36	55	39	29

Table A.7. (Continued)

Family	Charcoal- filtered	Ambient	Ambient + 40 ppb	Ambient + 80 ppb	Ambient + 160 ppb
41	38	26	29	19	21
42	53	39	64	55	54
43	35	17	35	32	26
44	34	26	33	27	18
45	48	37	53	52	49
46	29	15	36	34	19
47	33	24	30	39	19
48	28	22	22	35	27
49	34	26	44	29	24
50	24	29	37	27	25
51	48	46	53	54	41
52	87	62	76	67	59
53	53	31	40	36	40
54	31	23	36	39	36
55	28	25	32	50	21

Table A.8. Average diameter growth (mm) for 53 loblolly pine families for seedlings grown for 12 weeks in three ozone levels and irrigated with artificial rain at pH 4.5.

Family	Charcoal- filtered	Ambient	Ambient + 40 ppb	Ambient + 80 ppb	Ambient + 160 ppb
2	2.54			2.56	2.44
3	2.61			2.60	2.69
4	2.93			2.80	2.45
5	2.65			2.79	2.61
6	2.48			2.44	2.15
7	2.97			2.94	2.83
8	2.98			2.96	2.53
9	2.50			2.65	2.40
10	2.89			2.89	2.61
11	3.09	2.34	2.33	2.65	2.56
12	2.84	2.51	2.60	2.71	2.60
13	2.97	2.77	2.79	2.88	2.91
14	2.67	2.66	2.42	2.06	2.36
15	2.80	2.48	2.51	2.49	2.50
16	2.74	2.58	2.54	2.43	2.30
17	2.71	2.29	2.27	2.46	2.40
18	2.35	2.36	2.22	2.20	2.15
19	2.99	2.31	2.50	2.79	2.37
20	2.66	2.34	2.48	2.34	2.55
21	2.62	2.60	2.64	2.85	2.53
23	2.88	2.59	2.64	2.69	3.00
24	2.82	2.62	2.67	2.81	2.55
25	2.45	2.70	2.46	2.33	2.70
26	2.69	2.93	2.61	2.98	2.57
27	2.71	2.93	2.77	2.86	2.52
28	2.86	2.51	2.44	2.46	2.51
29	3.31	2.85	2.88	2.73	2.95
30	3.31	2.99	2.80	2.62	2.79
31	2.85	2.59	2.63	2.65	2.55
32	3.15	2.95	2.77	2.65	2.90
33	2.95	2.75	2.79	2.56	2.69
34	2.45	2.37	2.15	2.45	2.20
35	2.57	2.37	2.46	2.42	2.31
36	2.72	2.61	2.47	2.60	2.64
37	2.99	2.54	2.58	2.34	2.71
38	2.52	2.42	2.31	2.42	2.30
39	2.53	2.64	2.63	2.73	2.31
40	2.53	2.42	2.57	2.55	2.23

Table A.8. (Continued)

Family	Charcoal- filtered	Ambient	Ambient + 40 ppb	Ambient + 80 ppb	Ambient + 160 ppb
41	2.55	2.75	2.54	2.76	2.41
42	2.69	3.00	2.74	2.58	2.78
43	2.42	2.32	2.14	2.33	2.18
44	2.52	2.19	2.30	2.15	2.12
45	2.65	2.43	2.34	2.30	2.51
46	2.93	2.85	2.80	2.83	2.84
47	2.74	3.09	2.57	2.92	2.60
48	2.90	2.85	2.48	2.96	2.63
49	2.49	2.59	2.62	2.72	2.40
50	2.29	2.31	2.23	2.39	2.33
51	2.56	2.69	2.54	2.63	2.50
52	3.07	3.25	2.75	2.89	2.78
53	2.87	2.52	2.38	2.67	2.60
54	2.60	2.52	2.33	2.20	2.53
55	2.54	2.86	2.62	2.70	2.67

Table A.9. Averaged height growth (mm) over a 12 week period for 8 loblolly pine families exposed to three ozone levels under laboratory conditions.

Family	Charcoal- filtered	160ppb	320 ppb
2	14	22	24
3	36	35	48
4	61	48	47
5	20	18	24
6	17	30	23
8	23	35	37
9	80	72	59
10	36	28	36

Table A.10. Average diameter growth (mm) over a 12 week period for 8 loblolly pine families exposed to three ozone levels under laboratory conditions.

Family	Charcoal-filtered	160 ppb	320 ppb
2	1.23	1.36	1.36
3	1.09	1.09	1.14
4	1.34	1.32	1.09
5	1.15	1.34	1.16
6	1.31	1.43	1.20
8	1.33	1.72	1.50
9	1.95	1.54	1.36
10	1.18	1.31	1.24

Table A.11. Average family total weights of seedlings at two harvest times from the laboratory experiment (g)

Family	6-week harvest			12-week harvest		
	Charcoal- filtered	160 ppb	320 ppb	Charcoal- filtered	160 ppb	320 ppb
2	2.38	2.39	2.18	3.52	3.75	3.80
3	2.79	2.80	2.82	4.07	3.95	4.42
4	2.36	2.20	2.31	3.69	3.23	3.10
5	2.51	2.60	2.43	4.04	4.46	4.47
6	1.74	1.70	1.53	2.84	2.97	2.71
8	3.49	3.36	3.53	5.14	4.99	5.18
9	4.52	4.08	4.26	5.58	5.79	5.26
10	3.42	2.76	2.83	4.26	4.04	4.42

APPENDIX B.
STATISTICAL ANALYSES OF TREATMENT EFFECTS ON A FAMILY
BY-FAMILY BASIS FOR FIELD- AND LABORATORY-GROWN SEEDLINGS

Table B.1. Seedling diameter responses to ozone in the field.

Family	Source	DF	Type III ss	F value	PR > F
2	Block	2	0.364	1.27	0.2950
	Ozone	2	0.233	0.81	0.4528
	Diam 1	1	0.003	0.02	0.8848
	Error	30	4.298		
3	Block	2	1.040	2.58	0.0922
	Ozone	2	0.070	0.17	0.8422
	Diam 1	1	0.392	1.95	0.1732
	Error	30	6.037		
4	Block	2	1.580	5.06	0.0143
	Ozone	2	1.269	4.06	0.0297
	Diam 1	1	0.417	2.67	0.1146
	Error	25	3.903		
5	Block	2	0.997	1.99	0.1537
	Ozone	2	0.265	0.53	0.5936
	Diam 1	1	0.129	0.52	0.4776
	Error	30	7.494		
6	Block	2	0.679	4.92	0.0158
	Ozone	2	0.959	6.95	0.0040
	Diam 1	1	0.362	5.24	0.0307
	Error	25	1.726		
7	Block	2	0.365	0.71	0.5002
	Ozone	2	0.180	0.35	0.7081
	Diam 1	1	0.116	0.45	0.5062
	Error	30	7.716		
8	Block	2	0.127	0.26	0.7740
	Ozone	2	2.084	4.23	0.0244
	Diam 1	1	1.440	5.85	0.0221
	Error	29	7.137		
9	Block	2	1.548	2.28	0.1224
	Ozone	2	0.540	0.80	0.4620
	Diam 1	1	0.289	0.85	0.3644
	Error	26	8.828		
10	Block	2	0.137	0.31	0.7331
	Ozone	2	0.555	1.27	0.2967
	Diam 1	1	0.088	0.40	0.5317
	Error	27	5.890		
11	Block	2	1.262	2.68	0.0765
	Ozone	4	5.668	6.01	0.0004
	Diam 1	1	0.658	2.79	0.0997
	Error	65	15.329		
12	Block	2	1.853	3.10	0.0514
	Ozone	4	1.210	1.01	0.4070
	Diam 1	1	0.273	0.91	0.3426
	Error	68	20.311		

Table B. 1 (continued)

Family	Source	DF	Type III ss	F value	PR > F
13	Block	2	1.868	3.26	0.0443
	Ozone	4	0.750	0.65	0.6256
	Diam 1	1	0.775	2.70	0.1046
	Error	70	20.060		
14	Block	2	0.244	0.38	0.6853
	Ozone	4	3.227	2.51	0.0493
	Diam 1	1	0.714	2.22	0.1405
	Error	70	22.481		
15	Block	2	0.212	0.43	0.6534
	Ozone	4	1.006	1.02	0.4042
	Diam 1	1	0.027	0.11	0.7419
	Error	70	17.292		
16	Block	2	0.294	0.82	0.4439
	Ozone	4	1.547	2.16	0.0820
	Diam 1	1	0.765	4.28	0.0423
	Error	69	12.325		
17	Block	2	1.734	3.49	0.0365
	Ozone	4	2.164	2.18	0.0817
	Diam 1	1	0.023	0.09	0.7623
	Error	62	15.390		
18	Block	2	0.033	0.09	0.9182
	Ozone	4	0.528	0.69	0.6018
	Diam 1	1	0.721	3.77	0.0565
	Error	67	12.834		
19	Block	2	0.277	0.40	0.6747
	Ozone	4	2.808	2.01	0.1076
	Diam 1	1	0.005	0.01	0.9085
	Error	50	17.475		
20	Block	2	0.766	1.33	0.2708
	Ozone	4	0.853	0.74	0.5671
	Diam 1	1	0.990	3.44	0.0679
	Error	68	19.558		
21	Block	2	1.741	3.37	0.0403
	Ozone	4	0.897	0.87	0.4880
	Diam 1	1	0.101	0.39	0.5338
	Error	68	17.569		
23	Block	2	1.648	3.34	0.0402
	Ozone	4	2.450	2.48	0.0498
	Diam 1	1	0.060	0.24	0.6246
	Error	86	21.235		
24	Block	2	1.591	2.96	0.0588
	Ozone	4	0.913	0.85	0.4995
	Diam 1	1	0.359	1.33	0.2523
	Error	66	17.748		

Table B.1 (continued)

Family	Source	DF	Type III ss	F value	PR > F
25	Block	2	0.499	0.97	0.3857
	Ozone	4	1.636	1.58	0.1885
	Diam 1	1	0.001	0.00	0.9459
	Error	67	17.291		
26	Block	2	0.351	0.97	0.3834
	Ozone	4	1.885	2.62	0.0436
	Diam 1	1	3.868	21.48	0.0001
	Error	61	10.983		
27	Block	2	1.427	2.29	0.1085
	Ozone	4	1.873	1.51	0.2101
	Diam 1	1	0.827	2.66	0.1075
	Error	70	21.775		
28	Block	2	0.576	1.11	0.3362
	Ozone	4	2.519	2.42	0.0564
	Diam 1	1	2.972	11.42	0.0012
	Error	70	18.212		
29	Block	2	3.059	6.56	0.0025
	Ozone	4	2.272	2.43	0.0553
	Diam 1	1	0.444	1.90	0.1719
	Error	70	16.331		
30	Block	2	1.472	3.50	0.0358
	Ozone	4	2.326	2.76	0.0342
	Diam 1	1	0.002	0.01	0.9273
	Error	69	14.522		
31	Block	2	1.735	3.16	0.0494
	Ozone	4	0.741	0.67	0.6124
	Diam 1	1	0.022	0.08	0.7797
	Error	62	17.028		
32	Block	2	1.809	4.21	0.0174
	Ozone	4	1.572	1.83	0.1286
	Diam 1	1	5.266	24.51	0.0001
	Error	107	22.988		
33	Block	2	1.684	3.37	0.0389
	Ozone	4	1.115	1.12	0.3544
	Diam 1	1	0.016	0.07	0.7993
	Error	85	21.225		
34	Block	2	1.562	4.07	0.0218
	Ozone	4	1.104	1.44	0.2318
	Diam 1	1	0.603	3.14	0.0811
	Error	62	11.892		
35	Block	2	0.790	1.66	0.1969
	Ozone	4	0.710	0.75	0.5634
	Diam 1	1	0.203	0.85	0.3585
	Error	70	16.632		

Table B.1 (continued)

Family	Source	DF	Type III ss	F value	PR > F
36	Block	2	0.129	0.34	0.7113
	Ozone	4	1.038	1.37	0.2521
	Diam 1	1	3.052	16.15	0.0001
	Error	70	13.228		
37	Block	2	0.729	1.35	0.2663
	Ozone	4	1.866	1.73	0.1543
	Diam 1	1	17.253	63.80	0.0001
	Error	69	18.658		
38	Block	2	3.081	8.14	0.0007
	Ozone	4	0.467	0.62	0.6521
	Diam 1	1	0.055	0.29	0.5903
	Error	68	12.870		
39	Block	2	0.395	0.89	0.4136
	Ozone	4	1.507	1.70	0.1590
	Diam 1	1	0.276	1.25	0.2677
	Error	70	15.481		
40	Block	2	2.037	5.35	0.0069
	Ozone	4	1.292	1.70	0.1607
	Diam 1	1	0.092	0.48	0.4889
	Error	70	13.333		
41	Block	2	1.552	4.39	0.0160
	Ozone	4	1.716	2.43	0.0559
	Diam 1	1	1.258	7.12	0.0095
	Error	68	12.008		
42	Block	2	0.638	1.62	0.2047
	Ozone	4	1.481	1.89	0.1230
	Diam 1	1	0.021	0.11	0.7443
	Error	68	13.358		
43	Block	2	0.209	0.83	0.4390
	Ozone	4	1.001	1.99	0.1050
	Diam 1	1	0.268	2.13	0.1485
	Error	70	8.792		
44	Block	2	0.519	0.96	0.3860
	Ozone	4	2.042	1.89	0.1178
	Diam 1	1	0.115	0.42	0.5161
	Error	106	28.661		
45	Block	2	1.972	3.04	0.0565
	Ozone	4	0.866	0.67	0.6178
	Diam 1	1	0.018	0.05	0.8155
	Error	52	16.875		
46	Block	2	0.314	0.69	0.5068
	Ozone	4	0.216	0.24	0.9171
	Diam 1	1	1.445	6.32	0.0143
	Error	68	15.558		

Table B.1 (continued)

Family	Source	DF	Type III ss	F value	PR > F
47	Block	2	2.356	4.12	0.0205
	Ozone	4	2.685	2.35	0.0632
	Diam 1	1	0.155	0.54	0.4648
	Error	68	19.459		
48	Block	2	3.425	6.10	0.0037
	Ozone	4	1.940	1.73	0.1548
	Diam 1	1	0.066	0.23	0.6298
	Error	65	18.261		
49	Block	2	2.731	5.20	0.0079
	Ozone	4	1.342	1.28	0.2872
	Diam 1	1	0.759	2.89	0.0936
	Error	70	18.387		
50	Block	2	0.311	0.68	0.5099
	Ozone	4	0.246	0.27	0.8968
	Diam 1	1	1.318	5.77	0.0188
	Error	74	16.914		
51	Block	2	0.861	1.71	0.1862
	Ozone	4	0.796	0.79	0.5341
	Diam 1	1	0.645	2.56	0.1129
	Error	98	24.683		
52	Block	2	0.442	0.62	0.5425
	Ozone	4	2.957	2.07	0.0950
	Diam 1	1	0.469	1.31	0.2562
	Error	68	24.342		
53	Block	2	0.143	0.43	0.6514
	Ozone	4	2.077	3.14	0.0197
	Diam 1	1	0.289	1.75	0.1901
	Error	68	11.234		
54	Block	2	0.292	0.66	0.5201
	Ozone	4	1.185	1.34	0.2655
	Diam 1	1	0.337	1.53	0.2219
	Error	57	12.582		
55	Block	2	0.001	0.00	0.9981
	Ozone	4	0.787	0.80	0.5318
	Diam 1	1	0.043	0.18	0.6772
	Error	52	12.814		

Table B.2. Seedling height responses to ozone in the field.

Family	Source	DF	Type III ss	F value	PR > F
2	Block	2	1568.94	3.06	0.0619
	Ozone	2	169.75	0.33	0.7209
	Hgt 1	1	3228.77	12.58	0.0013
	Error	30	7697.34		
3	Block	2	691.79	1.00	0.3788
	Ozone	2	80.41	0.12	0.8904
	Hgt 1	1	665.80	1.93	0.1749
	Error	30	10346.86		
4	Block	2	3255.07	2.89	0.0742
	Ozone	2	375.85	0.33	0.7192
	Hgt 1	1	14.21	0.03	0.8750
	Error	25	14068.72		
5	Block	2	88.93	0.10	0.9030
	Ozone	2	289.94	0.33	0.7190
	Hgt 1	1	67.74	0.16	0.6958
	Error	29	12598.60		
6	Block	2	907.07	1.18	0.3233
	Ozone	2	4127.89	5.38	0.0114
	Hgt 1	1	440.30	1.15	0.2944
	Error	25	9595.48		
7	Block	2	1145.97	1.10	0.3450
	Ozone	2	64.49	0.06	0.9399
	Hgt 1	1	849.66	1.64	0.2108
	Error	30	15585.45		
8	Block	2	2478.05	1.81	0.1822
	Ozone	2	1313.63	0.96	0.3956
	Hgt 1	1	1323.47	1.93	0.1754
	Error	29	19888.04		
9	Block	2	2260.11	1.10	0.3488
	Ozone	2	583.25	0.28	0.7557
	Hgt 1	1	266.36	0.26	0.6154
	Error	26	26777.79		
10	Block	2	302.38	0.26	0.7735
	Ozone	2	2903.29	2.49	0.1024
	Hgt 1	1	1527.69	2.62	0.1175
	Error	26	15155.68		
11	Block	2	1468.55	1.47	0.2377
	Ozone	4	4611.21	2.31	0.0675
	Hgt 1	1	6971.95	13.95	0.0004
	Error	65	32493.88		
12	Block	2	370.46	0.23	0.7949
	Ozone	4	3254.71	1.01	0.4078
	Hgt 1	1	3452.63	4.29	0.0421
	Error	67	53890.33		

Table B.2 (continued)

Family	Source	DF	Type III ss	F value	PR > F
13	Block	2	119.22	0.22	0.8033
	Ozone	4	568.67	0.52	0.7185
	Hgt 1	1	1523.16	5.61	0.0206
	Error	70	18996.36		
14	Block	2	124.10	0.34	0.7151
	Ozone	4	1226.71	1.67	0.1681
	Hgt 1	1	2545.20	13.82	0.0004
	Error	68	12519.41		
15	Block	2	795.98	1.42	0.2485
	Ozone	4	3618.11	3.23	0.0172
	Hgt 1	1	4682.42	16.71	0.0001
	Error	70	19614.20		
16	Block	2	518.21	0.86	0.4273
	Ozone	4	2695.61	2.24	0.0739
	Hgt 1	1	3604.97	11.98	0.0009
	Error	67	20160.14		
17	Block	2	766.75	1.41	0.2519
	Ozone	4	2349.83	2.16	0.0839
	Hgt 1	1	2228.63	8.19	0.0057
	Error	62	16861.32		
18	Block	2	28.34	0.05	0.9486
	Ozone	4	2902.92	2.71	0.0375
	Hgt 1	1	573.48	2.14	0.1483
	Error	67	17965.04		
19	Block	2	1042.13	1.03	0.3633
	Ozone	4	6543.30	3.24	0.0192
	Hgt 1	1	302.92	0.60	0.4419
	Error	50	25210.71		
20	Block	2	356.61	0.62	0.5409
	Ozone	4	5544.87	4.82	0.0018
	Hgt 1	1	7141.10	24.84	0.0001
	Error	68	19549.73		
21	Block	2	920.16	1.53	0.2237
	Ozone	4	5063.17	4.21	0.0042
	Hgt 1	1	124.82	0.42	0.5215
	Error	68	20438.21		
23	Block	2	1863.87	1.36	0.2617
	Ozone	4	11166.09	4.08	0.0045
	Hgt 1	1	1532.19	2.24	0.1383
	Error	86	58865.36		
24	Block	2	7345.90	7.67	0.0010
	Ozone	4	4465.20	2.33	0.0651
	Hgt 1	1	6363.10	13.29	0.0005
	Error	65	31124.84		

Table B.2 (continued)

Family	Source	DF	Type III ss	F value	PR > F
25	Block	2	649.97	1.16	0.3208
	Ozone	4	4677.68	4.16	0.0045
	Hgt 1	1	2388.12	8.50	0.0048
	Error	67	18827.19		
26	Block	2	99.75	0.06	0.9399
	Ozone	4	8562.69	2.66	0.0409
	Hgt 1	1	7904.18	9.83	0.0026
	Error	61	49049.90		
27	Block	2	3349.66	1.35	0.2662
	Ozone	4	13881.81	2.79	0.0326
	Hgt 1	1	7457.26	6.00	0.0168
	Error	70	86929.21		
28	Block	2	494.19	0.96	0.3895
	Ozone	4	2064.56	2.00	0.1045
	Hgt 1	1	2582.71	9.99	0.0023
	Error	70	18096.08		
29	Block	2	313.16	0.20	0.8221
	Ozone	4	16337.24	5.12	0.0011
	Hgt 1	1	6223.82	7.81	0.0067
	Error	69	55000.38		
30	Block	2	3176.86	2.90	0.0617
	Ozone	4	9322.55	4.26	0.0039
	Hgt 1	1	1438.58	2.63	0.1096
	Error	69	37770.99		
31	Block	2	3602.77	3.29	0.0440
	Ozone	4	994.20	0.45	0.7695
	Hgt 1	1	186.75	0.34	0.5615
	Error	62	33982.52		
32	Block	2	1736.88	1.41	0.2498
	Ozone	4	3019.96	1.22	0.3060
	Hgt 1	1	20079.68	32.49	0.0001
	Error	107	66132.74		
33	Block	2	90.77	0.14	0.8727
	Ozone	4	7941.83	5.97	0.0003
	Hgt 1	1	624.61	1.88	0.1744
	Error	81	26946.87		
34	Block	2	995.52	1.56	0.2192
	Ozone	4	1892.89	1.48	0.2195
	Hgt 1	1	1588.92	4.97	0.0295
	Error	61	19510.65		
35	Block	2	1623.84	2.93	0.0599
	Ozone	4	548.85	0.50	0.7390
	Hgt 1	1	84.81	0.31	0.5817
	Error	70	19382.39		

Table B.2 (continued)

Family	Source	DF	Type III ss	F value	PR > F
36	Block	2	330.17	0.64	0.5322
	Ozone	4	2516.11	2.43	0.0561
	Hgt 1	1	3215.91	12.40	0.0008
	Error	69	17894.68		
37	Block	2	738.32	1.37	0.2602
	Ozone	4	4465.57	4.15	0.0045
	Hgt 1	1	5832.51	21.69	0.0001
	Error	69	18553.06		
38	Block	2	1613.79	1.98	0.1457
	Ozone	4	1144.73	0.70	0.5922
	Hgt 1	1	5528.02	13.60	0.0005
	Error	65	26430.08		
39	Block	2	1078.05	1.51	0.2287
	Ozone	4	5115.23	3.57	0.0104
	Hgt 1	1	3572.17	9.98	0.0023
	Error	70	25043.45		
40	Block	2	1269.69	1.37	0.2619
	Ozone	4	6563.23	3.53	0.0111
	Hgt 1	1	4336.32	9.33	0.0032
	Error	70	32537.94		
41	Block	2	544.20	0.81	0.4475
	Ozone	4	1193.18	0.89	0.4736
	Hgt 1	1	3587.87	10.73	0.0017
	Error	65	21726.45		
42	Block	2	5449.75	5.82	0.0047
	Ozone	4	3638.04	1.94	0.1134
	Hgt 1	1	6395.54	13.66	0.0004
	Error	67	31362.33		
43	Block	2	716.33	1.52	0.2253
	Ozone	4	2919.41	3.10	0.0208
	Hgt 1	1	4089.36	17.40	0.0001
	Error	68	15985.23		
44	Block	2	277.80	0.43	0.6498
	Ozone	4	3456.80	2.69	0.0349
	Hgt 1	1	6152.83	19.18	0.0001
	Error	105	33688.13		
45	Block	2	2648.96	2.14	0.1278
	Ozone	4	2357.68	0.95	0.4413
	Hgt 1	1	3430.52	5.55	0.0223
	Error	52	32169.54		
46	Block	2	2248.12	3.11	0.0509
	Ozone	4	4458.51	3.09	0.0214
	Hgt 1	1	1758.49	4.87	0.0307
	Error	67	24179.29		

Table B.2 (continued)

Family	Source	DF	Type III ss	F value	PR > F
47	Block	2	113.16	0.18	0.8341
	Ozone	4	2969.53	2.39	0.0601
	Hgt 1	1	21.20	0.07	0.7949
	Error	65	20222.07		
48	Block	2	12.82	0.03	0.9731
	Ozone	4	1506.18	1.60	0.1844
	Hgt 1	1	1.61	0.01	0.9343
	Error	64	15034.51		
49	Block	2	1645.23	1.99	0.1448
	Ozone	4	4235.79	2.56	0.0462
	Hgt 1	1	1046.20	2.53	0.1165
	Error	70	28986.42		
50	Block	2	253.73	0.38	0.6827
	Ozone	4	1563.21	1.18	0.3259
	Hgt 1	1	2078.72	6.29	0.0144
	Error	73	24133.85		
51	Block	2	2824.65	2.54	0.0840
	Ozone	4	3418.78	1.54	0.1972
	Hgt 1	1	5242.32	9.43	0.0028
	Error	97	53904.65		
52	Block	2	572.76	0.24	0.7863
	Ozone	4	6839.36	1.44	0.2302
	Hgt 1	1	894.53	0.75	0.3884
	Error	67	79512.98		
53	Block	2	90.40	0.10	0.9066
	Ozone	4	3400.01	1.85	0.1299
	Hgt 1	1	1285.55	2.79	0.0992
	Error	68	31290.51		
54	Block	2	588.46	0.97	0.3867
	Ozone	4	2401.26	1.97	0.1112
	Hgt 1	1	745.12	2.45	0.1233
	Error	57	17359.44		
55	Block	2	1182.96	1.34	0.2695
	Ozone	4	3771.76	2.14	0.0885
	Hgt 1	1	2528.49	5.75	0.0201
	Error	52	22872.76		

Table B.3. Ozone*rain interaction effects on seedling diameter.

Family	Source	DF	Type III ss	F value	PR > F
2	Block	2	1.734	4.08	0.0195
	Ozone	2	0.343	0.81	0.4486
	Rain	2	1.196	2.82	0.0643
	O * R	4	0.445	0.52	0.7183
	Diam 1	1	0.013	0.06	0.8066
	Error	108	22.939		
3	Block	2	1.493	3.17	0.0460
	Ozone	2	0.786	1.67	0.1933
	Rain	2	0.105	0.22	0.8004
	O * R	4	0.352	0.37	0.8269
	Diam 1	1	2.655	11.27	0.0011
	Error	108	25.447		
4	Block	2	5.342	13.37	0.0001
	Ozone	2	1.805	4.52	0.0136
	Rain	2	0.014	0.04	0.9649
	O * R	4	1.493	1.87	0.1231
	Diam 1	1	0.099	0.50	0.4824
	Error	88	17.585		
5	Block	2	1.175	2.34	0.1007
	Ozone	2	0.157	0.31	0.7313
	Rain	2	0.923	1.84	0.1632
	O * R	4	0.740	0.74	0.5675
	Diam 1	1	0.735	2.93	0.0897
	Error	108	27.049		
6	Block	2	3.579	13.15	0.0001
	Ozone	2	0.976	3.59	0.0313
	Rain	2	0.007	0.03	0.9743
	O * R	4	0.762	1.40	0.2396
	Diam 1	1	0.011	0.08	0.7772
	Error	100	13.608		
7	Block	2	1.544	3.02	0.0530
	Ozone	2	0.294	0.57	0.5646
	Rain	2	0.049	0.10	0.9079
	O * R	4	0.573	0.56	0.6921
	Diam 1	1	0.023	0.09	0.7643
	Error	104	26.579		
8	Block	2	0.581	1.14	0.3232
	Ozone	2	1.901	3.74	0.0272
	Rain	2	0.033	0.07	0.9370
	O * R	4	1.413	1.39	0.2434
	Diam 1	1	3.264	12.82	0.0005
	Error	102	25.959		

Table B.3 (continued)

Family	Source	DF	Type III ss	F value	PR > F
9	Block	2	3.146	5.31	0.0066
	Ozone	2	0.200	0.34	0.7145
	Rain	2	0.066	0.11	0.8949
	O * R	4	0.556	0.47	0.7585
	Diam 1	1	0.579	1.95	0.1654
	Error	93	27.554		
10	Block	2	2.313	2.91	0.0593
	Ozone	2	1.278	1.61	0.2055
	Rain	2	0.310	0.39	0.6778
	O * R	4	2.022	1.27	0.2861
	Diam 1	1	0.066	0.17	0.6839
	Error	94	37.335		
11	Block	2	2.978	6.09	0.0032
	Ozone	2	0.278	0.57	0.5675
	Rain	2	0.543	1.11	0.3331
	O * R	4	2.490	2.55	0.0439
	Diam 1	1	0.075	0.31	0.5807
	Error	101	24.684		
12	Block	2	2.146	3.94	0.0223
	Ozone	2	0.837	1.54	0.2197
	Rain	2	0.284	0.52	0.5953
	O * R	4	1.429	1.31	0.2703
	Diam 1	1	0.494	1.82	0.1807
	Error	109	29.690		
13	Block	2	1.222	2.62	0.0772
	Ozone	2	1.127	2.42	0.0939
	Rain	2	0.046	0.10	0.9056
	O * R	4	1.402	1.50	0.2060
	Diam 1	1	0.200	0.86	0.3561
	Error	110	25.642		
14	Block	2	0.987	2.07	0.1312
	Ozone	2	0.552	1.16	0.3184
	Rain	2	0.426	0.89	0.4121
	O * R	4	2.441	2.56	0.0425
	Diam 1	1	0.949	3.98	0.0486
	Error	113	26.963		
15	Block	2	2.589	5.43	0.0057
	Ozone	2	0.090	0.19	0.8278
	Rain	2	0.197	0.41	0.6633
	O * R	4	1.700	1.78	0.1377
	Diam 1	1	0.007	0.03	0.8613
	Error	109	25.998		

Table B.3 (continued)

Family	Source	DF	Type III ss	F value	PR > F
16	Block	2	1.449	2.99	0.0546
	Ozone	2	0.806	1.66	0.1947
	Rain	2	1.290	2.66	0.0747
	O * R	4	0.693	0.71	0.5844
	Diam 1	1	0.022	0.09	0.7623
	Error	111	26.942		
17	Block	2	0.373	1.12	0.3310
	Ozone	2	0.385	1.15	0.3194
	Rain	2	0.203	0.61	0.5463
	O * R	4	1.444	2.17	0.0783
	Diam 1	1	0.066	0.40	0.5291
	Error	100	16.667		
18	Block	2	1.143	2.79	0.0660
	Ozone	2	1.112	2.71	0.0710
	Rain	2	1.430	3.49	0.0340
	O * R	4	0.568	0.69	0.5990
	Diam 1	1	0.000	0.00	0.9941
	Error	111	22.771		
19	Block	2	1.123	1.70	0.1886
	Ozone	2	2.151	3.26	0.0434
	Rain	2	0.061	0.09	0.9112
	O * R	4	1.665	1.26	0.2916
	Diam 1	1	0.208	0.63	0.4291
	Error	82	27.046		
20	Block	2	0.163	0.26	0.7748
	Ozone	2	0.315	0.49	0.6112
	Rain	2	0.734	1.15	0.3190
	O * R	4	1.579	1.24	0.2976
	Diam 1	1	1.246	3.92	0.0503
	Error	110	34.980		
21	Block	2	0.034	0.07	0.9291
	Ozone	2	0.760	1.66	0.1943
	Rain	2	0.216	0.47	0.6240
	O * R	4	0.283	0.31	0.8711
	Diam 1	1	1.133	4.96	0.0280
	Error	107	24.443		
23	Block	2	0.120	0.22	0.8005
	Ozone	2	0.246	0.46	0.6339
	Rain	2	0.641	1.19	0.3072
	O * R	4	1.840	1.71	0.1523
	Diam 1	1	1.079	4.02	0.0474
	Error	111	29.818		

Table B.3 (continued)

Family	Source	DF	Type III ss	F value	PR > F
24	Block	2	0.208	0.45	0.6410
	Ozone	2	1.457	3.12	0.0480
	Rain	2	0.982	2.11	0.1268
	O * R	4	2.157	2.31	0.0622
	Diam 1	1	0.702	3.01	0.0856
	Error	109	25.432		
25	Block	2	7.029	11.62	0.0001
	Ozone	2	0.481	0.79	0.4544
	Rain	2	0.446	0.74	0.4805
	O * R	4	2.901	2.40	0.0546
	Diam 1	1	0.326	1.08	0.3013
	Error	110	33.281		
26	Block	2	1.157	2.28	0.1074
	Ozone	2	1.987	3.92	0.0230
	Rain	2	0.007	0.01	0.9869
	O * R	4	1.190	1.17	0.3267
	Diam 1	1	0.958	3.78	0.0547
	Error	99	25.082		
27	Block	2	1.711	2.99	0.0545
	Ozone	2	0.923	1.61	0.2043
	Rain	2	0.543	0.95	0.3906
	O * R	4	0.473	0.41	0.7991
	Diam 1	1	0.019	0.07	0.7961
	Error	113	32.387		
28	Block	2	0.785	1.34	0.2650
	Ozone	2	0.219	0.37	0.6885
	Rain	2	0.930	1.59	0.2082
	O * R	4	3.934	3.37	0.0122
	Diam 1	1	2.759	9.45	0.0027
	Error	109	31.833		
29	Block	2	0.729	2.21	0.1142
	Ozone	2	0.395	1.20	0.3057
	Rain	2	1.869	5.67	0.0045
	O * R	4	2.564	3.89	0.0054
	Diam 1	1	0.862	5.23	0.0240
	Error	113	18.626		
30	Block	2	0.288	0.72	0.4891
	Ozone	2	1.986	4.97	0.0086
	Rain	2	1.283	3.21	0.0441
	O * R	4	1.285	1.61	0.1775
	Diam 1	1	0.120	0.60	0.4403
	Error	113	22.596		

Table B.3 (continued)

Family	Source	DF	Type III ss	F value	PR > F
31	Block	2	0.477	0.77	0.4659
	Ozone	2	1.515	2.44	0.0921
	Rain	2	0.955	1.54	0.2194
	O * R	4	0.386	0.31	0.8697
	Diam 1	1	0.357	1.15	0.2857
	Error	97	30.052		
32	Block	2	3.707	7.31	0.0009
	Ozone	2	0.994	1.96	0.1440
	Rain	2	0.684	1.35	0.2622
	O * R	4	3.749	3.69	0.0065
	Diam 1	1	4.206	16.58	0.0001
	Error	174	44.137		
33	Block	2	5.565	15.30	0.0001
	Ozone	2	0.430	1.18	0.3105
	Rain	2	1.251	3.44	0.0356
	O * R	4	0.997	1.37	0.2490
	Diam 1	1	0.052	0.29	0.5925
	Error	111	20.189		
34	Block	2	1.244	3.15	0.0473
	Ozone	2	1.125	2.85	0.0628
	Rain	2	0.220	0.56	0.5744
	O * R	4	0.831	1.05	0.3848
	Diam 1	1	0.170	0.86	0.3557
	Error	99	19.558		
35	Block	2	0.425	0.74	0.4794
	Ozone	2	0.642	1.12	0.3306
	Rain	2	1.048	1.82	0.1661
	O * R	4	0.813	0.71	0.5888
	Diam 1	1	0.276	0.96	0.3294
	Error	114	32.762		
36	Block	2	2.262	4.56	0.0124
	Ozone	2	0.873	1.76	0.1767
	Rain	2	1.787	3.60	0.0304
	O * R	4	2.107	2.12	0.0823
	Diam 1	1	4.421	17.83	0.0001
	Error	113	28.014		
37	Block	2	0.228	0.55	0.5799
	Ozone	2	0.891	2.14	0.1224
	Rain	2	0.532	1.28	0.2828
	O * R	4	3.310	3.97	0.0047
	Diam 1	1	16.663	80.01	0.0001
	Error	113			

Table B.3 (continued)

Family	Source	DF	Type III ss	F value	PR > F
38	Block	2	0.818	1.10	0.3362
	Ozone	2	0.671	0.90	0.4081
	Rain	2	0.838	1.13	0.3274
	O * R	4	0.560	0.38	0.8247
	Diam 1	1	0.080	0.21	0.6440
	Error	111	41.223		
39	Block	2	2.190	4.11	0.0190
	Ozone	2	1.961	3.68	0.0284
	Rain	2	0.002	0.00	0.9971
	O * R	4	2.588	2.43	0.0521
	Diam 1	1	2.217	8.32	0.0047
	Error	108	28.773		
40	Block	2	1.797	3.07	0.0503
	Ozone	2	3.696	6.32	0.0025
	Rain	2	0.080	0.14	0.8723
	O * R	4	0.256	0.22	0.9275
	Diam 1	1	0.012	0.04	0.8386
	Error	108	31.560		
41	Block	2	0.453	1.30	0.2766
	Ozone	2	0.026	0.08	0.9269
	Rain	2	0.262	0.75	0.4736
	O * R	4	2.323	3.33	0.0128
	Diam 1	1	2.793	16.04	0.0001
	Error	110	19.160		
42	Block	2	0.486	0.87	0.4221
	Ozone	2	0.678	1.21	0.3010
	Rain	2	0.645	1.15	0.3190
	O * R	4	2.722	2.44	0.0514
	Diam 1	1	0.428	1.53	0.2183
	Error	109	30.443		
43	Block	2	0.415	1.41	0.2475
	Ozone	2	0.433	1.47	0.2334
	Rain	2	0.866	2.95	0.0563
	O * R	4	0.587	1.00	0.4108
	Diam 1	1	0.724	4.94	0.0283
	Error	114	16.729		
44	Block	2	0.973	2.24	0.1092
	Ozone	2	2.506	5.78	0.0037
	Rain	2	0.801	1.85	0.1609
	O * R	4	2.693	3.11	0.0169
	Diam 1	1	0.011	0.05	0.8222
	Error	169	36.631		

Table B.3 (continued)

Family	Source	DF	Type III ss	F value	PR > F
45	Block	2	0.511	0.76	0.4729
	Ozone	2	0.132	0.20	0.8228
	Rain	2	2.803	4.14	0.0193
	O * R	4	1.911	1.41	0.2371
	Diam 1	1	0.109	0.32	0.5715
	Error	84	28.421		
46	Block	2	0.590	1.15	0.3201
	Ozone	2	1.343	2.62	0.0775
	Rain	2	0.807	1.57	0.2119
	O * R	4	1.554	1.51	0.2028
	Diam 1	1	2.443	9.53	0.0026
	Error	111	28.466		
47	Block	2	0.125	0.26	0.7705
	Ozone	2	0.047	0.10	0.9074
	Rain	2	0.045	0.09	0.9114
	O * R	4	2.226	2.32	0.0613
	Diam 1	1	0.346	1.45	0.2319
	Error	107	25.644		
48	Block	2	0.520	0.85	0.4301
	Ozone	2	0.396	0.65	0.5257
	Rain	2	0.197	0.32	0.7251
	O * R	4	0.826	0.68	0.6105
	Diam 1	1	2.875	9.41	0.0027
	Error	109	33.320		
49	Block	2	0.600	1.30	0.2776
	Ozone	2	0.805	1.74	0.1801
	Rain	2	0.544	1.18	0.3121
	O * R	4	1.176	1.27	0.2854
	Diam 1	1	0.862	3.73	0.0561
	Error	113	26.134		
50	Block	2	0.473	0.95	0.3893
	Ozone	2	0.405	0.82	0.4451
	Rain	2	0.349	0.70	0.4973
	O * R	4	0.140	0.14	0.9664
	Diam 1	1	0.451	1.81	0.1811
	Error	97	24.083		
51	Block	2	0.448	0.72	0.4883
	Ozone	2	0.804	1.29	0.2772
	Rain	2	2.716	4.37	0.0143
	O * R	4	0.522	0.42	0.7941
	Diam 1	1	2.375	7.64	0.0064
	Error	156	48.508		

Table B.3 (continued).

Family	Source	DF	Type III ss	F value	PR F
52	Block	2	1.867	3.02	0.0529
	Ozone	2	0.174	0.28	0.7554
	Rain	2	0.193	0.31	0.7321
	O * R	4	0.666	0.54	0.7076
	Diam 1	1	0.491	1.59	0.2103
	Error	108	33.366		
53	Block	2	1.639	5.57	0.0049
	Ozone	2	0.183	0.62	0.5381
	Rain	2	1.354	4.60	0.0120
	O * R	4	0.734	1.25	0.2952
	Diam 1	1	0.568	3.87	0.0518
	Error	111	16.322		
54	Block	1	0.757	4.60	0.0351
	Ozone	2	0.129	0.39	0.6770
	Rain	2	0.015	0.05	0.9544
	O * R	4	0.797	1.21	0.3127
	Diam 1	1	0.704	4.28	0.0419
	Error	77	12.661		
55	Block	1	1.462	7.64	0.0073
	Ozone	2	0.326	0.85	0.4304
	Rain	2	0.428	1.12	0.3326
	O * R	4	0.874	1.14	0.3437
	Diam 1	1	0.285	1.49	0.2263
	Error	71	13.582		

Table B.4. Ozone * rain interaction effects on seedling height

Family	Source	DF	Type III ss	F value	PR > F
2	Block	2	542.09	1.11	0.3324
	Ozone	2	255.09	0.52	0.5939
	Rain	2	809.92	1.66	0.1945
	O * R	4	1443.08	1.48	0.2130
	Hgt 1	1	8272.01	33.96	0.0001
	Error	107	26060.98		
3	Block	2	4471.93	5.61	0.0048
	Ozone	2	3209.85	4.03	0.0206
	Rain	2	111.72	0.14	0.8694
	O * R	4	1384.46	0.87	0.4856
	Hgt 1	1	6760.54	16.96	0.0001
	Error	108	43054.29		
4	Block	2	14557.92	12.87	0.0001
	Ozone	2	1053.61	0.93	0.3978
	Rain	2	1088.58	0.96	0.3860
	O * R	4	1077.71	0.48	0.7530
	Hgt 1	1	2847.43	5.03	0.0274
	Error	88	49769.24		
5	Block	2	961.45	1.47	0.2346
	Ozone	2	446.13	0.68	0.5077
	Rain	2	2725.17	4.17	0.0182
	O * R	4	1620.02	1.24	0.2991
	Hgt 1	1	3115.76	9.53	0.0026
	Error	103	33670.36		
6	Block	2	2139.73	1.81	0.1696
	Ozone	2	267.57	0.23	0.7982
	Rain	2	1374.09	1.16	0.3177
	O * R	4	7448.92	3.15	0.0177
	Hgt 1	1	2890.12	4.88	0.0295
	Error	97	57428.30		
7	Block	2	436.05	0.33	0.7175
	Ozone	2	1101.93	0.84	0.4340
	Rain	2	434.65	0.33	0.7183
	O * R	4	2679.24	1.02	0.3990
	Hgt 1	1	2412.13	3.68	0.0577
	Error	103	67431.00		
8	Block	2	6903.36	8.34	0.0004
	Ozone	2	1069.65	1.29	0.2793
	Rain	2	2236.93	2.70	0.0719
	O * R	4	1948.87	1.18	0.3256
	Hgt 1	1	6214.07	15.01	0.0002
	Error	101	41813.58		

Table B.4 (continued)

Family	Source	DF	Type III ss	F value	PR > F
9	Block	2	4970.28	3.86	0.0247
	Ozone	2	1868.98	1.45	0.2399
	Rain	2	1229.19	0.95	0.3891
	O * R	4	3615.35	1.40	0.2394
	Hgt 1	1	5649.16	8.77	0.0039
	Error	91	58643.15		
10	Block	2	3968.68	4.19	0.0181
	Ozone	2	1706.79	1.80	0.1707
	Rain	2	3398.02	3.59	0.0316
	O * R	4	3658.42	1.93	0.1117
	Hgt 1	1	2899.21	6.12	0.0152
	Error	92	43557.10		
11	Block	2	35.98	0.04	0.9600
	Ozone	2	1564.83	1.77	0.1749
	Rain	2	2450.24	2.78	0.0670
	O * R	4	515.33	0.29	0.8824
	Hgt 1	1	8116.62	18.41	0.0001
	Error	99	43648.77		
12	Block	2	3406.96	2.43	0.0927
	Ozone	2	585.89	0.42	0.6594
	Rain	2	4663.28	3.33	0.0396
	O * R	4	1439.89	0.51	0.7258
	Hgt 1	1	5914.03	8.44	0.0045
	Error	108	75673.45		
13	Block	2	924.44	1.45	0.2380
	Ozone	2	1148.05	1.81	0.1691
	Rain	2	1561.04	2.46	0.0905
	O * R	4	810.46	0.64	0.6367
	Hgt 1	1	3508.83	11.04	0.0012
	Error	109	34632.99		
14	Block	2	46.92	0.16	0.8544
	Ozone	2	167.73	0.56	0.5709
	Rain	2	1583.20	5.32	0.0062
	O * R	4	1002.27	1.68	0.1589
	Hgt 1	1	3379.71	22.70	0.0001
	Error	111	16523.62		
15	Block	2	1636.07	2.26	0.1093
	Ozone	2	425.38	0.59	0.5575
	Rain	2	1054.15	1.46	0.2377
	O * R	4	3064.99	2.12	0.0837
	Hgt 1	1	4558.42	12.59	0.0006
	Error	107	38732.11		

Table B.4 (continued)

Family	Source	DF	Type III ss	F value	PR > F
16	Block	2	733.42	1.38	0.2558
	Ozone	2	172.33	0.32	0.7237
	Rain	2	910.18	1.71	0.1851
	O * R	4	1933.30	1.82	0.1302
	Hgt 1	1	4064.39	15.30	0.0002
	Error	109	28956.24		
17	Block	2	2070.39	2.59	0.0797
	Ozone	2	1982.17	2.48	0.0886
	Rain	2	958.35	1.20	0.3052
	O * R	4	756.50	0.47	0.7547
	Hgt 1	1	5285.02	13.24	0.0004
	Error	100	39902.48		
18	Block	2	58.94	0.11	0.8983
	Ozone	2	170.28	0.31	0.7341
	Rain	2	384.17	0.70	0.4990
	O * R	4	1270.84	1.16	0.3339
	Hgt 1	1	706.90	2.57	0.1115
	Error	111	30484.41		
19	Block	2	807.42	0.64	0.5308
	Ozone	2	2195.33	1.74	0.1828
	Rain	2	678.68	0.54	0.5869
	O * R	4	3028.08	1.20	0.3185
	Hgt 1	1	1991.15	3.15	0.0797
	Error	82	51869.33		
20	Block	2	8.87	0.01	0.9867
	Ozone	2	1013.69	1.54	0.2202
	Rain	2	1225.03	1.86	0.1615
	O * R	4	1363.58	1.03	0.3941
	Hgt 1	1	6200.24	18.78	0.0001
	Error	105	34669.42		
21	Block	2	55.32	0.10	0.9054
	Ozone	2	316.57	0.57	0.5678
	Rain	2	1501.41	2.70	0.0719
	O * R	4	2410.92	2.17	0.0777
	Hgt 1	1	4100.01	14.74	0.0002
	Error	106	29485.50		
23	Block	2	1237.42	0.91	0.4060
	Ozone	2	2474.47	1.82	0.1673
	Rain	2	2886.02	2.12	0.1249
	O * R	4	7789.01	2.86	0.0267
	Hgt 1	1	3272.44	4.81	0.0304
	Error	111	75569.05		

Table B.4 (continued)

Family	Source	DF	Type III ss	F value	PR > F
24	Block	2	3803.82	4.39	0.0147
	Ozone	2	1421.11	1.64	0.1990
	Rain	2	679.41	0.78	0.4593
	O * R	4	1513.41	0.87	0.4828
	Hgt 1	1	7180.08	16.56	0.0001
	Error	107	46379.28		
25	Block	2	75.09	0.08	0.9241
	Ozone	2	2023.74	2.13	0.1241
	Rain	2	1524.74	1.60	0.2060
	O * R	4	1305.32	0.69	0.6027
	Hgt 1	1	6743.54	14.19	0.0003
	Error	104	49422.09		
26	Block	2	1009.06	0.80	0.4538
	Ozone	2	1759.24	1.39	0.2543
	Rain	2	5778.30	4.56	0.0128
	O * R	4	355.04	0.14	0.9669
	Hgt 1	1	3555.83	5.61	0.0198
	Error	98	62074.88		
27	Block	2	1538.72	0.71	0.4916
	Ozone	2	2039.67	0.95	0.3909
	Rain	2	3188.92	1.48	0.2318
	O * R	4	8877.98	2.06	0.0906
	Hgt 1	1	9864.33	9.16	0.0031
	Error	111	119486.50		
28	Block	2	284.36	0.63	0.5371
	Ozone	2	245.32	0.54	0.5847
	Rain	2	94.76	0.21	0.8123
	O * R	4	2455.06	2.70	0.0344
	Hgt 1	1	1786.72	7.85	0.0060
	Error	109	24794.80		
29	Block	2	847.21	0.63	0.5368
	Ozone	2	1984.45	1.47	0.2354
	Rain	2	1079.75	0.80	0.4531
	O * R	4	10286.66	3.80	0.0062
	Hgt 1	1	2794.40	4.13	0.0446
	Error	111	75160.43		
30	Block	2	320.27	0.30	0.7413
	Ozone	2	2819.74	2.64	0.0756
	Rain	2	275.18	0.26	0.7731
	O * R	4	7292.85	3.42	0.0112
	Hgt 1	1	1959.05	3.67	0.0579
	Error	112	59747.52		

Table B.4 (continued)

Family	Source	DF	Type III ss	F value	PR > F
31	Block	2	5983.11	4.80	0.0103
	Ozone	2	210.75	0.17	0.8447
	Rain	2	156.13	0.13	0.8824
	O * R	4	972.01	0.39	0.8155
	Hgt 1	1	243.15	0.39	0.5338
	Error	96	59845.85		
32	Block	2	2311.80	1.74	0.1791
	Ozone	2	2394.25	1.80	0.1686
	Rain	2	1039.48	0.78	0.4596
	O * R	4	4918.11	1.85	0.1219
	Hgt 1	1	17007.35	25.55	0.0001
	Error	173	115137.18		
33	Block	2	1095.81	1.83	0.1647
	Ozone	2	907.36	1.52	0.2237
	Rain	2	1.65	0.00	0.9972
	O * R	4	5706.70	4.77	0.0014
	Hgt 1	1	306.09	1.02	0.3137
	Error	109	32569.23		
34	Block	2	475.49	0.55	0.5771
	Ozone	2	876.81	1.02	0.3646
	Rain	2	292.97	0.34	0.7122
	O * R	4	2300.97	1.34	0.2615
	Hgt 1	1	3729.87	8.67	0.0040
	Error	97	41716.23		
35	Block	2	1642.90	2.06	0.1320
	Ozone	2	183.31	0.23	0.7948
	Rain	2	1448.30	1.82	0.1671
	O * R	4	2373.30	1.49	0.2101
	Hgt 1	1	19.59	0.05	0.8249
	Error	113	45014.94		
36	Block	2	54.39	0.09	0.9108
	Ozone	2	65.56	0.11	0.8936
	Rain	2	448.32	0.77	0.4653
	O * R	4	1668.42	1.43	0.2276
	Hgt 1	1	897.25	3.08	0.0818
	Error	111	32296.88		
37	Block	2	356.62	0.56	0.5743
	Ozone	2	110.93	0.17	0.8410
	Rain	2	1202.18	1.88	0.1575
	O * R	4	6680.67	5.22	0.0007
	Hgt 1	1	12261.11	38.33	0.0001
	Error	113	36147.75		

Table B.4 (continued)

Family	Source	DF	Type III ss	F value	PR > F
38	Block	2	806.66	0.75	0.4752
	Ozone	2	2592.22	2.41	0.0949
	Rain	2	1302.78	1.21	0.3022
	O * R	4	2196.19	1.02	0.4004
	Hgt 1	1	9897.12	18.39	0.0001
	Error	107	57595.67		
39	Block	2	571.19	0.62	0.5413
	Ozone	2	698.35	0.75	0.4726
	Rain	2	197.37	0.21	0.8082
	O * R	4	3678.49	1.99	0.1016
	Hgt 1	1	4398.51	9.51	0.0026
	Error	106	49035.90		
40	Block	2	678.73	0.78	0.4632
	Ozone	2	703.25	0.80	0.4506
	Rain	2	907.69	1.04	0.3582
	O * R	4	4682.34	2.67	0.0359
	Hgt 1	1	2938.51	6.71	0.0109
	Error	107	46850.52		
41	Block	2	1185.20	2.24	0.1114
	Ozone	2	531.21	1.01	0.3696
	Rain	2	273.95	0.52	0.5971
	O * R	4	2335.61	2.21	0.0731
	Hgt 1	1	1445.52	5.47	0.0213
	Error	102	26954.90		
42	Block	2	8634.64	7.80	0.0007
	Ozone	2	580.73	0.52	0.5935
	Rain	2	879.90	0.79	0.4545
	O * R	4	3401.58	1.54	0.1970
	Hgt 1	1	8304.35	14.99	0.0002
	Error	109	60369.90		
43	Block	2	29.88	0.05	0.9559
	Ozone	2	765.26	1.16	0.3187
	Rain	2	1863.17	2.81	0.0644
	O * R	4	638.36	0.48	0.7490
	Hgt 1	1	6775.83	20.46	0.0001
	Error	110	36429.06		
44	Block	2	775.08	1.22	0.2976
	Ozone	2	49.84	0.08	0.9245
	Rain	2	3712.48	5.85	0.0035
	O * R	4	3410.78	2.69	0.0332
	Hgt 1	1	5820.28	18.34	0.0001
	Error	164	52048.47		

Table B.4 (continued)

Family	Source	DF	Type III ss	F value	PR > F
45	Block	2	2793.30	3.01	0.0547
	Ozone	2	1441.14	1.55	0.2177
	Rain	2	2683.00	2.89	0.0611
	O * R	4	4812.74	2.59	0.0423
	Hgt 1	1	4303.96	9.27	0.0031
	Error	84	38981.48		
46	Block	2	1685.37	1.95	0.1472
	Ozone	2	5904.42	6.84	0.0016
	Rain	2	405.44	0.47	0.6267
	O * R	4	3354.28	1.94	0.1088
	Hgt 1	1	2156.19	4.99	0.0275
	Error	107	46215.92		
47	Block	2	205.31	0.19	0.8250
	Ozone	2	808.64	0.76	0.4708
	Rain	2	1544.41	1.45	0.2395
	O * R	4	3724.74	1.75	0.1454
	Hgt 1	1	206.21	0.39	0.5352
	Error	101	53808.87		
48	Block	2	888.55	1.90	0.1544
	Ozone	2	158.37	0.34	0.7134
	Rain	2	658.46	1.41	0.2490
	O * R	4	901.60	0.96	0.4302
	Hgt 1	1	129.84	0.56	0.4577
	Error	108	25243.74		
49	Block	2	812.24	1.91	0.1529
	Ozone	2	173.95	0.41	0.6653
	Rain	2	566.83	1.33	0.2679
	O * R	4	1199.66	1.41	0.2351
	Hgt 1	1	1533.99	7.21	0.0083
	Error	112	23817.22		
50	Block	2	693.59	0.86	0.4250
	Ozone	2	756.80	0.94	0.3934
	Rain	2	58.20	0.07	0.9302
	O * R	4	810.41	0.50	0.7326
	Hgt 1	1	4499.42	11.20	0.0012
	Error	96	38564.52		
51	Block	2	3380.11	1.51	0.2251
	Ozone	2	1774.80	0.79	0.4554
	Rain	2	4174.09	1.86	0.1592
	O * R	4	6529.48	1.45	0.2188
	Hgt 1	1	3753.31	3.34	0.0694
	Error	154	172841.54		

Table B.4 (continued)

Family	Source	DF	Type III ss	F value	PR > F
52	Block	2	71.20	0.04	0.9637
	Ozone	2	528.31	0.27	0.7608
	Rain	2	4365.93	2.27	0.1088
	O * R	4	11292.16	2.93	0.0242
	Hgt 1	1	43.80	0.05	0.8316
	Error	107	103109.81		
53	Block	2	320.54	0.34	0.7112
	Ozone	2	1283.22	1.37	0.2588
	Rain	2	2665.51	2.84	0.0626
	O * R	4	4243.63	2.26	0.0670
	Hgt 1	1	2178.05	4.65	0.0333
	Error	108	50630.17		
54	Block	1	83.38	0.26	0.6113
	Ozone	2	369.75	0.58	0.5638
	Rain	2	302.23	0.47	0.6256
	O * R	4	297.70	0.23	0.9193
	Hgt 1	1	3972.50	12.41	0.0007
	Error	76	24335.92		
55	Block	1	3.48	0.01	0.9351
	Ozone	2	1338.15	1.28	0.2839
	Rain	2	536.87	0.51	0.6000
	O * R	4	3183.26	1.53	0.2044
	Hgt 1	1	1276.20	2.45	0.1224
	Error	69	35996.37		

Table B.5. Seedling diameter responses to ozone in CSTR studies.

Family	Source	DF	Type III ss	F value	PR F
2	Rep	3	0.723	3.58	0.0359
	Ozone	2	0.090	0.67	0.5256
	Diam 1	1	0.002	0.03	0.8699
	Error	17	1.145		
3	Rep	3	0.271	0.58	0.6376
	Ozone	2	0.232	0.74	0.4912
	Diam 1	1	1.678	10.74	0.0044
	Error	17	2.656		
4	Rep	3	0.135	0.74	0.5413
	Ozone	2	0.225	1.85	0.1879
	Diam 1	1	0.587	9.65	0.0064
	Error	17	1.033		
5	Rep	3	0.291	1.40	0.2770
	Ozone	2	0.162	1.17	0.3350
	Diam 1	1	0.301	4.34	0.0527
	Error	17	1.178		
6	Rep	3	0.229	1.30	0.3076
	Ozone	2	0.233	1.98	0.1687
	Diam 1	1	0.030	0.51	0.4847
	Error	17	0.999		
8	Rep	3	0.134	0.43	0.7377
	Ozone	2	0.423	2.01	0.1665
	Diam 1	1	1.555	14.78	0.0014
	Error	16	1.684		
9	Rep	3	0.221	0.27	0.8437
	Ozone	2	1.501	2.78	0.0900
	Diam 1	1	0.029	0.11	0.7458
	Error	17	4.584		
10	Rep	3	0.010	0.06	0.9805
	Ozone	2	0.154	1.32	0.2942
	Diam 1	1	0.532	9.14	0.0081
	Error	16	0.931		

Table B.6. Seedling height responses to ozone in CSTR studies.

Family	Source	DF	Type III ss	F value	PR > F
2	Rep	3	175.57	0.61	0.6181
	Ozone	2	332.53	1.74	0.2097
	Hgt 1	1	325.04	3.39	0.0853
	Error	15	1436.18		
3	Rep	3	703.43	1.31	0.3054
	Ozone	2	796.68	2.23	0.1402
	Hgt 1	1	140.59	0.79	0.3885
	Error	16	2862.27		
4	Rep	3	185.06	0.16	0.9227
	Ozone	2	1144.08	1.47	0.2577
	Hgt 1	1	224.94	0.58	0.4575
	Error	17	6615.81		
5	Rep	3	319.59	0.52	0.6725
	Ozone	2	142.86	0.35	0.7094
	Hgt 1	1	1.44	0.01	0.9340
	Error	16	3257.52		
6	Rep	3	107.61	0.26	0.8503
	Ozone	2	699.29	2.57	0.1055
	Hgt 1	1	2.70	0.02	0.8896
	Error	17	2309.14		
8	Rep	3	612.55	1.20	0.3419
	Ozone	2	621.31	1.83	0.1940
	Hgt 1	1	1437.11	8.48	0.0107
	Error	15	2542.07		
9	Rep	3	15.01	0.01	0.9992
	Ozone	2	1647.85	1.14	0.3445
	Hgt 1	1	1.29	0.00	0.9669
	Error	17	12337.63		
10	Rep	3	144.37	0.14	0.9316
	Ozone	2	359.60	0.54	0.5919
	Hgt 1	1	1.84	0.01	0.9415
	Error	17	5651.41		

APPENDIX C

THREE TECHNIQUES FOR MEASURING PHOTOSYNTHESIS OF LOBLOLLY
PINE SHOOTS: COMPARISONS BETWEEN TECHNIQUES AND
THEIR RELATIONSHIP TO SEEDLING GROWTH

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C.1 INTRODUCTION

Measurements of the exchange of CO₂ (photosynthesis) and water vapor (transpiration) are important indicators of a plant's physiological status and are widely utilized in studies of plant stress. A variety of methodologies are available for measuring these gas-exchange processes. Laboratory gas-analysis systems based on open or closed gas-flow designs encompassing a range of complexity (Jarvis et al. 1971) have been used in plant research since the development of infrared CO₂ analyzers. Techniques utilizing ¹⁴CO₂ labeling (Voznesenskii et al. 1971, Michael et al. 1985), together with scintillation counting systems to assay the radioactivity have also been employed. Recently, commercially available and truly portable gas-exchange systems have become available for routine use in field monitoring of crop and forest plant responses to stress.

Within the larger parent study described in preceding sections, three different techniques were used to assess CO₂ exchange (net photosynthesis) of two families of loblolly pine (Pinus taeda L.) in response to combinations of ozone and acid rain chemistries under field or laboratory exposure conditions. The three systems included a laboratory open gas-exchange system equipped with a temperature-controlled cuvette (Siemens system), a portable-gas exchange system (Licor 6000/6050), and a ¹⁴CO₂ labeling technique (isotope labeling). The objective of this research note is to compare the results obtained with each technique quantitatively and qualitatively and to assess the usefulness of all three techniques as indicators of final seedling dry matter and/or treatment-induced changes in dry matter.

C.2. METHODS

C.2.1. Plant Material and Exposure Conditions

Loblolly pine seedlings from two families [family 8" from Gates county North Carolina (Weyerhaeuser Company 8-80) and "family 9" from Beaufort county North Carolina (Weyerhaeuser Company 8-130)] were grown

and treated as described in Sect. 1. Ozone and acid rain exposure conditions were as described in Sect. 1.

C.2.2 Techniques for Measuring Seedling CO₂ Exchange Rates

C.2.2.1 Siemens Technique

Whole-shoot CO₂ exchange rates (CERs) were measured as a function of photosynthetic photon flux densities (PPFDs). Measurements of CER-PPFD relationships were made during a 1-week period following the last ozone treatment. One seedling from each treatment was measured daily in a random order (three or four seedlings per day), until all seedlings had been measured (three or four seedlings per family-treatment combination). Whole-shoot CERs were measured in an open infrared gas-analysis system (Koch et al. 1968) at six levels of PPFD (0, 33, 60, 410, 800, and 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Four high-pressure sodium vapor lamps (General Electric, Lucalox LU400/BU) were used to illuminate the plants during measurements, and combinations of neutral density filters (Lee Filters; Andover England; #209, 210, and 211) were used to produce the range of PPFDs. Shoots were sealed in the cuvette of the gas-analysis system between 0830 and 0930 eastern daylight time and allowed to acclimate under a PPFD of 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ before starting CER measurements. Cuvette temperatures were 25 ± 2 °C for all measurements. Needle temperatures, measured with a hypodermic thermocouple inserted into the needle, were within 2 ± 1 °C of cuvette temperatures. CO₂ concentrations of air exiting the cuvette (i.e. the effective ambient CO₂ concentrations) were between 330 and 350 ppm. Air flow through the cuvette was maintained at approximately 10 L/min. Approximately 2 h was required to generate a single CER-PPFD curve for each shoot. Dry weight of needles was obtained after drying the needles for 2 d at 65° C. Calculations of CER in units of $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ were made as described by Long and Hallgren (1985) using total needle mass to normalize data between seedlings.

Estimates of light-saturated CER (P_{max} - $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$), light compensation point (LCP - $\text{mol photons m}^{-2} \text{ s}^{-1}$), and CER at zero PPFD (dark respiration, R_d - $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$) were obtained from the

following equation [modified from the original (Hanson et al. 1987)] and nonlinear regression techniques:

$$\text{CER} = P_{\max} \left[1 - \left(1 - \frac{R_d}{P_{\max}} \right) \left(1 - \frac{\text{PPFD}/\text{LCP}}{\text{LCP}} \right)^{xx} \right] \quad (1)$$

The parameter "xx" in the equation is a constant that allows a better fit to the "whole-shoot" data. The regressions were run on the pooled data for all seedlings of an individual treatment. The predicted value of CER at PPFD = 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was used in the comparison between techniques.

C.2.2.2 Licor 6000/6050

CO_2 exchange rates were measured with a portable photosynthesis system equipped with a 4000 cm^3 leaf cuvette (LI-6000; Licor Inc., Lincoln, Nebr.). The CO_2 analyzer and leaf cuvette of this apparatus formed a closed, infrared gas-analysis system. The CO_2 analyzer was calibrated at the beginning and end of each measurement period. During operation, cuvette air was pumped sequentially through a magnesium perchlorate drying column, a mass flow meter, and a nondispersive infrared gas analyzer before being returned to the leaf cuvette. Air flow through the analyzer was adjusted to maintain leaf cuvette relative humidity near ambient levels at the time of measurement. CO_2 concentrations in the cuvette dropped by up to 20 ppm over the 50- to 60-s measurement period. Prior to measurements, cuvette CO_2 concentration was allowed to equilibrate to ambient greenhouse levels (350 to 370 ppm). CO_2 exchange rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was calculated automatically by the system's computer using an arbitrary projected leaf area of 100 cm^2 . Subsequent to all measurements, the mass of needles enclosed in the cuvette was obtained after drying at 65° C for at least 2 d and CER was normalized by needle weight and finally expressed in units of $\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$.

Incident PPFD reaching leaves during measurements ranged from 500 to 700 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ and typified fluxes experienced by the individual leaves during growth in the greenhouse. The PPFD was provided by a mixture of ambient light and supplemental light from a single high-

pressure sodium vapor lamp (General Electric, Lucalox - LU400/BU). Leaf temperatures were within $\pm 1^\circ$ of ambient ($26 \pm 2^\circ$ C) during measurements.

C.2.2.3 ISOTOPE Labeling

Four-month-old loblolly pine seedlings were exposed to ^{14}C -enriched CO_2 after 13 weeks of treatment. A 90 x 60 x 72 cm wood and clear Teflon chamber was used to expose the plants to ^{14}C -enriched CO_2 (360 ppm CO_2 , 0.736 MBq/L). High-pressure sodium vapor lamps (General Electric, Lucalox LU400/BU) provided PFDs of 500 to 600 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ at the top of the seedlings. The $^{14}\text{CO}_2$ gas (0.736 MBq/L) was delivered into the 0.389 m^3 chamber at a flow rate of 0.10 L/s for 30 s yielding an approximate specific activity of the air equal to 5.68 kBq/L. A small fan within the chamber ensured circulation of the gas. After the initial 30 s $^{14}\text{CO}_2$ injection was halted, and the air was circulated an additional 90 s (total exposure time ~ 120 s). Then the chamber was vented. The plants were removed from the chamber after exposure, and a representative sample of foliage (approximately 0.02 g dry weight) was collected from each seedling. The foliage sample was immediately frozen with liquid nitrogen, stored at -20° C, and dried in a forced-draft oven at 70° C. The dry needles were oxidized using a Packard Model 306 Tri-Carb sample oxidizer. After oxidation, the released CO_2 was trapped in scintillation cocktail and counted in a Packard Tri-Carb 460C automatic liquid scintillation counting system which yielded results in disintegrations per minute (dpm).

The following equation and assumptions were used to convert dpm/needle mass (g) to units of $\mu\text{mol CO}_2 \text{g}^{-1} \text{s}^{-1}$ (Michael et al. 1985):

$$\text{CER} = \frac{\text{PM} * [\text{CO}_2] * 1.18}{\text{SA} * \text{LM} * t}$$

where

dpm = disintegrations per minute,

$[\text{CO}_2]$ is the concentration of CO_2 in the air ($\mu\text{mol/L}$),

1.18 is a discrimination factor accounting for differences in diffusion and biochemical reaction of $^{14}\text{CO}_2$ versus $^{12}\text{CO}_2$,

SA = specific activity of the exposure gas (dpm/L),

LM = needle mass in grams,

t = time in seconds.

C.3 RESULTS AND DISCUSSION

C.3.1 Comparisons Between Techniques

The three measurement techniques did not yield the same quantitative values for net photosynthesis, but qualitative similarities with respect to family or treatment effects were reflected by all three techniques (Table C. 1). The SIEMENS system rates were consistently higher than both the LICOR 6000 and the ISOTOPE techniques (40% and 66% higher, respectively). Within a technique, the measurements indicated that family 9 seedlings had higher photosynthetic rates than family 8, but the trend was not as clear for the $^{14}\text{CO}_2$ technique. Furthermore, for family 8 seedlings under charcoal filtered conditions (14 ppb ozone), both the SIEMENS system and the LICOR 6000 detected an increase in net photosynthesis along a decreasing pH gradient (see Sect. 4 for more information). Seedling photosynthetic rates as measured by all three techniques consistently showed no statistically significant response to ozone at PPFD = $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Note: net photosynthesis under saturating light was impacted by ozone -- Sect. 4). The exact reasons for differences in measured photosynthetic rates between techniques are not known, but variable control over cuvette environmental conditions and mutual shading of needles during measurements may have been involved. The SIEMENS system cuvette had a constant temperature regime (25°C), well-stirred conditions, a uniform distribution of needles under a uniform light source, and an open gas-exchange system, which may have provided the optimum conditions for CO_2 exchange. The LICOR cuvette

Table C.1. Comparative photosynthetic rates of *Pinus taeda* L. seedlings using three different techniques ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$).

Treatment ^b	SIEMENS	LICOR 6000	¹⁴ CO ₂
($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$)			
Field study, (family 8)			
<u>Ozone pH</u>			
14 3.3	0.096	0.059	0.029
14 4.5	0.088	0.054	0.025
14 5.2	0.066	0.040	0.026
167 3.3	--	0.049	0.036
167 4.5	0.078	0.052	0.022
167 5.2	--	0.056	0.037
Field study, family 9			
14 3.3	--	0.064	0.034
14 4.5	--	0.083	0.031
14 5.2	--	0.063	0.041
167 3.3	--	0.071	0.034
167 4.5	--	0.073	0.034
167 5.2	--	0.070	0.034
(95% C.I.)	(0.023)	(0.017)	(0.011)
Laboratory study, Family 8			
<u>Ozone</u>			
0	0.093	0.059	0.033
160	0.095	0.063	0.038
320	0.102	0.061	0.035
Laboratory study, family 9			
0	0.137	0.067	0.044
160	0.120	0.071	0.037
320	0.115	0.065	0.042
(95% C.I.)	(0.027)	(0.008)	(0.006)
<u>Number of plants (n=)</u>	3 or 4	7 or 8	5 or 6

^aAn open-flow laboratory system (SIEMENS), a portable system (LICOR), and a ¹⁴CO₂ labeling technique (¹⁴CO₂).

^bData are presented for seedlings exposed (12 weeks) to various combinations of acid rain and ozone in either field or laboratory conditions. All data were measured at, or extrapolated to a photosynthetic photon flux density (PPFD) of 600 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$.

^cMean ozone concentrations are expressed in ppb.

also had well-stirred conditions, but temperature and CO₂ were not steady, and shoot positioning in its cuvette may have resulted in greater amounts of mutual shading between needles. Of these factors, additional mutual shading probably resulted in the lower rates of net photosynthesis observed with the LICOR. Mutual shading of needles and potential errors in our ability to predict the actual specific radioactivity (Bq/L) of the exposure gas are probably reasonable explanations for the low photosynthetic rates observed with the ¹⁴CO₂ technique.

Although the quantitative measurements of photosynthesis did not agree between techniques, the correspondence of the qualitative trends in the data collected from each technique indicate that each is a useful tool for observations of plant physiological status.

C.3.2. Relationship of Trends in Net Photosynthesis to Growth Parameters

Photosynthetic rates of individual families measured by the three techniques were not highly correlated with final seedling growth variables or changes in the variables over time (Table C.2).

There are a variety of reasons why variables of seedling growth might not reflect measured photosynthetic rates. Typical measures of photosynthesis are made at points in time and, as such, lack the ability to account for the integrated CO₂ flux taking place during growth. Measurements of shoot photosynthesis do not account for mitochondrial respiration of roots or stem and leaf tissue respiration in the dark. Changes in plant dry weight resulting from tissue or organ abscission may also not be reflected by net photosynthesis measurements. Finally, instantaneous photosynthetic rates may reflect the ontogenetic status of the plant, but not the integrated status of the plant's dry matter accumulation. That is existing leaves of actively growing plants may exhibit enhanced photosynthetic rates (Hanson et al. 1988).

While individual family rates of net photosynthesis were not correlated with the variables of seedling growth, combined LICOR data

from families 8 and 9 showed strong correlations with photosynthetic rates (Table C.2). Negative correlations between photosynthesis and (1) final seedling mass, (2) diameter growth, and (3) volume growth indicated that the smallest seedlings had the highest rates of photosynthesis. This unexpected pattern could indicate that the smaller family 9 seedlings had a higher respiration rate at night or in tissues not measured (i.e., roots), or that the rates of photosynthesis were measured just prior to a flush of root growth and as a result may have been "enhanced" (see previous paragraph). Another plausible explanation may be that the degree of mutual shading was reduced in the smaller seedlings due to a smaller amount of foliar biomass being included in the measurement cuvette. At any rate the variability of these relationships illustrates the inherent difficulty in relating point measurements to an accumulative process such as growth. Both temporal variations in gas exchange rates due to normal phenological changes as well as treatment induced responses in the amount and activity of foliage are potentially complicating factors in drawing direct correlations between physiological activity at one point in time with growth. On the other hand, one can look at treatment induced differences in growth parameters in relationship to treatment induced changes in gas exchange rates as an evaluation of the degree to which the measured rates indicate that a shift in growth has occurred or many occur. As we show in Table 4.6, the responses of seedling height, diameter, and biomass to ozone treatment level as measured with the Siemens system were consistently (85% of the time) in the same direction (positive or negative) as responses of net photosynthesis. Thus these measures may be more useful in documenting that changes have occurred rather than in predicting the absolute level of response. More measurements in time, longer response times for growth analyses, and more dramatic growth responses will be needed to more effectively test the strength of these relationship however. Further description of the many considerations in relating shoot level measurements to tree growth can be found in Appendix D.

Table C.2. Correlation coefficients (R^2) of linear regressions between variables of growth and photosynthetic rates measured by three techniques^a.

Technique/ Family ^b	Growth Variable				
	Total Mass	Height Growth	Diameter Growth	Volume Change	Root/Shoot Ratio
SIEMENS					
F8	0.38(-) ^c	0.01	0.06	0.03	0.20
F9	--	--	--	--	--
LICOR					
F8	0.31(-)	0.05	0.04	0.14(-)	0.15
F9	0.04	0.12	0.01	0.22	0.18
Combined	0.70(-)	0.51	0.41(-)	0.66(-)	0.54
¹⁴ CO ₂					
F8	0.06	0.20	0.02	0.00	0.01
F9	0.13	0.00	0.23	0.07	0.06
Combined	0.21(-)	0.24	0.12(-)	0.27(-)	0.11

^aAn open-flow laboratory system (SIEMENS), a portable system (LICOR), and a ¹⁴CO₂ labeling technique (¹⁴CO₂).

^bMeasurements were made on seedlings from two seed sources: family 8 (F8) and family 9 (F9). Data are only presented for the field study.

^cValues followed by parentheses indicate negative correlations between net photosynthesis and the growth variable.

The net photosynthetic measurements described in this appendix are valuable techniques enabling us to isolate families having different photosynthetic capacities, and are useful in describing the responses of plants to applied treatments. However, our evidence suggests that instantaneous estimates of net photosynthesis may not provide an accurate indication of a seedling's total potential for carbon (dry matter) gain. Because the ozone and acid rain treatments had little statistically verifiable impact on growth of family 8 and 9 seedlings in this experiment (Sect. 3), the data may not have provided enough variation in plant sizes and weights to observe a clear relationship between net photosynthesis and variables of seedling growth. Measurement approaches that account for CO₂ exchange more frequently (i.e., day and night), and on a greater number of tissues (e.g., leaves, stems, and roots) would undoubtedly provide a more accurate picture of a plant's growth response to external stress (Dutton, 1988; Heichel 1971, McCree 1983, Proctor et al. 1976, Schwartzkopf 1985).

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APPENDIX D.
THE USE OF BRANCH CHAMBER MEASUREMENTS IN EVALUATING
WHOLE-PLANT RESPONSES TO AIR POLLUTANTS*

S. B. McLaughlin

*Reprinted from W. E. Winner and L. B. Phelps (eds), Responses of Trees to Air Pollution: The Role of Branch Studies. Proceedings of an EPA/USDA Workshop, Nov. 5-6, 1987, Boulder, Colorado. EPA/USDA, 1988.

The Use Of Branch Level Measurements In Evaluating Whole Plant
Responses To Air Pollutants 1,2,3

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Abstract. The use of branch exposure or branch monitoring techniques to study responses of large trees to air pollutants offers experimental advantages that must be weighed against some potentially significant limitations. Advantages include ease of pollutant exposure and monitoring physiological changes that can advance our current understanding of responses of carbon and water relations of foliage to well defined pollution stress regimes. Disadvantages are the uncertainties regarding the degree to which branch level responses represent responses to be expected when whole trees, including root systems, are exposed to chronic pollution stress. The influence of pollutants on carbon allocation including photosynthesis, respiration, and translocation represents one area of information need that can be productively addressed at the branch level to provide information of relevance to understanding how large trees respond to pollutants under field conditions.

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2. Publication No. _____, Environmental Sciences Division, ORNL.
3. Manuscript Citation: S. B. McLaughlin, 1988. The use of branch level measurement in evalutain whole plant responses to air pollutants, pp 165-185, in Response of Trees to Air Pollution - The Role of Branch Studies ed by W. E. Winner and L. B. Phelps. Proceedings of a workshop, Nov. 5-6 1987, Boulder, CO. USEPA/USDA Forest Service, 248 p.

Recent interest in branch exposure techniques has stemmed from the need to better evaluate canopy-level influences of ambient pollutants on larger forest trees, which may differ metabolically from the seedling trees that have principally been utilized to date. The logistical advantages of pollutant exposure and physiological monitoring at the branch level are obvious, however the degree to which branch level studies can adequately represent the spatial and physiological complexity of a larger tree canopy is a valid concern. If one's objective is to characterize canopy level responses to atmospheric pollutants then the relative exposure, sensitivity, and response of different portions of the canopy and, for conifers, differences in response of different needle age classes within the crown become important. The phenology of canopy growth patterns, determinant or indeterminate, may also be an important consideration in the timing of leaf susceptibility to air pollutants (Coleman 1986) and the extent to which episodic stresses are translated into reductions in seasonal carbon assimilation (Taylor and Norby 1984). In the absence of proven methodologies for controlling exposure levels and measuring physiological responses of large forest trees, branch exposure techniques offer one alternative for addressing these information needs. Their principal utility is perhaps as a tool to provide insights into the physiological principles underlying whole canopy responses to pollutant stress.

A wide variety of biochemical and cytological measurements can be made at the branch level that provide valid insights into alterations of leaf function, however the parameters that have highest potential for describing tree level effects are changes in carbon, or water relations. With respect to water relations, changes in transpiration or stomatal behavior in relationship to leaf water potential reflect alterations in control of the balance of water loss relative to water supply. While detecting adverse effects of pollutants on branch water status would provide evidence for significant whole plant effects under natural exposures, the fact that root function is unlikely to have been disturbed by exposing only a portion of the canopy to pollutants

represents an ameliorating effect on potential disruption of water supply to pollutant-stressed foliage. Studies on nutrient relations using branch chambers would be of less value since the combined effects of wet and dry deposition become increasingly important in addition to the obvious significance of root systems in nutrient supply.

The most relevant response that can be studied at the branch or canopy level is carbon economy. Measurements of photosynthesis, dark respiration, and allocation to maintenance processes are potentially useful indicators of whole plant carbon economy (McLaughlin, 1987) and can all be addressed meaningfully on individual branches. The remaining portions of this paper focus on (1) the relevance of measurements of these parameters to evaluating whole plant responses and (2) results of two studies that indicate that branch or canopy level studies can meaningfully address whole tree responses.

Carbon Allocation Processes As Indicators Of Whole Plant Response

Photosynthesis. The exchange of carbon, both photosynthetic uptake and respiratory losses, by foliage of forest trees has been an obvious focal point in many studies aimed at evaluating tree growth potential (Shaedle 1975). With respect to air pollutant impacts, changes in photosynthesis particularly have figured prominently in efforts to understand the concentration threshold for physiological responses (Botkin et al. 1972), characterize differences in sensitivity among genotypes of the same species (Boyer et al. 1986, Eckert and Houston 1980) or evaluate comparative sensitivity across a variety of different species (Reich and Amundson 1985, Reich 1987). Understandably most of the research to date has been conducted under controlled laboratory conditions. As efforts shift increasingly to the field in attempts to evaluate effects of current pollutant deposition on forested landscapes, additional considerations become important in efforts to evaluate risks to forest systems posed by current or projected pollutant levels. Among these are:

1. Are ambient levels of pollutants sufficiently high to cause impairment of gas exchange processes?
2. What is the significance of typically short term measurements of gas exchange rates to seasonal changes in carbon assimilation capacity of the tree?
3. What is the resilience of a tree's photosynthetic systems following pollutant stress episodes that diminish photosynthetic capacity?
4. How can measured changes in gas exchange rates of foliage be integrated over space within the canopy to adequately describe changes in whole-canopy production capacity?

With respect to ambient pollutant concentrations, current evidence indicates that the principal pollutant occurring at phytotoxic levels in both Western Europe and the United States is ozone (Skelly 1980, McLaughlin 1985). A review of several controlled field and laboratory studies on effects of ozone on crop and tree species (Reich and Amundson 1985), showed that ambient levels of ozone occurring in the eastern U.S. are typically high enough to reduce rates of net photosynthesis of all seven species tested. Reductions in growth were linearly related to reductions in P_n at different O_3 levels. The effects of exposures on growth ranged from a minimum of about 20% for tree seedlings to about 60% for crops.

At present we have relatively little information on the response and recovery cycle of net photosynthesis (P_n) to successive ambient ozone episodes of varying length and frequency. The length and frequency of respites between significant exposures may be an important determinant of responses of forests to chronic pollutant exposures (Taylor and Norby, 1985). Measurements of P_n at any point in time, while they may provide important information on the integration of exposure effects to that time, may not adequately describe the past or future kinetics of the photosynthetic system. Boyer et al. (1986) indicate that P_n of white pine recovered following exposure to 0.05 ppm (6 h/d) but decreased more rapidly on each successive day of exposure suggesting progressive impairment of the photosynthetic system. The manner in which response and recovery systems operate over time to

determine seasonal influences on carbon assimilation capacity is an important issue that can be approached at the branch level with branch exposure systems.

In the San Bernardino Forest in Southern California where ozone levels are among the highest for forested ecosystems in the United States, several aspects of photosynthetic production of sapling ponderosa pine trees were adversely affected by exposure to ambient concentrations (Coyne and Bingham, 1981). These included reduced P_n , reduced stomatal conductance, reduced carboxylation capacity and premature senescence of older needles, and decreased recovery of stomatal function following winter depression. In this study, high initial photosynthetic activity of current year foliage was associated with high sensitivity to gaseous pollutants. This direct relationship of P_n rate appears to be a general property of plant sensitivity to gaseous pollutants based on controlled fumigation studies (Reich and Amundson 1985, Boyer et al. 1986, Oleksyn and Bialobok 1986). The relationship between P_n rate and sensitivity was inverse in older needles, however, due to incomplete recovery of stomatal function following wintertime depression (Coyne and Bingham, 1981).

Thus, P_n has two dimensions: (1) as an indicator of carbon assimilation rate, the initial basal rate may be directly related to sensitivity to O_3 uptake and hence the potential for P_n reduction in the presence of O_3 ; and (2) as an indicator of longer term capacity for the integration of pollutant and other stresses over the life of the foliage, decreases in P_n reflect sensitivity to deterioration of the integrity of photosynthetic systems.

It should be noted that compensatory factors may partially offset the effects of stress on photosynthetic systems. The capacity of foliage of some plants to respond to a decrease in source to sink ratio by increasing P_n efficiency may be an important characteristic determining tree resilience to foliar damage (McLaughlin and Shriner 1980). Mann et al. (1982) suggest that such a mechanism may be involved in the absence of a detectable photosynthetic depression of

foliage of ozone sensitive white pine trees growing in the field in east Tennessee.

Dark respiration. To date relatively little emphasis has been placed on pollutant-induced effects on dark respiration. However, stimulation of dark respiration is an expected consequence of plant repair mechanisms (McLaughlin and Shriner 1980) and may deplete as much or more carbon from available energy pools as reduced photosynthesis. Increased dark respiration may be particularly significant when coupled with reduced rates of Pn. Barnes (1972) detected reduction of photosynthesis (-10 % average) and stimulation of dark respiration (+33 % average) in seedlings of three species of southern pines exposed to 15 pphm O₃ under laboratory conditions. McLaughlin et al. (1982) found that dark respiration was stimulated approximately 50 % while photosynthesis was reduced only 7% in mature field grown white pine trees showing high apparent sensitivity to ambient levels of ozone in east Tennessee. Obviously, increased emphasis on dark respiration is warranted in research aimed at accurately quantifying pollutant impacts on total assimilate supply.

Translocation. There is good evidence that air pollutants may exert significant effects on plant productivity by altering partitioning of dry matter between plant parts (Manning 1978, Oshima 1979, Tingey 1978, and Tingey et al. 1976). One important aspect of altered distribution of biomass is the rate of transport of assimilates from the canopy to competing sinks for those assimilates within the plant. Several recent studies under both laboratory (Jones and Mansfield 1982, McLaughlin and McConathy 1983, Noyes 1980, Teh and Swanson 1982, and Tingey 1978) and field conditions (McLaughlin et al. 1982) have indicated that carbohydrate translocation may be both sensitive to exposure to air pollutants and useful as a general indicator of pollution related stress.

The transport of assimilates away from production centers to points of utilization within the tree represents an integrative step in the carbon utilization cycle that may be examined either at the branch level, or at the whole plant level. At the branch level shifts in

translocation from foliage may occur either as a consequence of interference of pollutants with the loading of the phloem with assimilates or as a consequence of increased assimilate demand by foliage. A review of studies with several plant species indicates that internal costs of maintaining leaf functions are high (McLaughlin and Shriner 1980). These maintenance costs would be expected to be increased by exposure to pollutants at levels high enough to cause metabolic or cytologic injury.

Where the interest is in the transport and utilization of photoassimilates over time or across tissue types, the use of carbon-14 and radiochemical techniques provides a technique by which the fate of photoassimilated carbon may be budgeted (McLaughlin et al. 1982). Although nonspecific in terms of individual biochemical fractions, this technique provides a convenient way to examine the net effects of many processes associated with the carbon transport and utilization by tissues stressed by air pollutants.

Branch Level Studies And Tree Growth Relationships

Two examples of branch level studies are provided to demonstrate the utility of branches as functional units for study and to illustrate diverse approaches to the use of branches or portions of tree canopies as indicators of tree level responses to ambient pollutant levels. The first presents data derived from a previously published study on photosynthesis, dark respiration and carbon allocation of white pine exhibiting varying degrees of response to apparent ambient ozone damage (McLaughlin et al., 1982). The latter presents preliminary data from a within canopy gas exchange system being developed to monitor continuous photosynthetic responses to daily and seasonal fluctuations in ambient ozone levels.

Carbon allocation by pollutant stressed white pine branches. To determine the physiological relationship between carbon allocation patterns, differences in sensitivity to visual foliar symptoms typical of ozone damage, and reduced radial growth rates of field grown white pine McLaughlin et al (1982) examined seasonal changes in carbon

economy. The study focused on seasonal differences in allocation of C-14 labelled photosynthate of lower branches labelled four times during the growing season. From an examination of differences in photosynthetic uptake, translocation of photosynthate away from tagged branches and incorporation into associated woody and foliar tissues up to 5 years in age, the authors concluded that the more sensitive trees were reflecting the cumulative effects of chronic reductions in photosynthate available for export from branches. These effects included reduced photosynthetic tissue due to premature senescence of older needles, decreased photosynthetic activity, increased respiratory losses, and increased retention of photosynthate in foliage.

From the combined seasonal data and respiration measurements made in the laboratory on detached branches at the end of the growing season one can calculate what the combined effects of these multiple processes might be in terms of the level of photosynthate available for export from these branches (Table D.1). Such a budget demonstrates the influences of multiple processes that can be measured at the branch level of carbon economy of the branch, but it also emphasizes that the total effect is a result of multiple processes, not merely photosynthetic rate. The combined data from these multiple processes, when expressed as a net reduction in carbon export from the study branch to the larger branch and bole tissues, show a reduction of translocatable photosynthate of approximately 58 %. When this reduction in available photosynthate to the main stem is compared to the 66 % reduction in radial growth between sensitive and resistant trees, it provides some encouragement that the branch is a suitable unit of study for evaluating the basis and dimensions of larger scale growth responses.

Canopy level gas exchange. In response to a need to provide measurements of changes in the carbon assimilation rates of whole canopies of soybeans exposed to air pollutants in open top chambers, a system has been developed that uses boundary layer gas exchange rates at the leaf surface to characterize gas exchange processes on a continuous basis during the growing season. The system (McConathy and

Table D.1. A comparison of carbohydrate production and utilization by field grown white pine trees exhibiting varying degrees of apparent sensitivity to chronic air pollution stress (see McLaughlin et al. 1982 for details)

Sensitive Trees As A Percentage Of Resistant Trees

Weight of foliage per branch ¹	- 20 %
Photosynthetic rate ²	- 7 %
Respiration of foliage ³	+ 48 %
Internal allocation of carbon ⁴	+ 12 %
Calculated availability of photosynthate for translocation to bole ⁵	- 58 %
Measured radial growth ⁶	- 66 %

¹Based on the average weight and average length of retention of needles of various age classes

²Based on seasonal rates of C-14 uptake

³Based on measurements in the laboratory made on detached branches in August

⁴Based on seasonal retention of C-14 photosynthate by foliage

⁵Calculated from measurements 1-4 to consider production and in situ utilization of photosynthate.

⁶Measured difference in annual increase in basal area of tree rings.

McLaughlin, 1987) utilized a set of ten 0.3 mm tubes connected to a single manifold to characterize relative diurnal and seasonal changes in the exchange of gases in the leaf air interface. As a way of integrating processes across the plant canopy and following changes in those processes for the same canopy across the growing season in relationship to changes for other canopies in different treatments, this system can provide useful information on the cumulative effects of treatment conditions on gas exchange processes. Figure D.1 describes the principal components of the system originally tested in the field on soybean canopies. Figure D.2 represents the types of data routinely collected with this system when comparisons are made between charcoal filtered and non filtered conditions. In addition to the comparative diurnal trends in temperature, wind speed, solar radiation, and exchange of CO₂ and H₂O, the importance of wind speed is demonstrated by the influence of initiating fan circulation in the morning on net CO₂ differential .

Results of CO₂ exchange measurements on five days during the 1984 growing season are shown in Table D.2. These daily mean data describe relative photosynthetic rates of 12 soybean canopies representing three rain treatments (4 chambers each) with 6 chambers having charcoal filtered air and 6 non-filtered air. Standard errors include the variability attributed to across - treatment differences within each considered treatment mean.

The data show late season reductions in both the level and duration of photosynthesis for plants grown in ambient air and higher rainfall acidities (McConathy and McLaughlin, 1986). Reductions in relative photosynthetic rate were more pronounced as the acidity level increased and, like the effects of filtration, became most apparent only toward the end of the growing season.

This technique is dependent on boundary layer processes and hence is influenced by wind speed. In an open top chamber system wind speed is dominated by the introduced air from the blower system and hence these measurements should not be significantly influenced by ambient winds. However for larger trees in the ambient environment the

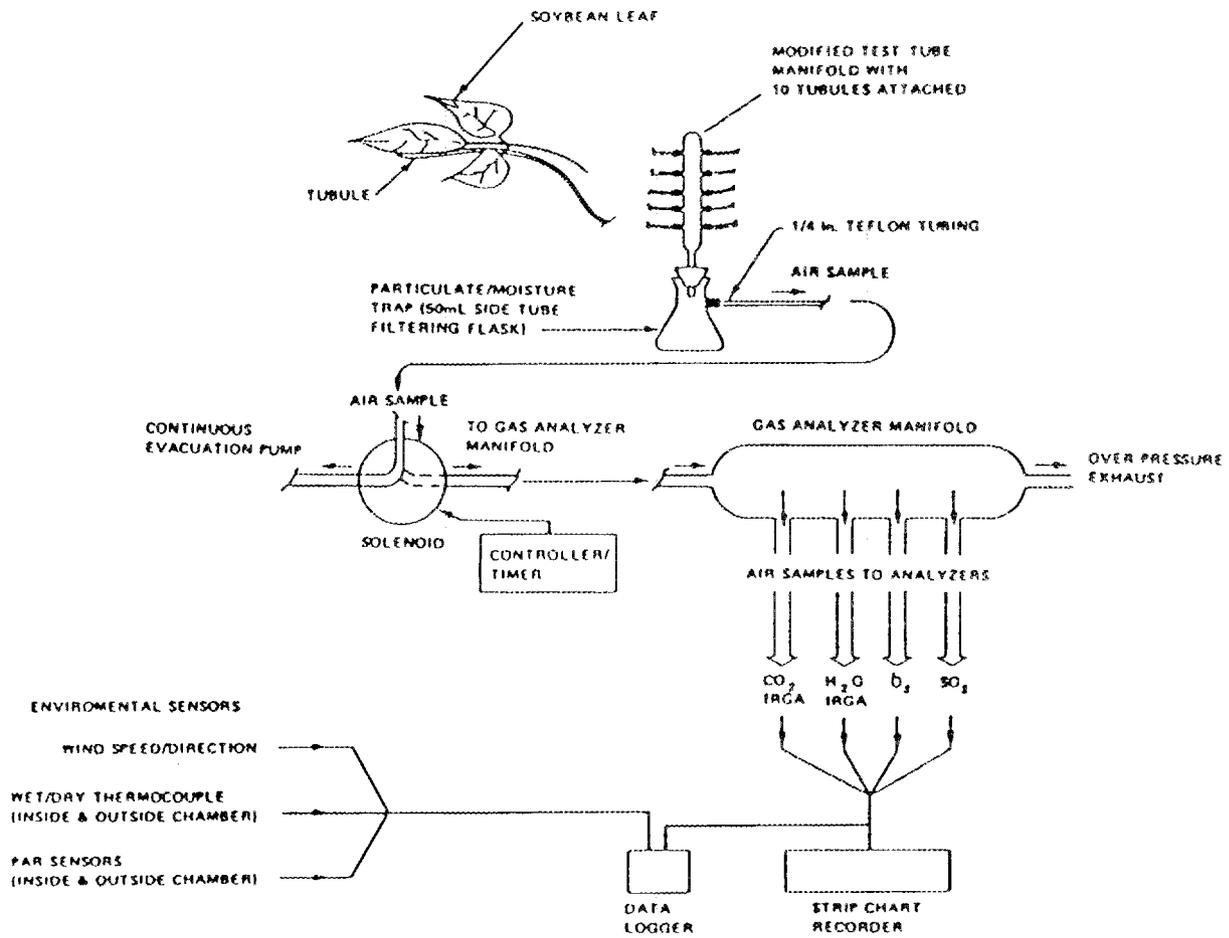


Figure D.1. Schematic diagram of principal components in the canopy gas exchange sampling system.

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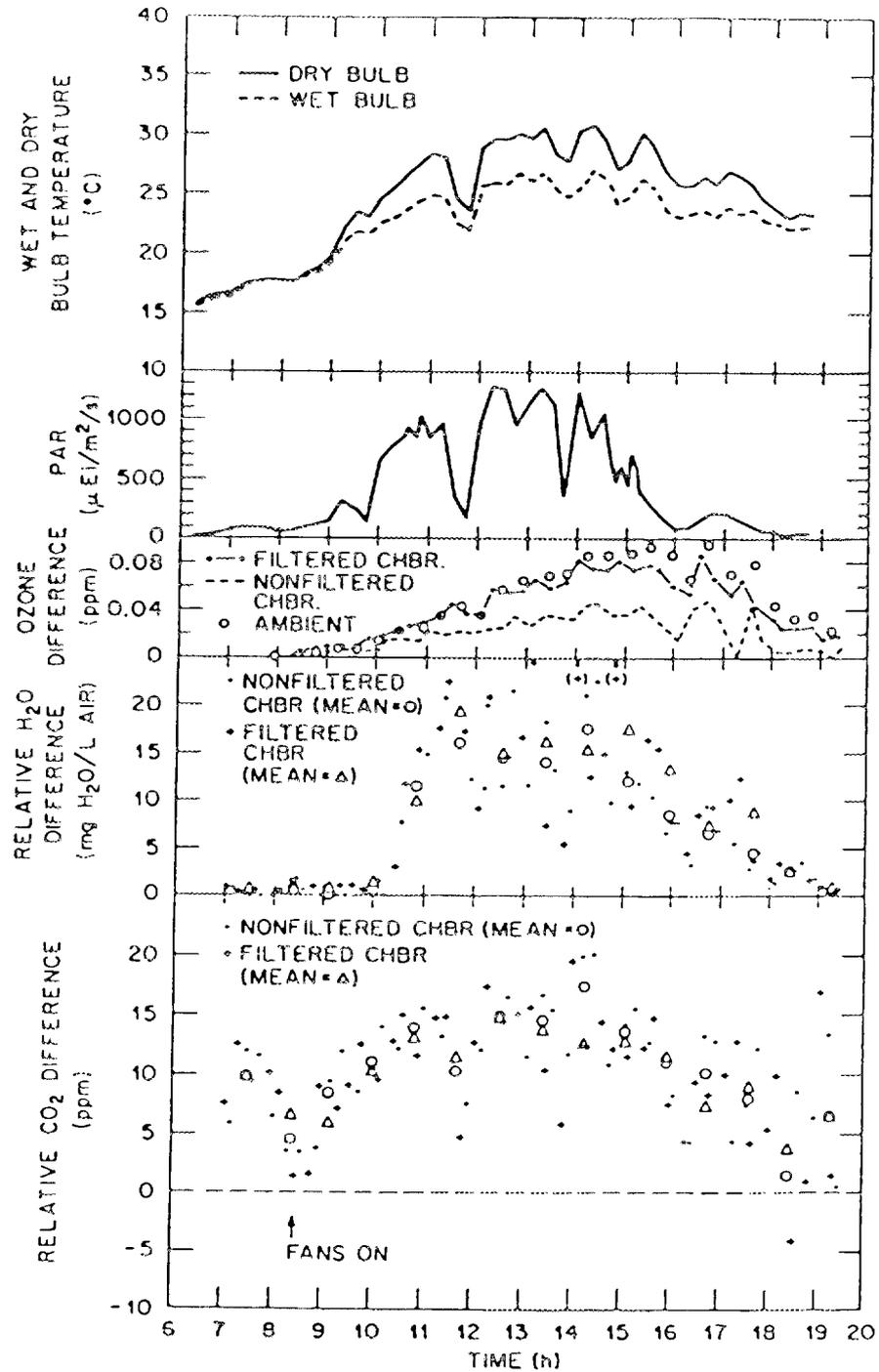


Figure D.2. Diurnal patterns in principal variables measured on a daily basis showing comparisons in relative CO₂ exchange between soybean canopies grown in field chambers in charcoal filtered and non filtered air.

Table D.2. Daily means of CO₂ exchange of soybean canopies in 12 open top chambers for six days during the 1984 growing season. Data are means of 6 chambers (2 in each of the three rain pH treatments) for filtered or nonfiltered treatments or for 4 chambers (2 each in filtered or nonfiltered air) for each of the three rain pH treatments. Standard errors of the mean are indicated.

	Sampling Date				
	7-28	7-29	8-25	8-26	9-23
	1				
	Relative Photosynthetic Rate				
Filtered	10.0 +0.9	7.7+1.2	10.0+1.6	6.6+2.7	7.1+0.6
Nonfiltered	10.4 +1.1	8.7+1.4	6.1+1.8	3.0+2.3	4.3+0.9
PH 5.2	8.5+1.2	7.1+1.0	11.6+1.8	9.8+4.0	10.2+1.3
PH 4.2	12.5+1.0	9.1+1.4	6.4+1.1	2.7+0.9	5.3+0.7
PH 3.2	9.5+1.2	8.3+1.6	6.2+2.5	1.9+3.4	1.6+0.8

¹CO₂ difference in ppm between reference air and sample air pulled from 10 within-canopy positions at a total flow of approximately 1.2 liters per minute.

naturally varying wind speeds can introduce an important source of external noise into the measured signal. For conifers the geometry of needles changes the boundary layer configuration further reducing the signal for physiologically active gases such as CO₂.

To overcome this limitation, a small lightweight glass cuvette (Figure D.3) has been designed that is attached to the tubule and mounted on individual branches to measure continuous gas exchange from needles of specific age classes and from different positions within the canopy. As with the tubule system, ten sampling positions are physically aggregated and hence mathematically averaged with a sampling manifold. The present system has the capability to collect data from up to 12 manifolds at different sampling positions. Data collection, reduction and chamber switching programs are controlled by an IBM computer.

Cuvette dimensions are 4 cm in length and 2 cm in diameter so that, in contrast to the previous example with broadleaves, one has a defined length of foliage within the cuvette at any time. At full sunlight, two fascicles of loblolly pine (typically 6 needles) within each cuvette are sufficient to provide a maximum CO₂ drawdown of 15-25 ppm within the 1.0 to 1.5 lpm air stream pulled through a typical cuvette system. Initial testing with these cuvettes showed no significant within-cuvette temperature increase, excellent stability within the freely moving canopy and no long term visual impairment of needle function or appearance. Comparisons of rates of photosynthesis measured with a Licor 6200, indicated that rates determined from this system, are about half the Licor rates. This calculation assumes that the cuvettes are closed to wind incursion at the ends and the foliage inside the cuvette is contributing equally along its entire cuvette length. In practice an operational cuvette length can be determined empirically and used to define effective needle lengths and hence photosynthetic rates in terms comparable to those measured with closed systems.

Results of an analysis of photosynthetic activity of current year, upper canopy foliage from two 5 m tall loblolly pine trees for one day



Figure D.3. A 4 cm X 2 cm glass cuvette has been designed to reduce air turbulence and provide a more constant boundary layer environment around the subtended needle fascicles contained within the photosynthetic cuvette.

during the 1987 growing season are shown in Figure D.4. Future plans for this system involve using a charcoal filtered air stream to reduce the ozone levels in one canopy and ambient air to provide similar air flow to the other to allow evaluation of the daily and seasonal kinetics of photosynthetic responses to be followed. Additionally canopy level responses would be followed in relation to sapling growth rates in charcoal filtered and nonfiltered open top chambers using families of loblolly pine that range from very sensitive to insensitive to ambient levels of ozone.

Summary

Branch level studies offer many possibilities for obtaining important information on the effects of pollutant stress on physiological processes of forest trees. The primary consideration in designing branch chambers and conducting studies at the branch level should be the desired resolution and potential uses of the data to be obtained. While attempting to obtain environmental conditions within chambers that are close to those in the unconfined canopy is a worthy objective, it is not reasonable to consider branch studies as an adequate source of information for an understanding of whole tree function, particularly the water and nutrient cycles that are so significantly influenced by the root system. A diversity of approaches should be encouraged to provide levels of sophistication of design that match the sequence of information needs in the exploratory - confirmatory research cycle.

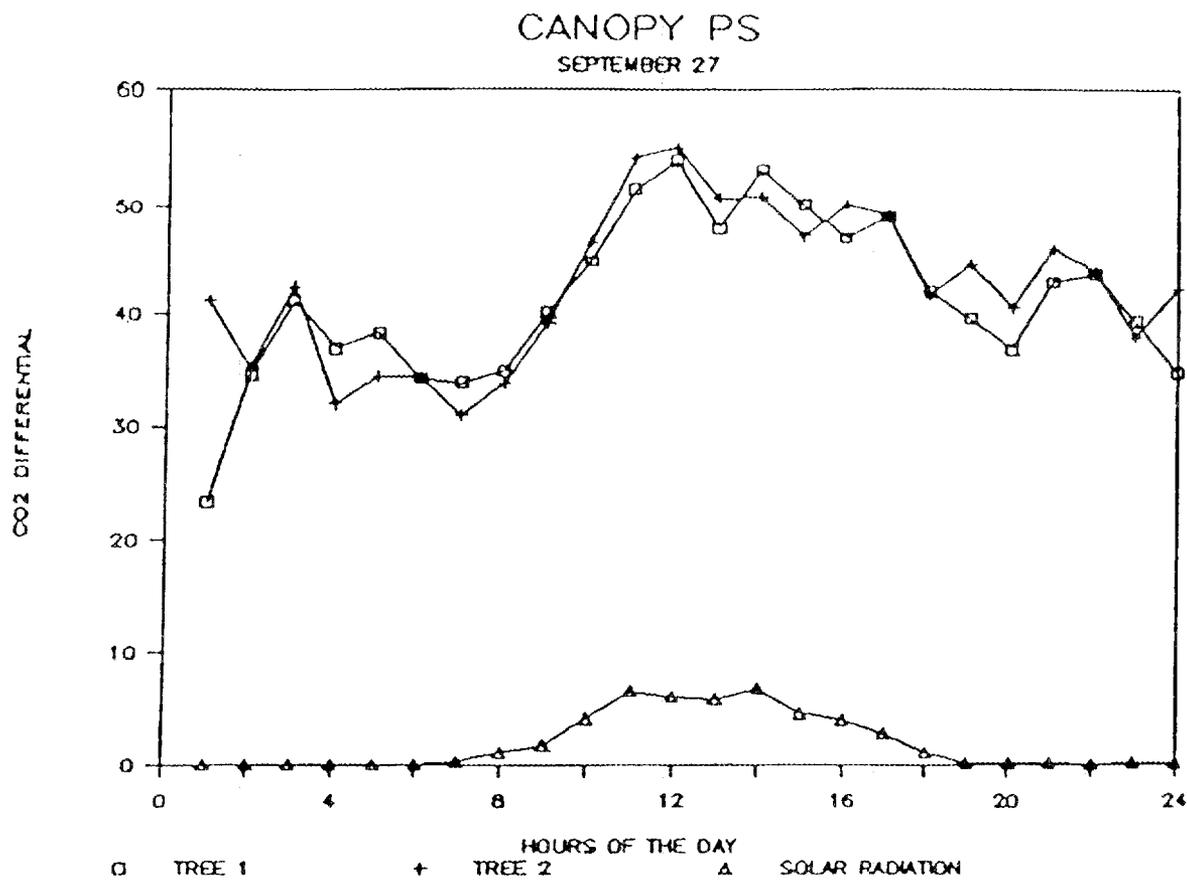


Figure D.4. Raw data showing relative CO₂ exchange within the canopies of two 5 m tall loblolly pine trees during September of 1987. The X-axis represents recorder scale units where a change of one unit represents a CO₂ differential of 1 ppm. The scale was adjusted initially so that 35 units represents approximately 0 ppm differential.

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APPENDIX E.
PROJECT QUALITY ASSURANCE

E.1. INTRODUCTION

This section addresses the considerations that were implemented to ensure and document the quality of the data being obtained for this project. Many of these considerations are covered in the text. In those cases, reference to the location of that material will be made with only summary comments regarding principal findings included here.

As a part of the national Forest Response Program this project was from it's initiation under the programmatic guidance of the Quality Assurance (QA) Program. This interaction took several forms:

1. participation of staff in workshops designed to suggest standard measurement protocols,
2. a site visit by a QA team to observe project protocol and make suggestions for improvements where warranted,
3. staff interactions with other investigators working out procedural details of current methods, and,
4. Observation of procedural protocols previously established for seedling cultural practices. These protocols were designated initially as formats for ensuring standard procedures for growing seedlings across the three sites involved in the initial laboratory studies.

There were three primary areas in which QA required special attention and documentation: plant cultural practices, delivery and monitoring pollutant dose, and measurements of plant growth and physiology.

E.2. PLANT CULTURAL PRACTICES

Cultural practices used in these experiments were modeled after the "Seed, Seedling, and Design Protocol For Determining Response of Loblolly Pine to Air Pollutants" supplied to participating laboratories in the Southern Commercial Cooperative in February of 1986. This document described the seed selection process as well as recommended cultural conditions. This protocol was followed as outlined and documented in Sects. 2 and 3 with the following exceptions.

E.2.1 Seedling Fertilization

Because of the large number of seedlings initially produced (over 12,000), hand application of liquid fertilizer to each individual container in the nursery was deemed impractical, so a commercial liquid spraying device that mixed the appropriate stock solution with water was used to apply the fertilizer to the seedlings. Fertilization was in each case followed immediately by a light water spray to wash the surface deposited nutrients from the foliage.

Similarly in the field, a slow-release, commercially available fertilizer was used to deliver a continuous supply of fertilizer complete with micronutrients to avoid hand-watering the approximately 10,000 seedlings in their holding pallets. To test the effects of slow-release fertilizer pellets versus the standard liquid fertilizer regime used in the greenhouse/continuously stirred tank reactor (CSTR) studies, a greenhouse comparison test was run for 12 weeks in parallel with those studies. The results, shown in Table E-1, indicate that the application of liquid fertilizer every 2 weeks induced comparable rates of diameter growth in the three families tested, but stimulated height growth by approximately 37% on the average. Since seedlings actually grew faster in the field studies compared to the greenhouse/CSTR conditions, it can be concluded that the observed faster growth under those conditions was in fact a response to more optimum radiation and temperature conditions since watering rates were similar between the two regimes.

E.2.2 Measurement Protocol

The slow-release fertilizer and the potting medium used produced a soil surface that was irregular and subject to significant change as a reference point for height measurements over time because of wetting, droplet impaction, and redistribution of the mix. For this reason, seedling height was measured from the level of the top of the seedling container to the base of the terminal bud of the dominant shoot. Because seedling potting depth was variable, the use of pot-rim level as a reference created a bias towards calculating a disproportionately high relative growth rate (height growth versus initial height) for any

Table E-1. Mean height and diameter growth for seedlings of three loblolly pine families grown for 12 weeks in the greenhouse under two fertilizer regimes^a

Family	Liquid Fertilizer ^b		Slow Release Fertilizer ^c	
	Height	Diameter	Height	Diameter
1	1.73	1.76	1.61	1.84
6	3.04	2.15	2.32	2.07
7	2.05	1.77	1.77	1.74

^aGrowth ratio as final/initial after 12 weeks. Mean percent height difference, liquid vs pellet = + 37%. Mean diameter difference, liquid vs pellet < - 1%.

^bLiquid fertilizer: 88-13-21 ppm in solution as NH_4NO_3 , Na_2HPO_4 , and KCl applied once every 2 weeks as recommended.

^cPelletized fertilizer: 17-6-10 with micronutrients from Agriform applied at a rate of 11 g to the surface of each pot at the initiation of field studies.

seedlings for which the potting surface was deeper than the typical 2-cm depth and for this reason height growth data were analyzed as total height growth. Initial height was used as a covariate in statistical analyses, however, as discussed in Sect. 3. It should be noted that potting depth was recorded for all of the families that were harvested for dry-mass determinations in the final harvest so that true values for relative height growth could be calculated for those approximately 1000 seedlings. We have not considered this to be necessary for purposes of the present analyses.

Diameter growth measurements were made approximately 2 mm below the base of the cotyledonary node after examining the extent of stem taper in this area and determining that variable potting depth of some seedlings and the logistics of accessing and measuring all seedlings in a timely and consistent manner would reduce the quality of the data obtained by the suggested root collar measurements of stem diameter. Stems were found to have little taper below the cotyledonary node. This minimized errors due to estimation of the 2-mm reference point. A summary of measurement errors for weight and diameter is provided in Sect. 2. Figures E.1 and E.2 show the nature of the variability between measurements taken by seven individuals after six weeks of exposure.

E.3 POLLUTANT DOSE

The methods of dispensing and monitoring pollutant dose, both acid rain and ozone, are discussed in Sect. 2. There were several considerations in evaluating the performance of the pollutant distribution and monitoring system utilized in the field studies. These included the levels of pollutants monitored within the chambers, the efficiency of the sampling lines in delivering an accurate picture of actual concentrations of ozone in the chambers, calibration of the monitoring instruments, and variability of dose delivered within and between chambers.

HEIGHT MEASUREMENT COMPARISONS

SEPT. 19, 1986

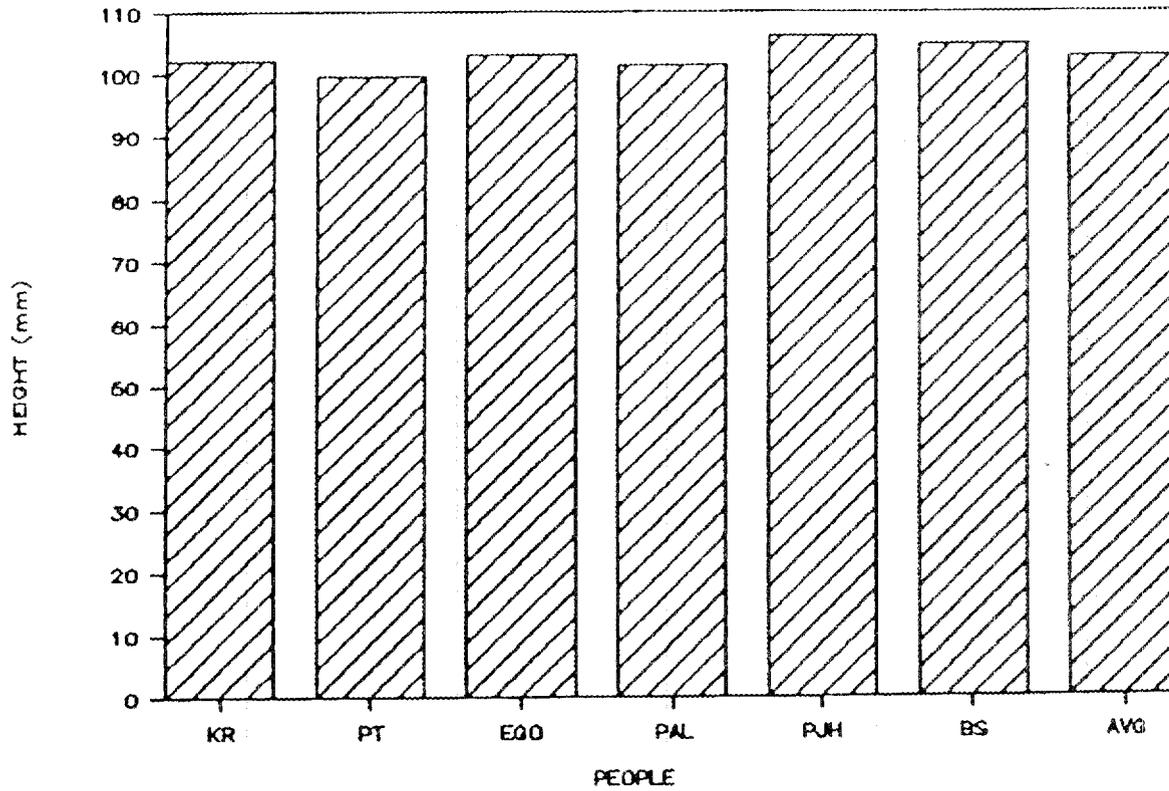


Fig. E.1. Mean Height measurements obtained by seven individuals measuring a standard set of eight loblolly pine seedlings six weeks after the initiation of the twelve week exposure sequence.

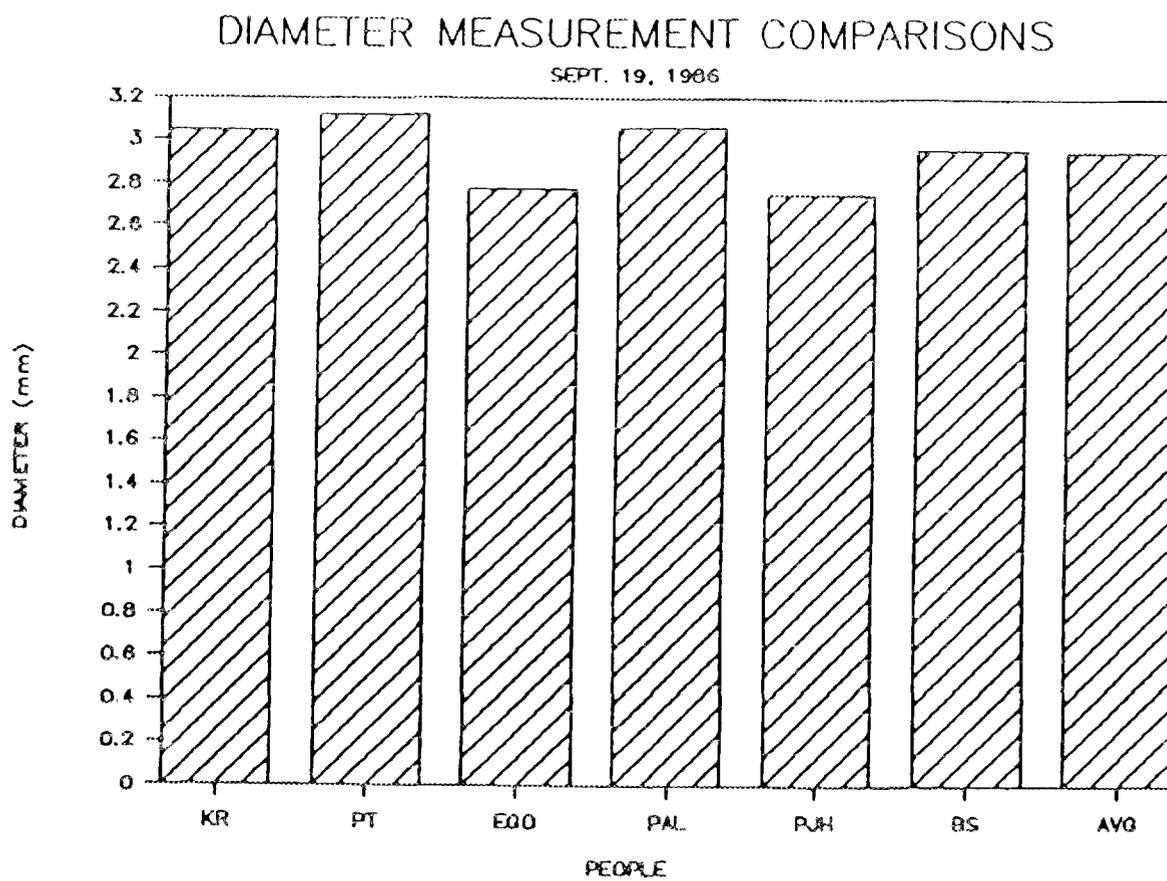


Fig. E.2. Mean diameter measurements obtained by seven individuals measuring a standard set of eight loblolly pine seedlings six weeks after the initiation of the twelve week exposure sequence.

E.3.1 Dispensing and monitoring ozone: Field Studies

Ozone was distributed to individual chambers by a computer-controlled pneumatic valve system that was started up manually to reduce controller oscillations during the transition from ambient background concentrations to the 40-, 80-, or 160-ppb add-on levels specified. The kinetics of a typical daily concentration regime for ambient concentration and each of the ozone additions are shown in Figure E-3. While additions at the 40- and 80-ppb levels provide rather steady state additions to the specified ambient levels, it is apparent in Fig. E-3(c) that at the highest addition level there was about a 10% overshoot of the desired ozone concentration. Desired mean concentration levels were rather well replicated however, as noted in Table 2.3 (Sect. 2) and were typically within ± 15 ppb of the desired mean addition level. Both variability among chambers at the same treatment level (Table 2.3) and variability at test positions within an individual test chamber were very low (Section 2, Fig. 2.3).

The accuracy of ozone concentrations monitored in these experiments was checked both by periodic calibration of the three monitors used in this shared time system (Sect. 2, Table 2.1) and by checking the efficiency of sample lines with a standard ozone addition made at the chamber end. Instrument calibrations indicated very close agreement between introduced and monitored levels of ozone with actual concentrations typically being reproduced by values that averaged ± 1 ppb at the low end and ± 5 ppb at the high end (a 2 to 3% error). Variability across 12 sampling positions within a representative chamber (Sect. 1, Fig. 1.3) was quite low, with a coefficient of variation of 5%.

E.3.2 Dispensing and Monitoring Ozone: Laboratory Studies

Ozone provided in the laboratory studies was generated by irradiating an oxygen supply stream. Control of ozone levels was manual and data were recorded on a strip chart recorder and reduced manually as noted in Sect. 2. Results of instrument calibration on eight dates are included in Sect. 2. Accumulative doses for the 160-

3 SEPT. 1986

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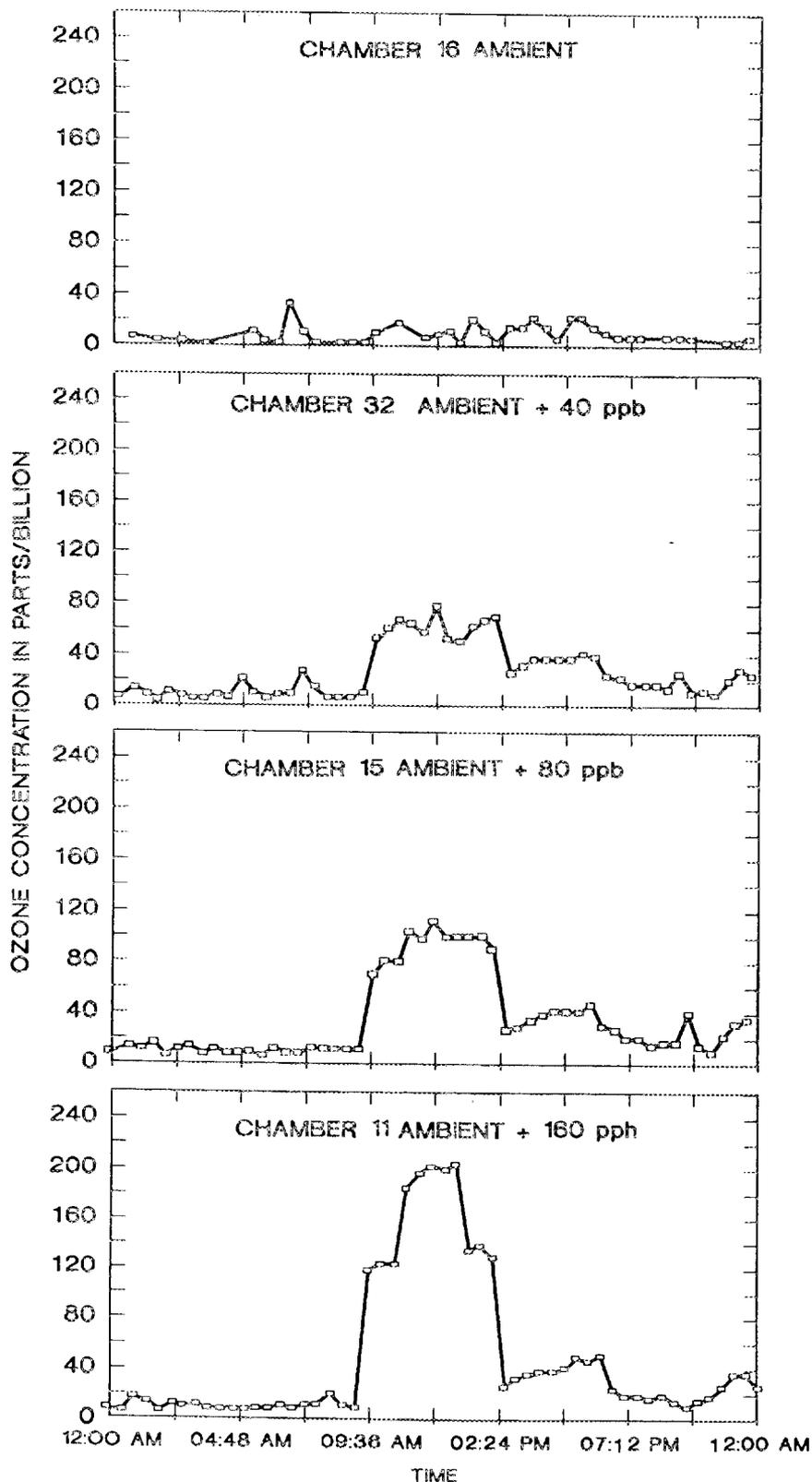


Fig. E.3. Kinetics of typical daily exposure concentrations for ambient (A), and ambient plus 40 ppb, 80 ppb, and 160 ppb controlled exposures to ozone (B, C, and D, respectively).

and 320-ppb treatments were 40 ppm•h and 80 ppm•h, respectively. These levels were only approximately 10% below the target concentrations of 46 ppm•h and 92 ppm•h set forth as initial objectives.

One QA lapse occurred early in the lab experiments when an ethylene supply tank ran low on pressure and caused the ozone monitor to read abnormally low concentrations in the chambers. By manually increasing the ozone flow into the chambers the technician was able to maintain the desired recorder levels; however, based on the recorded flow settings we estimate that chamber ozone levels may have exceeded target concentrations by as much as 100%. Little visible injury and no apparent growth effects were produced by this exposure short term episode however.

Calibration of ozone monitors used in both field and laboratory studies was accomplished using a Permacal 8500 unit that was cross-calibrated against the calibration system maintained by the TVA Air Quality Laboratory in Knoxville, Tennessee.

E.3.3 Acid Rain Doses

Two aspects of the acid deposition were of primary interest in these experiments: the range in precipitation chemistry and the volume of water delivered to the chambers across the system. Statistics on pH of the rain simulants were shown in Table 2.5 and Fig. 2.9 (Sect. 2). These data indicate that while there was overlap of treatment ranges three distinct pH treatments were obtained. Although actual mean values were more acidic than desired at the extremes, they were quite comparable at the intermediate level. The concentrations achieved represent a close approximation to the range of mean ambient pH of rainfall across the southeastern region at the two higher pH levels and the level of episodic "worst case" events at the lower end. Mean ionic concentrations from two rain events are compared with target concentrations in Table E.2. Mean ionic concentrations for two rain events for the CSTR are also shown.

Rainfall volume was of interest in these studies not only because of its role in delivering the treatment dose, but also because

Table E.2. Ionic concentrations of rain simulants (mg/L)

	Mg ⁺⁺	K ⁺	Na ⁺	NO ₃ ⁻	SO ₄ ⁻²	Cl ⁻	NH ₃	Ca ⁺⁺	SO ₄ :NO ₃
Field study, actual concentrations									
5.2	0.097	<.1 ^a	0.93	1.47	1.77	0.86	0.50	0.27	1.20
4.5	0.055	<.1	0.66	1.35	2.32	0.20	0.32	0.20	1.72
3.3	0.058	<.1	0.51	3.02	8.05	0.17	0.34	0.23	2.66
Field study, target concentrations ^b									
5.0	0.035	0.03	0.17	0.61	1.48	0.11	0.26	0.16	2.43
4.3				1.30	3.20				2.46
3.5				6.00	15.00				2.50
CSTR study, actual concentrations									
	0.088	<.1 ^a	<.50 ^a	2.98	4.55	0.31	0.59	0.51	1.53
CSTR study, Target concentrations ^b									
	0.035	0.03	0.17	1.30	3.20	0.11	0.26	0.16	2.46

^adetection unit.^bfrom Irving (1985).

seedlings were grown above the soil level and hence did not receive other significant sources of moisture. The mean volume of rainfall delivered to chambers in each of the three blocks for eight representative events during the twelve week period of exposure is shown in Table E.3. From these data it is apparent that there was no consistent pattern of bias in the amount of rainfall delivered across the three blocks and no basis for concern that block responses were significantly influenced by varying levels of deposition of prescribed doses.

For the CSTR study, plants were randomly located on the two rain tables over time so that any variations in volume received was minimized.

E.4 MEASUREMENT OF PLANT GROWTH AND PHYSIOLOGY

E.4.1 Plant Growth

The large number of seedlings utilized in the field studies necessitated the use of 12 different people during the various measurement operations throughout the growing season. Variability of measurement tendencies between observers was a source of concern and for this reason both a record of who did the measuring on individual pallets and the performance of observers on specified seedlings were documented. Measurers were given instructions on standard measurement protocol initially and were instructed to initial the specific pallets within each chamber that they measured. In most cases there were two or more trained measurers per chamber and measuring and data recording duties were rotated at least once during measurement of the six pallets per chamber. Diameter was measured with a digital, battery-powered caliper using the long axis of the pallet to orient the measurement along a constant plane. Calipers were electronically zeroed several times per hour; however, instrument drift was negligible.

Variability of measurements was determined during each measurement date by having each observer measure a standard set of plants. On one date, the same group of plants was remeasured three times during the day to determine precision as well as accuracy of the measurement team

Table E-3. Rainfall volumes delivered to each of three blocks for eight dates (cm depth)^a

Treatment date	8/8	8/15	8/19	8/22	8/20	9/12	9/23	10/10
Block I	2.51	1.24	1.14	1.12	1.12	1.09	1.57	1.04
Block II	3.37	1.37	1.02	1.12	1.30	0.89	1.70	1.02
Block III	2.87	1.37	1.14	1.16	1.24	0.91	2.00	0.91

^aSummed rainfall depths for blocks I, II, III were 10.83 cm, 11.79 cm, and 11.60 cm respectively across the eight dates.

members. Figures E-1 and E-2 were included to demonstrate typical variability in height and diameter measurements by individuals on the same group of plants on September 19, 1986, at the midpoint of the study. Coefficients of variation for all measurement periods averaged 3.8% for height and 5.6% for diameter. Variability in repetitive measurements by the same individuals is shown in Table E-3. Average coefficients of variation were 5.4% for height and 2.1% for diameter.

E.4.2 Physiological Measurements

Photosynthetic measurements were obtained by three different approaches during these studies: Siemens Sirigor, Licor, and ^{14}C labelling techniques. The comparability of measurements between techniques for measurements on the same subsets of plants has been covered in Appendix C.

Starch analyses were developed in collaboration with other investigators in the cooperative and were accompanied by measurements of starch recovery from commercially available standard samples. From these measurements it was determined that recovery of starch was approximately 95%.

Nutrient analyses were performed at the University of Georgia Soil Testing Laboratory (see Sect. 2) and were accompanied by a set of standard samples supplied by the Cooperative QA staff. Results of these analyses indicated that most of the analyses from the Georgia laboratory were within the acceptable limits for most of the nutrients (see attached letter). The only exceptions were the Mn, Fe, and Al analyses, which were lower than the prescribed limits of variability by the Cooperative. The discrepancy for these nutrients was likely due in large part to the sample preparation as noted by the results of subsequent analyses summarized in the follow-up letter by Dr. Robert Isaac of the Georgia Laboratory.

OAK RIDGE NATIONAL LABORATORY

OPERATED BY MARTIN MARIETTA ENERGY SYSTEMS, INC.

POST OFFICE BOX X
OAK RIDGE, TENNESSEE 37831

November 11, 1987

Dr. Robert Isaac
Soil Testing and Plant Analysis Laboratory
University of Athens
2400 College Station Road
Athens, Georgia 30605-3698

Dear Dr. Isaac:

We recently sent some samples of loblolly pine foliage to your laboratory for nutrient analyses. Included were four samples containing NBS pine and citrus foliage which were to serve as cross-lab standards. We were satisfied with the results of the analyses. However, the Forest Response Program's Quality Assurance Officer has indicated that the Mn, Fe, and Al values obtained by your personnel for the pine sample were lower than the NBS means by more than 3, 3 and 2 respective NBS standard deviations. These results are shown on the attached sheet.

We do not require any further action on your part, but wished to communicate this discrepancy to you. At present, we are simply flagging these data as not meeting our Data Quality Objectives. However, I welcome any comments or suggestions regarding these differences.

Sincerely,



Mary Beth Adams
Environmental Sciences Division
Building 1506, MS-034

MBA:llc

cc: Sandy McLaughlin

Attachment

Foliar Inorganic Analyses
Comparison with NBS Standards

	<u>Reported Values</u>	<u>NBS Values</u>
Mn	534.6	675±15
Fe	107.4	200±10
Al	472.7	545±30

REFERENCES

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