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Forest Response Program

Major Program Output #3

United States
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SEEDLING RESPONSE TO SULFUR, NITROGEN, AND ASSOCIATED POLLUTANTS

by

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ABSTRACT

In 1986, the National Acid Precipitation Assessment Program (NAPAP) established the Forest Response Program (FRP) to assess the effects of acidic deposition and associated pollutants on forests. Seedling exposure studies were initiated to determine acute effects of simulated acid deposition, ozone, and sulfur dioxide, and to identify hypothesized mechanisms by which these effects might alter tree condition and hence result in forest decline. From data available as of December, 1988, altered post-exposure growth and imbalance in above- and below-ground responses to sulfur dioxide indicated changes in carbon allocation patterns. Simulated acid precipitation reduced frost hardiness of red spruce seedlings at pH 3.0 and led to higher rates of foliar tissue mortality during extreme cold. Loblolly pine showed root and stem growth decreases at ozone levels 80 ppb and higher. Of western conifers, only ponderosa pine showed consistent growth decreases due to ozone. Many treatment effects did not show up until the following growing season. The influences of different exposure methods, exposure durations, and experimental material are discussed, as are statistical considerations and quality control of treatments. Further results will be presented in a future FRP report and in NAPAP State of Science/Technology documents.

1 NOTICE

The information in this document has been funded wholly or in part by the United States Environmental Protection Agency. It has been subjected to the Agency's peer and administrative review, and it has been approved for publication as an EPA document.

This document is a preliminary interpretation of the EPA-USFS Forest Response Program's existing and accumulating data on the response of seedlings to controlled exposures of simulated acidic deposition, sulfur dioxide, and ozone. Many of the exposure studies had not been concluded at the time these data were requested. Consequently, some estimates and interpretations are subject to change as experiments conclude and more data become available.

2 EXECUTIVE SUMMARY

2.1 Purpose of the Document

This document, Major Program Output #3, was prepared at the request of the Environmental Protection Agency (EPA) and the USDA Forest Service as an internal report. Its purpose is to summarize seedling exposure research conducted in the Forest Response Program (FRP). The data used in this document were those available from FRP research efforts as of December, 1988, and are intended to provide input to policy decisions. Forthcoming results from continuing studies will be integrated with other research efforts in future reports of the FRP and state-of-science documents for the National Acid Precipitation Assessment Program (NAPAP).

2.2 Rationale for Seedling Research

In 1986, the FRP was directed by NAPAP to assess the effects of acidic deposition and associated pollutants on forests. Hypothesized mechanisms were identified by which a pollutant might affect tree condition, and hence result in forest decline. While recognizing that seedlings have both advantages and limitations, seedling exposure studies were initiated as the quickest way to determine acute effects of pollutants and mechanisms by which these effects operate.

2.3 Assessing Data Quality

The assessment of data quality has not been completed for all projects. When that task is accomplished, the primary use of the results will be to evaluate the relevance of the data quality objectives to see if those specifications can be realistically attained. The achievement of an average target value is relative to the accompanying fluctuations. The purpose of that information is not to reject data from a given project, but is intended to help the principal investigator monitor the system and make necessary corrections to keep the system under control. However, information such as magnitude and direction of the charted treatment fluctuations will be considered in a subjective way, when evaluating the biological data from a project. Final data quality analyses will be contained in a separate data quality document currently in preparation, and updated in MPO #4.

2.4 Summary of Principal Findings

The main body of this report describes results from individual FRP seedling exposure studies. The major findings of these studies regarding short-term effects (i.e., one exposure season) of sulfur dioxide, simulated acidic deposition, and ozone on seedlings, as well as possible long-term implications, are as follows:

Sulfur Dioxide: Two projects examined visible effects of sulfur dioxide, and three examined growth effects. No visible injury was observed in response to concentrations as high as 66 ppb. Increased above-ground growth due to sulfur dioxide occurred for Engelmann spruce, white fir, western redcedar, and Douglas-fir, relative to a control treatment. Douglas-fir, ponderosa pine, and lodgepole pine showed reductions in root biomass and root/shoot ratios. Compared to a control, bud elongation was increased by higher sulfur dioxide levels (up to 66 ppb base level) for ponderosa pine, Douglas-fir, western hemlock, and western redcedar. The altered post-exposure growth and imbalance in above- and below-ground responses indicate that changes in carbon allocation patterns occurred. Under chronic exposure, survival or eventual tree productivity could potentially be negatively impacted. Sulfur dioxide was not tested with red spruce or southern pines, and although planned for eastern hardwoods, experimentation problems precluded a clear study of sulfur effects.

Simulated Acid Deposition: The clearest effect of simulated acid deposition was a reduction of frost hardiness at pH 3.0 and higher rates of foliar tissue mortality during extreme cold for red spruce seedlings. Most species that were tested at pH levels below 3.0 showed some visible injury. Across all species, there were no conclusive short-term effects of simulated acid deposition by itself on seedling growth. However, growth of black cherry was decreased by pH 3.0 versus 4.2. Furthermore, increased above-ground growth coupled with no apparent effects on below-ground biomass in western conifers at pH 2.1 compared with pH 5.6 indicates that changes in carbon allocation patterns occurred.

Ozone: The direct effect of ozone varied from physiological changes in the foliage of red spruce to suppressed growth of loblolly pine, ponderosa pine, and some hardwood species. Eastern hardwood species showed visible injury with ozone of 70 ppb or higher. Yellow-poplar, yellow birch, sweetgum, red maple, white ash, and black cherry appeared to be the most sensitive species tested. Among western conifers, white fir, subalpine fir, ponderosa pine, and western hemlock also showed visible injury in response to ozone at 70 ppb. Despite considerable differences in experimental designs and procedures, there was a pattern of root and stem growth decreases at ozone near 80 ppb or higher for loblolly pine. At intermediate levels (40 to 80 ppb) results were more variable; it was not uncommon for growth rate to be greater at intermediate levels than in charcoal-filtered air. In the West, only ponderosa pine showed consistent decreases across several growth variables due to ozone; most other species showed increased growth rates at levels less than 100 ppb. At this point in time, there are no data with which to address the anomaly of increased seedling growth at these levels (approximately 1.5 times ambient). Among the hardwoods tested, levels of ozone 70 ppb and higher decreased growth for black cherry, white oak, red maple, and yellow birch; yellow poplar, white ash, and red oak displayed no response at the same levels. Cumulative decreases in net photosynthetic rate in response to ozone were found for loblolly pine. Red spruce did not exhibit consistent decreases in net photosynthesis. However, damage to foliar mesophyll cells, decreased photosynthetic pigments, and seasonal changes in photosynthesis in red spruce in response to ozone at 40 ppb and higher suggest an increased potential for winter injury to red spruce.

Pollutant Interactions: There is preliminary evidence of some anion x pH interactions, where sulfur-based acids caused greater foliar injury than nitrogen-based acids at the same pH. Although there are some indications of ozone x acid deposition interactions, the interpretations of these interactions are inconclusive at this time. Any further information that becomes available will be incorporated in MPO #4.

Species Sensitivity: Relative species sensitivity was tested only in western conifers and in eastern hardwoods. Visible injury and growth changes indicate that ponderosa pine was the most sensitive, and western redcedar the least sensitive, of species exposed to expected pollutant scenarios in the west. Results of visible injury to eastern hardwoods from one year of exposure indicate that black cherry was the most sensitive of species exposed to either acid precipitation or ozone.

Long-term Implications: Although there are currently no data on the long-term effects of multi-year exposures on seedlings, differential responses in above- versus below-ground biomass to short-term pollutant exposures indicate long-term problems for seedlings. Under chronic exposure, survival or eventual tree productivity could be affected.

2.5 Recommendations for Future Research

One product of these seedling exposure studies has been the identification of issues that qualify the above results and that should be addressed in new and continuing studies involving tree material. Although other issues are likely to arise before the FRP studies are concluded, major issues identified to date are:

Species/Plant Variability: A major source of variation in plant response lies in the plant material itself. Virtually all the analyses demonstrated the large variation in growth that can be attributed to large differences in initial size (height and/or diameter). Careful selection and randomization of plants prior to treatment would help to alleviate this nonuniformity. The large variability and range of responses to pollutant exposures among loblolly pine families show the importance of reducing that variability if results of different experiments are to be compared.

Choice of Response Variables: The value of studying mechanisms for change in tree conditions is already apparent and is summarized in the Conclusions and Recommendations of the report. Currently, there is insufficient information on changes in below-ground biomass. Therefore, future studies should include measurements on both above-ground and below-ground (i.e., at least root biomass) changes from the same plant.

Microclimate Characterization: Differences in seedling responses among sites, or among years at a given site, cannot be correctly interpreted without knowing corresponding spatial and temporal differences in factors such as light, temperature, and humidity.

Duration of Experiments: Most of the initial studies were designed to evaluate effects over short periods of exposure (e.g., 12 to 16 weeks). In one study designed to test post-exposure effects, growth differences due to treatment were reflected in bud elongation (i.e., reduced shoot growth) in the growing season following the season of treatment. While useful information can be obtained from short-term exposures, most effects can be better evaluated with multiple-year exposures. In addition, the variability of results among studies is typically reduced with longer exposures. Results from ongoing multi-year exposure studies in southern pines and red spruce will be forthcoming in the next two years.

Statistical Power: Power should be considered when planning research and computed at the conclusion of each project. In the absence of formal significance due to low power, evidence of treatment effects may still be present in the form of trends or patterns that should not be overlooked.

Repeatability of Experiments: Any experiment worth doing once should be considered for replication, regardless of the treatment duration (i.e., single-year versus multi-year exposures) or the statistical significance of the initial outcome. The ability to replicate an experiment in time or place is critical to confirmation of results.

3 INTRODUCTION

3.1 Forest Response Program

The Forest Response Program (FRP) is a research initiative under Task Group V of the National Acid Precipitation Assessment Program (NAPAP). The FRP is responsible for determining the actual and potential effects of acid deposition and its associated pollutants on trees, forests, and forest ecosystems of the United States (Schroeder and Kiester, 1989). The FRP is jointly administered by the US Environmental Protection Agency (EPA) and the USDA Forest Service. Operationally, it consists of a National Management Staff, six Research Cooperatives, a Quality Assurance/Quality Control (QA/QC) Staff, and the Synthesis and Integration (S&I) Project. The six research cooperatives are the Spruce-fir Research Cooperative, Southern Commercial Forest Research Cooperative, Eastern Hardwoods Research Cooperative, Western Conifers Research Cooperative, National Vegetation Survey, and Atmospheric Exposure Cooperative.

The objective of the FRP is to address three broad questions related to environmental policy:

Policy Question #1: Is there significant forest damage in North America caused by acidic deposition, alone or in combination with other pollutants?

Policy Question #2: By what mechanisms does acidic deposition, alone or in combination with other pollutants, contribute to forest damage in North America?

Policy Question #3: What is the dose-response relationship between acidic deposition, alone or in combination with other pollutants, and forest damage in North America?

3.2 Synthesis and Integration

The FRP addresses these policy questions and regulatory needs through many diverse projects. Therefore, the FRP must ensure that the technical information developed in the six Research Cooperatives is coordinated across the FRP to understand the effects of pollutants on major forest species. The Synthesis and Integration Project (S&I) provides the technical focus for the FRP.

S&I produces the program-wide output documents that provide answers to scientific questions relevant to the policy questions listed above. S&I synthesizes and integrates results across individual experiments wherever possible when answering these questions. In order to conduct a thorough analysis of results across the program, investigators' analyses of their data are made available to S&I. An additional role for S&I is to review FRP studies and provide suggestions for further analyses.

The FRP research that addresses the three policy questions is being synthesized in five Major Program Output documents (MPOs). The MPOs and their expected completion dates are as follows:

MPO #1: Evaluation of the extent and magnitude of recent changes in forest condition (9/89)

MPO #2: Evaluation of the role of non-air pollution factors in growth reduction and visible decline (9/89)

MPO #3: Seedling response to sulfur, nitrogen, and associated pollutants (4/89)

MPO #4: Evaluation of the roles of sulfur, nitrogen, and associated pollutants in forest decline (12/89 and 9/90)

MPO #5: Projection of responses under alternative deposition scenarios (12/89 and 9/90)

The purpose of this document (MPO #3) is to summarize the seedling exposure research conducted in the FRP. The seedling studies reported here thus contribute to Policy Question #2. MPO #4 will also contribute to answering Policy Question #2 by adding to the knowledge in MPO #3. Policy Question #1 will be addressed in MPO #1 and #2, and Policy Question #3 will be addressed in MPO #5.

3.3 The Role of Mechanisms as Scientific Questions in the FRP

In order to investigate Policy Question #2, scientific questions were outlined that hypothesize specific mechanisms by which a change in tree condition might occur. Change in tree condition includes visible injury to the foliage, change in growth, or death. Table 1 lists these hypothesized mechanisms, and Figure 1 illustrates how the mechanisms relate to tree growth. One single mechanism is unlikely to be the sole cause of a change in forest condition for the following reasons:

(a)Tree growth is an interrelated set of processes. For example, toxicity to roots or mycorrhizae (S.Q.2.1a and S.Q.2.1b in Table 1) will have an effect upon both water and nutrient uptake. The effect of foliar leaching (S.Q.2.1c in Table 1) upon forest growth will also depend upon the rate and amount of uptake of nutrients. Similarly, susceptibility to insects and disease is influenced by both nutrient status and water status of trees.

(b)Pollutants across the United States change in time and space. A change in the ratio between nitrogen and sulfur compounds, as acid precursors, seems likely and could have a major impact upon uptake of water and nutrients.

Complete measurement of all mechanisms in mature trees across a wide range of acidic deposition and pollutant conditions is not technically possible. It is the objective of S&I to identify conditions under which one mechanism may be dominant, as well as to assess how mechanisms may operate in combination to effect, for example, a change in growth. This integration must take the interrelations of the growth process into account. Data on soil processes and mature trees are being collected as part of the FRP and as a part of related programs such as the Electric Power Research Institute's Integrated Forest Study (Lindberg and Johnson, 1989). The FRP seedling research focuses on Scientific Questions 2.3 and 2.4, and to a lesser extent on Question 2.2.

Figure 1 illustrates an integrating model of tree growth. This model has been adopted because it demonstrates plausible interactions of the various processes as well as the possible results of modifications in one or more of these processes. For example, if repairing pollutant-damaged tissue increases respiration, less energy will be available for root and foliage maintenance and growth, even if photosynthetic rate remains constant. Reduced root growth can affect photosynthesis via disruptions of water and nutrient fluxes. Reduced foliage area will result in a diminished capacity to fix carbon and a decrease in energy available for all plant processes. Therefore, it is apparent that the three principal components of growth, photosynthesis, water uptake, and nutrient uptake, are interdependent.

Table 1. Scientific Questions (SQ 2.X) in the Forest Response Program of mechanisms by which a change in tree condition may occur.

Sulfur and nitrogen compounds and other air pollutants may affect trees and forests by the mechanisms of:

SQ2.1a.	Direct toxicity to roots, mycorrhizae, or soil microbial populations by mobilized metals in acidified soil water;
SQ2.1b.	Nitrogen toxicity to mycorrhizae;
SQ2.1c.	Increased leaching of soil nutrients resulting in reduced nutrient availability.
SQ2.2.	Increased leaching of nutrients from foliage.
SQ2.3.	Altered photosynthesis, respiration, and carbon allocation patterns (e.g., morphology) with possible induction of water or nutrient stress.
SQ2.4.	Delayed cold hardening or premature break in dormancy resulting in increased winter injury.
SQ2.5.	Disruption of reproduction or regeneration.
SQ2.6.	Alteration of susceptibility to insects and pathogens.

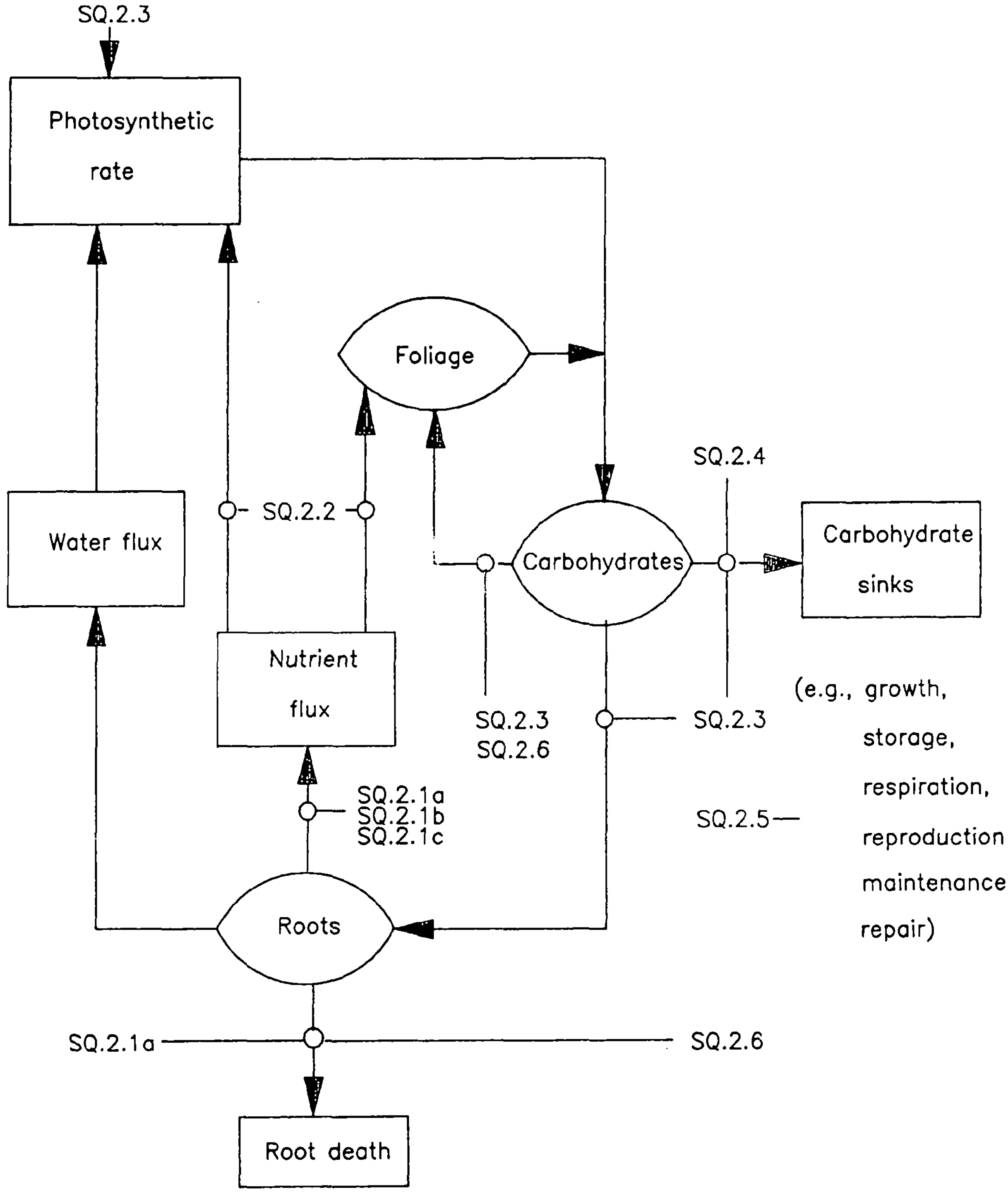


Figure 1: Diagram of basic processes and their interactions resulting in tree growth. Processes are represented by boxes and interactions by arrows. Functional components are represented by ellipses. The Scientific Questions (Table 1) ask how changes in these processes may affect growth.

4 METHODS AND MATERIALS

4.1 Choice of Experimental Material

Results from seedling studies, such as visible or latent seedling response, are the major source of information currently available to address Scientific Questions 2.2, 2.3, and 2.4 posed in Table 1. Information on seedlings is important because seedlings are representative of mature trees in many physiological processes, and seedling establishment is necessary for future forests. In this report, the term seedling refers to trees small enough (e.g., height generally less than 1 meter) to be housed in standard open-top chambers.

The consensus within the FRP is that while seedlings do have limitations, the use of seedlings is a cost-effective way to obtain information through exposure studies, particularly given the short time frame of the program (three years). In comparison with larger trees, seedlings are easier to manipulate and present fewer sampling problems in experimental designs, particularly those that require growth chamber fumigation systems. The size of seedling plant material allows mechanisms to be measured over a range of experimental treatments often infeasible with mature trees.

4.2 General Methods

Twenty-four studies, currently in various stages of completion, have been funded by the FRP to quantify seedling responses to simulated acid deposition, sulfur dioxide, and ozone. Twelve conifer species, twelve hardwood species, and 100 commercially important families of loblolly pine are being tested. This section describes the general methods employed across the studies. It should be noted that methods among studies varied and may have affected the particular results observed. An overview of the experimental approaches used by the four research cooperatives is given in Tables 2, 3, 4, and 5.

Seedlings were grown from germinated seeds obtained from known seed sources. These sources were: 1) specific regions of forest occurrence for spruce fir; 2) tree nurseries for the eastern hardwoods; 3) commercial and research seed orchards of loblolly pine; and 4) regions of forest occurrence and forest-tree nurseries for western conifers. Most seedlings were planted in individual containers. Rooting media were typically composed of commercial mixtures (e.g., peat, vermiculite, perlite), although soil representing a forested site was sometimes used. Ages of seedlings at time of treatments ranged from 12 weeks to 4 years; the majority were 2 years or younger at the beginning of the studies. Seedlings were grown under non-stressed conditions with adequate nutrients, water, and light. In some cases, seedlings were screened prior to treatments and seedlings of atypical growth form were rejected.

To apply the treatments, seedlings were placed in chambers that provided for delivery of simulated precipitation and allowed some modification of the air space around the seedlings. Two types of chambers were used, and choice of chamber type involved a trade-off between experimental control and replication of realistic conditions. Completely enclosed chambers, referred to as continuously-stirred tank reactors (CSTRs) in greenhouses or laboratories allowed for higher precision in the application of gaseous treatments. Open-top chambers located outdoors were used in cases where some exclusion of ambient air, but exposure to sunlight, humidity, and normal air temperatures was desired. The outdoor chambers may or may not have had rainfall exclusion devices, depending upon experimental objectives.

Table 2. Overview of Spruce-fir seedling exposure research by project number with principal investigator (* indicates regime used by each project).

	Study Description	SF22 FERET	SF32 GREENWOOD 1988	SF32 GREENWOOD 1989	SF06 JACOBSON	SF07 JENSEN 1988	SF07 JENSEN 1987	SF31 KOHUT	SF10 McLAUGHLIN 1987	SF10 McLAUGHLIN 1988	SF13 SEILER ozone only	SF13 SEILER ozone & acid rain	SF27 THORNTON	SF14 UNSWORTH (a)	SF14 UNSWORTH (b)	SF16 WEINSTEIN
STUDY TYPE	Controlled Field	•	•	•	•			•					•	•	•	•
	Controlled Laboratory					•	•		•	•	•	•				
EXPOSURE REGIME	Artificial	•	•	•		•	•		•	•	•	•				
	Modified Ambient				•			•								•
	Simulated Ambient															
	Ambient Profile												•		•	
FACILITIES	Open-Top Chambers	•	•	•												•
	Rain Exclusion				•			•						•		
	Air/Rain Exclusion												•		•	
	CSTR (Laboratory)					•	•				•	•				
	CSTR (Greenhouse)					•										
	Growth Chambers								•	•						
	Branch Chambers															
POLLUTANT	Ozone		•	•		•	•	•			•	•				•
	Acidic Rain					•	•	•				•		•		
	Acidic Mist				•											
	Sulfur Dioxide						•								•	
	Nitrogen Dioxide														•	
	Nitric Acid								•							
	Hydrogen Peroxide									•						
PLANT MATERIAL	Seedlings		•	•	•	•	•	•	•	•	•	•	•	•	•	•
	Saplings							•								
	Mature Trees															
	Grafts	•	•	•												
SPECIES	Red Spruce		•	•	•	•	•	•	•	•			•	•	•	•
	Fraser Fir	•									•	•				
	Balsam Fir			•												

Table 3. Overview of Eastern Hardwoods seedling exposure research by project number with principal investigator (* indicates regime used by each project).

Study Description		EH01 DAVIS 1987 study	EH01 DAVIS 1988 study	EH04 SKELLY	EH06 JENSEN 1987 study	EH06 JENSEN 1988 study with Ohio corridor gradient	EH06 JENSEN 1988 study with Michigan gradient	EH06 JENSEN 1989 study with Michigan gradient	EH06 JENSEN Karnosky (both 1988 & 1989)
STUDY TYPE	Controlled Field			•			•	•	•
	Controlled Laboratory	•	•		•	•		•	
EXPOSURE REGIME	Artificial	•	•		•	•	•	•	
	Modified Ambient								•
	Simulated Ambient								
	Ambient Profile			•					
FACILITIES	Open-Top Chambers			•			•	•	•
	Rain Exclusion						•	•	
	Air/Rain Exclusion								
	CSTR (Laboratory)								
	CSTR (Greenhouse)		•		•	•		•	
	Growth Chambers	•							
	Branch Chambers								
POLLUTANT	Ozone	•	•		•	•	•	•	•
	Acidic Rain	•	•		•				
	Acidic Mist								
	Sulfur Dioxide		•		•	• (sulfate)			
	Nitrogen Dioxide							• (nitrate)	
	Nitric Acid								
	Hydrogen Peroxide								
	Ambient			•					
PLANT MATERIAL	Seedlings	•	•	•	•	•	•	•	•
	Saplings								
	Mature Trees								
	Grafts								
SPECIES	European Beech				•				
	Sweetgum	•			•				
	Hickory spp.				•				
	Red Oak spp.	•	•	•	•				
	White Oak spp.	•			•	•			
	Black Oak					•			
	Yellow-Poplar	•	•	•	•				
	White Ash	•			•				
	Red Maple	•	•	•	•		•		
	Sugar Maple				•		•	•	•
	Black Cherry	•	•	•					
	E. White Pine				•				
	Trembling Aspen						•	•	•
	Flowering Dogwood					•			
	Chestnut Oak					•			
	Yellow Birch	•							

Table 4. Overview of Southern Commercial Pine seedling exposure research by project number with principal investigator (* indicates regime used by each project).

	Study Description	SC14 FLAGLER	SC02 FONG	SC13 JOHNSON	SC06 KRESS	SC15 LOCKABY	SC12 McGREGOR	SC04 McLAUGHLIN		SC05 REINERT	SC07 RICHARDSON
STUDY TYPE	Controlled Field	*		*	*	*	*		*		
	Controlled Laboratory		*					*		*	
EXPOSURE REGIME	Artificial		*					*		*	
	Modified Ambient	*		*	*	*	*		*		
	Simulated Ambient										
	Ambient Profile										
FACILITIES	Open-Top Chambers	*		*	*	*	*		*		
	Rain Exclusion	*		*	*	*	*				
	Air/Rain Exclusion										
	CSTR (Laboratory)							*			
	CSTR (Greenhouse)									*	
	Growth Chambers		*								
	Branch Chambers										
POLLUTANT	Ozone	*	*	*	*	*	*	*	*	*	*
	Acidic Rain	*		*	*	*	*		*	*	*
	Acidic Mist										
	Sulfur Dioxide										
	Nitrogen Dioxide										
	Nitric Acid										
	Hydrogen Peroxide										
	Ambient										
PLANT MATERIAL	Seedlings	*	*	*	*	*	*	*	*	*	*
	Saplings										
	Mature Trees										
	Grafts										
SPECIES	Loblolly Pine		*		*	*		*	*	*	*
	Shortleaf Pine	*					*				
	Slash Pine			*							

Table 5. Overview of Western Conifer seedling exposure research by project number with principal investigator (* indicates regime used by each project).

Study Description		WC20 HOUPIS		WC09 MILLER	WC08 HOGSETT	WC07 TURNER
STUDY TYPE	Controlled Field	*	*	*	*	
	Controlled Laboratory					*
EXPOSURE REGIME	Artificial					*
	Modified Ambient	*	*			
	Simulated Ambient			*	*	
	Ambient Profile					
FACILITIES	Open-Top Chambers	*		*	*	
	Rain Exclusion			*	*	
	Air/Rain Exclusion					
	CSTR (Laboratory)					
	CSTR (Greenhouse)					
	Growth Chambers					*
	Branch Chambers		*			
POLLUTANT	Ozone	*	*	*	*	
	Acidic Rain					
	Acidic Mist			*	*	*
	Sulfur Dioxide			*	*	
	Nitrogen Dioxide					
	Nitric Acid					
	Hydrogen Peroxide					
	Ambient					
PLANT MATERIAL	Seedlings	*		*	*	*
	Saplings					
	Mature Trees		*			
	Grafts					
SPECIES	Subalpine Fir			*		
	Ponderosa Pine	*	*	*	*	
	W. Redcedar				*	
	Douglas-Fir			*	*	*
	Englemann Spruce			*		*
	W. Hemlock				*	
	Lodgepole Pine				*	
	White Fir			*		

The most prevalent treatments included simulated acid precipitation and ozone applied alone or in combination. One to six levels of acidity were used, ranging from pH 2.1 to 5.6. Simulated acid precipitation typically consisted of a chemical composition that reflected rainfall chemistry of the study area (sulfur-to-nitrogen ratios were typically 2:1). In this document, terms such as acidity, acid, or acid deposition refer to the hydrogen ion concentration plus the chemical composition of the simulated precipitation.

One to six levels of ozone concentration were used, ranging from 0 to 320 ppb. Charcoal filtering can remove up to 100% of ozone in ambient air. Therefore, CSTRs can attain 0 ppb treatments while open-top chambers never quite approach 0 ppb due to mixing of filtered air and ambient air through the open tops. Ozone concentrations in open-top chambers receiving charcoal-filtered air are typically 30% to 50% of ambient concentrations. In addition to acidity and ozone, one project varied the ratio of sulfur to nitrogen in the precipitation, while others applied treatments of sulfur dioxide. Finally, several studies tested for interactions of acid precipitation and/or ozone with winter injury or interactions with water stress. A range of treatments by pollutants and levels of exposure for a selected number of studies is given in Table 6.

Treatments were applied to the seedlings during periods of active above-ground growth over intervals varying from ten weeks to seven months. Multiple-year exposures are also being carried out. However, this document is generally limited to results from a single season of exposures. As such, some of the conclusions are tentative and may change as more data are obtained.

Simulated precipitation was applied as rain, mist, or fog. Usually precipitation was applied to both foliage and rooting medium in a pattern reflecting historical trends for a specific region. In some cases, precipitation was applied only to saturate the foliage; in such cases, the rooting medium received controlled watering. Ozone was applied over regulated time intervals, usually during daylight hours. Applications varied among studies, but were of two general types. The first, more simple type was a square-wave regime where a constant concentration of ozone was applied over a definite time interval during the day. In more sophisticated designs, ozone applications followed the monitored ambient concentrations for the region, where ozone concentrations typically increased to a mid-afternoon peak then decreased until dusk.

Response variables measured on the seedlings are presented as either effects or mechanisms. Effects represent a change in seedling condition and in this document include visible effects or growth changes. Mechanisms are the processes by which effects are manifested. The mechanisms examined include carbon allocation, winter injury, and foliar leaching, reflecting Scientific Questions 2.2, 2.3, and 2.4 posed in Table 1. In this document, carbon allocation is used as a general term that includes growth, morphology, and general physiology, including photosynthesis and respiration. In the results, growth is discussed separately as an observable effect, whereas photosynthesis (carbon fixation) and physiological responses are discussed as mechanisms that may affect growth.

Given the short time frame of the program, effects and mechanisms were examined simultaneously in the FRP research, as opposed to sequentially. While it was generally accepted that pollutants at some level affect plants, the goal of the FRP was to quantify these effects on trees while also identifying mechanisms of the effects, particularly those with relevance for policy.

The actual response variables measured were in some studies quite numerous. Some variables were measured several times during the treatments, while others were measured only at the termination of treatments. Visible effects included foliage discoloration (chlorosis, necrosis), foliage loss (senescence), or whole-tree subjective classification. Growth effects involved some measure of seedling biomass (linear measures of branches or roots, diameter of stem, or mass of various components). Carbon allocation involved measures of photosynthetic rates, respiration rates, tissue damage assessed microscopically, or tissue chemistry (sugars, starch and non-struc-

Table 6. Treatment levels used in a cross section of seedling studies of the Forest Response Program.

PI (Project #)[1]	Acidity (pH)	Ozone (ppb)[2]	Sulfur dioxide (ppb)
Jacobson (SF06)	2.5, 3.5, 4.5		
	2.8, 3.5, 4.2		
	2.6, 3.4, 4.2		
Jensen (SF07)	3.5, 4.0, 4.5	CF, 150(6h), 150(6h)+70(18h)	
	3.0, 4.2	CF, 50(12h), 100(12h)+50(12h), 150(12h)+100(12h)	
Seiler (SF13)	3.0, 4.3, 5.6	0, 100	
		<20, 50, 100	
Unsworth (SF14)	2.5, 2.7, 3.0, 3.5, 4.0, 5.0		
Weinstein (SF16)		CF, A, 2A, 3A, 4A (A=40, 7hm)	
Kohut (SF31)	3.1, 4.1, 5.1	CF, A, 0.5A, 1.5A, 2A (A=38, 12hm)	
Davis (EH01)	3.0, 4.2	0, 75, 150	
Jensen (EH06)	3.0, 3.5, 4.2	CF, 70, 150	0, 20
Fong (SC02)		CF, 160, 320	
McLaughlin (SC04)	4.3	CF, 160, 320	
	3.3, 4.5, 5.2	CF, A, A+40, A+80, A+160 (A=36, 12hm)	
Reinert (SC05)	3.3, 4.3, 5.3	0; 80, 160, 240, 320	
	4.3	0, 80, 160, 240, 320	
Kress (SC06)	3.5, 5.2	CF, A, 1.5A, 2.25A, 3A (A=45, 12hm)	
Turner (WC07)	2.1, 3.1, 5.6		
Hogsett (WC08)	2.1, 3.1, 5.6	CF, 1.6B, 1.8B (B=53, 7hm)	CF, B, .33B, .67B (B=66)
Miller (WC09)	2.1, 3.1, 5.6	CF, B, 1.6B, 1.8B (B=53, 7hm)	CF, B, .33B, .67B (B=66)

[1] PI = Principal Investigator
Project # = the number assigned to a study within FRP research cooperatives:
- SC = Southern Commercial
- SF = Spruce-fir
- EH = Eastern Hardwoods
- WC = Western Conifers

[2] CF = charcoal-filtered air
- A = ambient air at the research site
- B = base profile; designed to simulate a potential exposure regime using a worst-case ambient in the West
- h = hours
- hm = hour mean

tural carbohydrates, photosynthetic pigments, or enzymes). Winter injury was examined as an interacting stress; in these cases, seedling responses were measured after treated seedlings were allowed to over-winter at ambient temperatures or after tissues were exposed to simulated frosts. Foliar leaching involved some measure of solution chemistry of throughfall or of solutions in which treated tissues were leached. Refer to the project summaries in Appendix A for further details on measurements taken at each study site.

4.3 Statistical Methods

All studies were designed to test hypotheses statistically. Building on exposure studies of crops in the National Crop Loss Assessment Network (NCLAN), the experimental designs were generally a variation of split-plot or randomized blocks. Most studies also incorporated repeated measurements (usually five or more intervals) of total plant height and root collar diameter. Data were analyzed via analysis of variance (ANOVA), analysis of covariance (ANCOVA), or regression techniques.

Important statistical issues identified by S&I for the seedling exposure experiments are: design and analysis, relevance, combining results across experiments, and statistical power. These issues were the subject of several workshops held in 1988 by C.E. Peterson and W.G. Warren with statisticians and principal investigators associated with FRP seedling studies. In both the workshops and subsequent contacts, discussions focused on how designs fit objectives (testing for sensitivity, exposure-response, mechanisms), and whether the model and proposed analyses would take full advantage of available covariates, time intervals, etc. A short discussion of these issues follows.

4.3.1 Design and Analysis

An important function of FRP Work Plans and Quality Assurance Plans is to specify both the objective and the experimental design with the proposed statistical procedure for the exposure studies. For instance, are seedling studies intended to screen species or families for sensitivities to pollutant exposures, help build an exposure-response (E/R) model, or to study mechanisms? Has an E/R model been selected a priori common to all sites or is the testing of alternative models an intended use of the data? In the case of ANOVA or ANCOVA, are the statistical models completely specified (i.e., fixed and random effects identified)? What uncontrollable factors are not measured or accounted for? Questions such as these must be addressed in order to evaluate the contribution of each individual project and to identify common objectives among the experiments when attempting to combine individual results. When considering the possible geographical inference from combined results, it is also important to determine whether the experimental sites were chosen purposively or randomly.

4.3.2 Relevance

The relevance of seedling experimental material for making inferences requires careful consideration. At a minimum, seedling studies provide information for the evaluation of hypotheses of changes in seedling condition that are applicable to seedling populations. Ideally, information might also indicate hypotheses for the study of mature trees. However, direct extrapolation from seedlings to mature trees is not intended with this research.

4.3.3 Combining Results

Although experimental results in forest ecology have not generally been used for regional assessments, the results from many ongoing and planned studies of forest response to acid deposition are expected to play a significant role in future decision-making. The practice of combining results from several studies has not been fully addressed in forest ecology with statistical rigor, although the notion was formally introduced some time ago to researchers (Fisher, 1932). Combining results from individual studies has the potential to increase the power of conclusions drawn from the individual experiments. Consequently, an element of regionalization can be brought into the evaluation of results.

The methodology for actually combining results of several experiments may take the form of combining probabilities or test statistics (e.g., Fisher, 1932). However, combining results from the types of exposure studies carried out by the FRP may also involve the use of regression (e.g., Heagle, Heck, Rawlings, and Philbeck, 1983; Rawlings, and Cure, 1985; Rawlings, Lesser, and Dassel, 1988).

This challenge is an ongoing concern for the FRP (e.g., Warren, 1987). However, it must be emphasized that many experiments with widely different variations in objectives and designs may not be comparable, and in this case individual results should probably not be combined. Thus, the primary goal has been to evaluate the seedling studies as they actually were carried out to identify potential problems with treatments or experimental procedures that could either cloud the interpretation of an individual experiment or hinder the combination of results, and to use this information as feedback to principal investigators.

4.3.4 Statistical Power

Power of the statistical test, that is, the probability of detecting a consequential change in condition, should be considered for all controlled exposure studies at both the initiation and conclusion of the research (e.g., Rawlings, 1986). Power is a function of experimental design, including sample size and experimental (uncontrolled) error, where sample size refers to both the number of seedlings used per treatment combination and the number of replicates (commonly chambers). Each scientist should compute both the confidence limits for differences attributed to pollutant exposures and the realized power. A simple abbreviated example of a power curve computed by Project WC08 for ponderosa pine seedlings is included in Appendix B.

4.4 Assessment of Data Quality

The goals for data quality assessment in forestry research are to provide procedures that reduce random and systematic error in treatment application and response measurement, and to evaluate data accuracy and precision. To achieve these goals, quantification of the inherent variability associated with both the treatment application and response variable measurement is necessary. In this section, the variability inherent in the technology used to apply exposure treatments as well as the variability of the experimental measures collected are discussed. A detailed summary of quality control information for individual projects cited in this report is available from the FRP Program Manager.

In the following discussion, data quality is represented graphically on control charts and frequency histograms. Control charts indicate when a measurement process has exceeded predetermined statistical limits of variability. Limits on control charts represent the upper and lower bounds for these estimates. Data quality objectives (DQOs) for upper and lower control limits have been set by FRP investigators as their best estimate of the bounds within which the process is in statistical control. Warning limits were set at half the data quality objective (see Figure 2 for an example). Data for acidic precipitation and gaseous exposures at the research sites have been plotted as fluctuations about target concentrations. The objectives were to identify variation about the target as well as the source of that variation, and to evaluate the appropriateness of the data quality objectives. A consistent trend outside of the warning limits indicates that the process is not in control and an adjustment to the process is typically required.

Frequency histograms are also used to summarize large number of observations over time to identify trends in the data. This technique can be used to show the frequency distribution about a target, such as pH and ozone concentration, or to plot the coefficient of variation of analytical data.

4.4.1 Acid Precipitation and Gaseous Exposure Techniques

The difficulties inherent in conducting controlled exposures with mature trees have focused exposure-response research on seedlings. Growth chambers, continuously-stirred tank reactors (CSTRs), and open-top chambers represent seedling exposure technologies ranging from highly controlled but artificial environments to less controlled yet more ambient environments. All three techniques require that ambient conditions be modified to apply experimental treatments.

Growth chambers permit maximum environmental control with some trade-offs; the small size of the chambers dictates a limited number of seedlings, and the cost necessitates a limited number of chambers, allowing little or no replication. Lack of replication may prevent the use of standard statistical analyses to define treatment effects; thus, it may be only possible to infer biological trends. Growth chambers are intended to control environmental parameters such as light, temperature, and humidity. However, laminar air flow within the chambers and the associated pollutant delivery systems may not be able to attain the same degree of treatment control for gases, as illustrated in Figure 2.

The lack of treatment control in growth chambers may be due to chamber design that mimics the air flow over a tree canopy and that may cause improper gas mixing in the air stream. The chambers also have significant horizontal and vertical gas concentration gradients above and within the tree canopy. Gas concentration gradients in growth chambers were eliminated in CSTRs by the use of a mixing impeller to improve the internal mixing of gases. Tighter control of the gas treatments can be achieved, but it is often at the cost of control of environmental variables. There are usually no environmental control systems associated with CSTRs. In the greenhouse, the CSTR environment is affected by the seasonal ambient conditions (Figure 3). CSTRs located in laboratories combine the tight control of the gas delivery system with better environmental controls. The artificial light, temperature, and humidity control of the laboratory does differ significantly from the greenhouse environment.

Although CSTRs are designed for thorough mixing of gases throughout the chamber, the systems for delivering the gas to the chamber are unique to individual sites. Research projects using CSTRs had varied amounts of manual control over the gas delivery systems. Adjustments to the manual system that changes the gas concentrations within the CSTR are based on operator judgement. The manual system can achieve uniform control of the treatments that meet the data quality objective (Figure 4). However, the variation inherent in a manually controlled system is evident in Figure 5. Equipment malfunctions and operator error are additional sources of treatment variability. These two factors may affect the treatment results (Figure 6).

Open-top chambers are an alternative to the laboratory or greenhouse controlled environments. Although significant differences exist between the chamber and ambient environments, this technology is currently the best for controlled exposure of large numbers of trees in the field. Air temperature increases of 2 to $3^{\circ}C$, light reduction of 10%, and both higher and lower relative humidity have been observed in chambers when compared to ambient conditions. Modifications to the original open-top design have had a large impact on the variability of environmental conditions and treatment applications. The additions of a constricting baffle and rain exclusion covers to the top of the chamber have minimized ingression of ambient air into the top of the

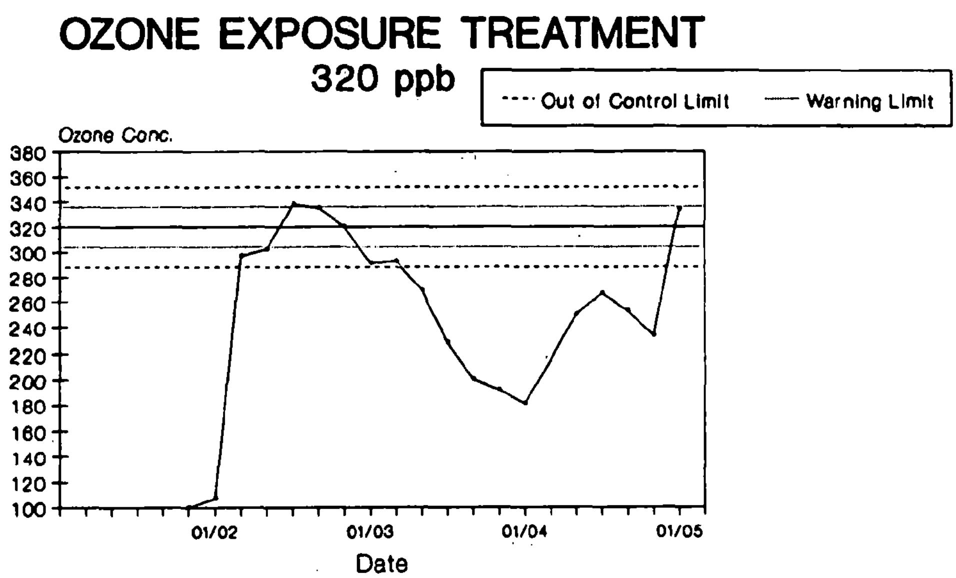
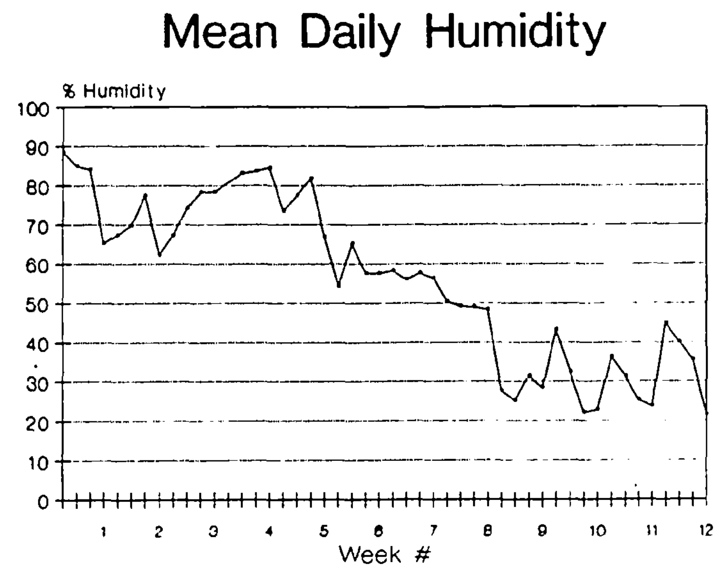


Figure 2: Ozone treatment in growth chamber with laminar air flow.



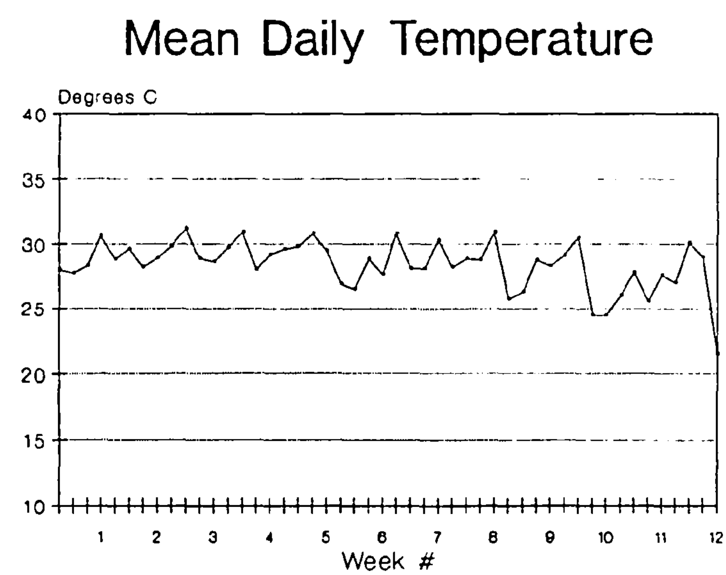


Figure 3: CSTR temperature and relative humidity during 12 weeks of exposure in a greenhouse.

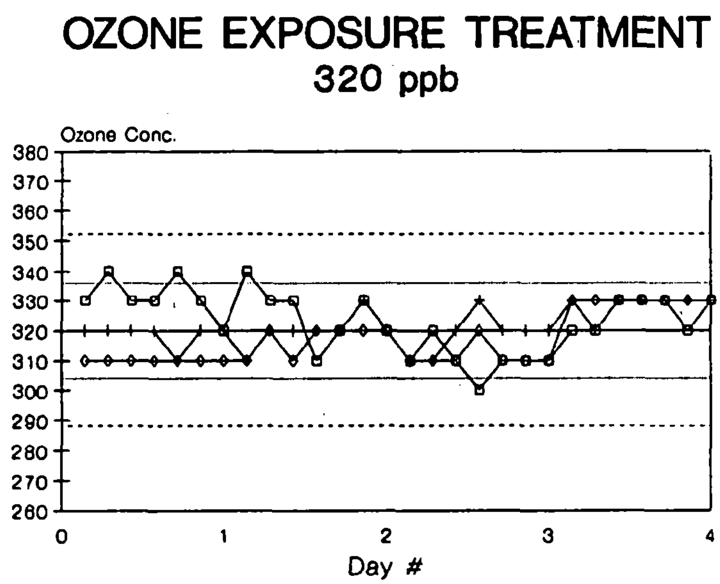
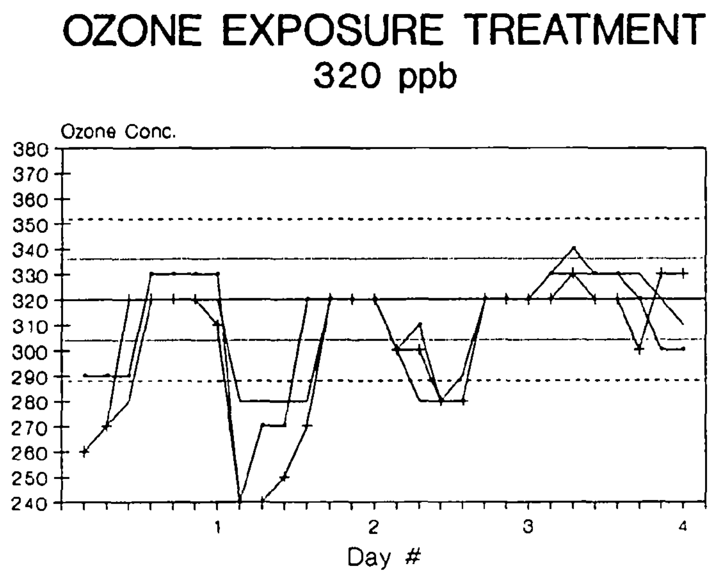


Figure 4: Accuracy of treatment applications in three CSTRs.



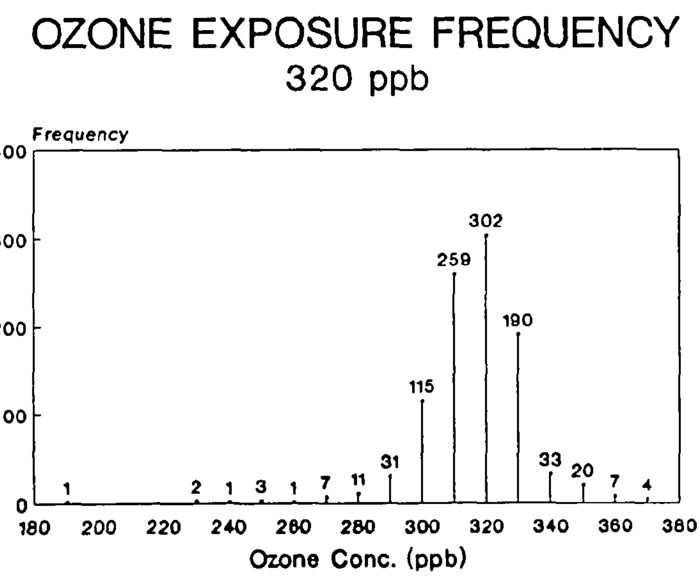


Figure 5: Control chart showing variability of treatment application during one week and a frequency histogram summarizing the precision of treatment application over 12 weeks.

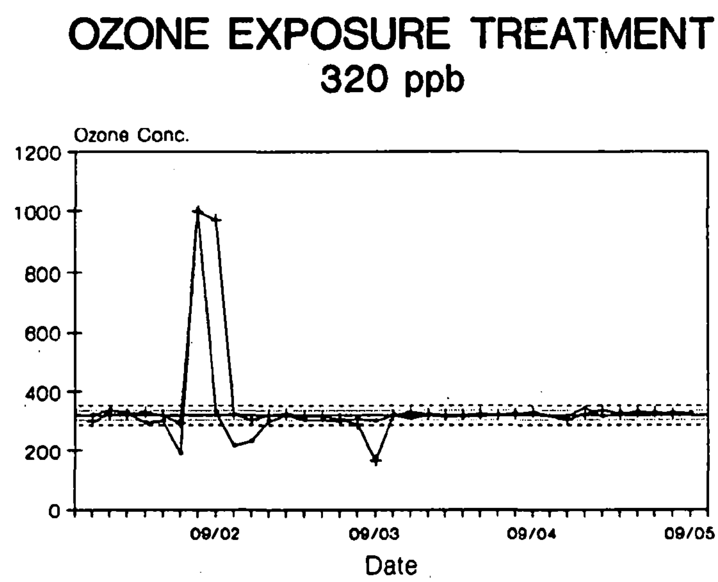


Figure 6: Example of equipment failure during treatment application.

chamber and can improve filtering efficiency from 50% to 80%. However, the improved efficiency is attained at the cost of increases in variability of environmental measurements from ambient.

The large number of open-top chambers at individual research sites requires computer controls for treatment application. Time sharing of air quality monitors and custom computer software programs have significant impacts on the accuracy and precision of treatment application. Although the technology is capable of applying fairly uniform treatments over time, environmental factors, such as wind speed and direction, can affect the treatments (Figure 7). Analysis of quality control data indicated significant variability among sites using the same exposure methodologies. The variability was site dependent with most attributed to the exposure technology used at the site. The study type, exposure regime, facility, and pollutant also contributed to this variability, although these variance components have not been estimated.

Acidic precipitation treatments, rain or mist, are used at most sites. Treatments are formulated in tank batches and distributed to individual chamber application systems. Batch mixing allows for high precision in treatments being applied to large numbers of plots, but operator error can account for fluctuation around target concentrations (Figure 8). Realistic DQOs should be technology-specific and derived from previously collected experimental data. Those DQOs established in the Quality Assurance Methods Manuals were a best estimate of precision and, in many cases, were not based on experimental data. Most DQOs were attained by almost all sites for acidic precipitation, but not for gaseous pollutants.

4.4.2 Growth and Physiology Response Variables

To estimate accuracy and precision for growth and physiology measurements, it is best to separate variables measured by traditional analytical techniques from those requiring more subjective interpretation. The dry weight of a tissue or its nitrogen content can be determined by analytical techniques that follow a standard operating procedure. Using National Bureau of Standard weights and tissue samples, an estimate of accuracy and precision can be determined and the investigator can follow the DQO criteria for accepting or rejecting data. For variables such as height and rate of photosynthesis these criteria are not as easy to apply.

Natural variability in plant growth makes height and diameter determinations subjective. Analysis of repeated measurement data indicated that the precision of diameter measurements generally fell outside the DQO. Height was measured more precisely (Figure 9). Data from sites with saplings and mature trees showed the opposite trend.

The rate of photosynthesis is an example of an instantaneous measurement of a dynamic system that does not permit repeated measurements for precision estimates. When interpreting data it is important to be aware of the inherent variability of these measurements and determine whether the data should be viewed as relative or absolute values.

4.4.3 Summary

Traditionally, variation in the response variables has received some consideration when accounting for experimental error, while the inherent variability associated with treatment application has been ignored or assumed to be zero. For research cited in this report, variability in treatment application could be a major source of experimental error. Factors contributing to experimental error include:

System malfunctions: The bimodal distribution of exposure in Figure 10 suggests that a system failure occurred on numerous occasions during the experiment. Sometimes treatment concentrations can vary enough to actually overlap other treatment levels. As shown in Figure 2, in one case

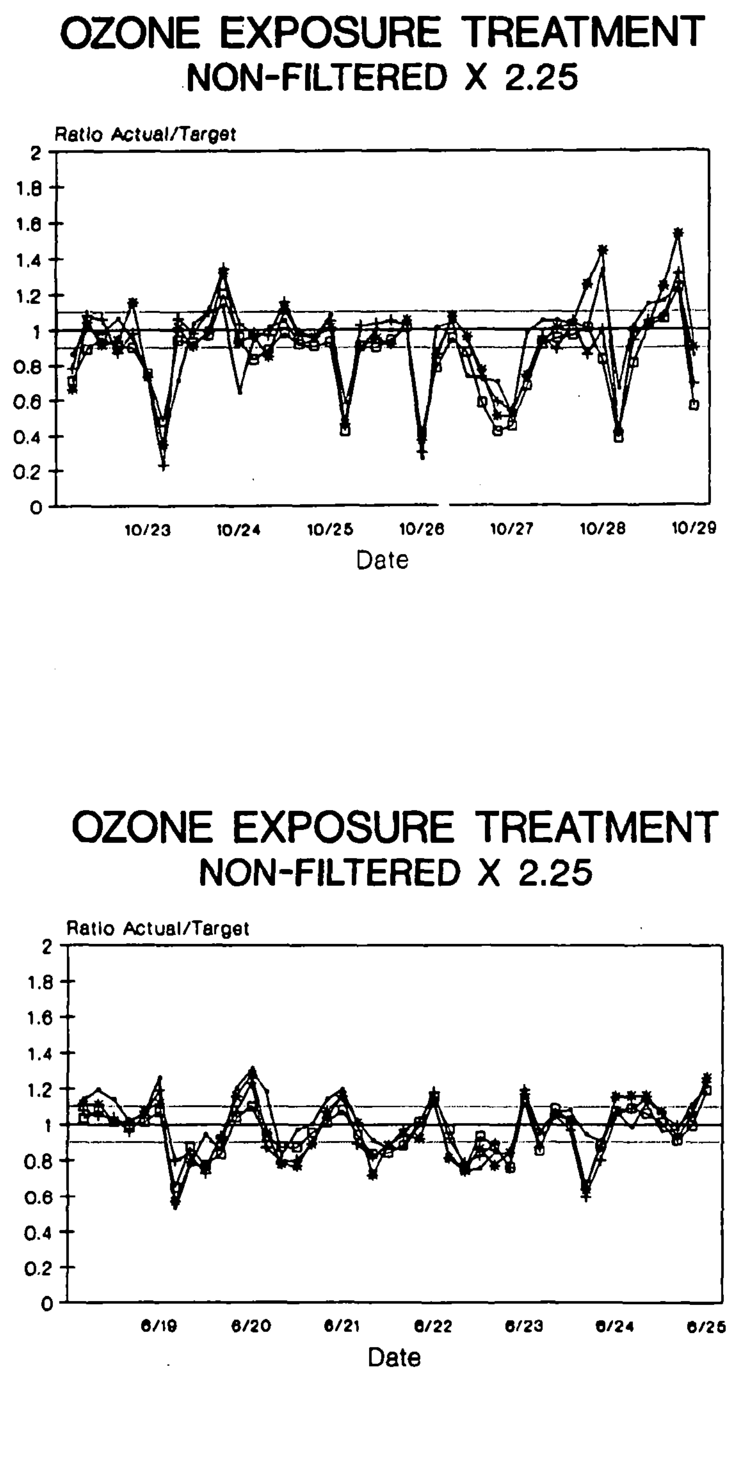
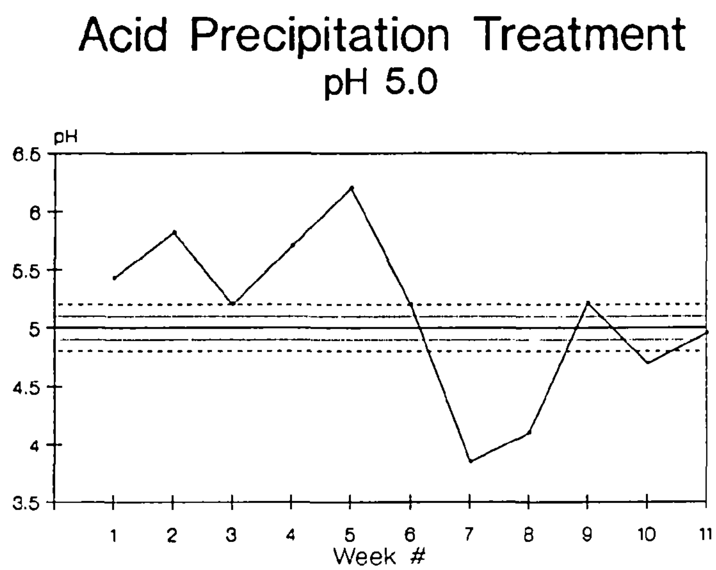


Figure 7: Comparison of two exposure weeks in open-top chambers with differing amounts of ambient air ingression.



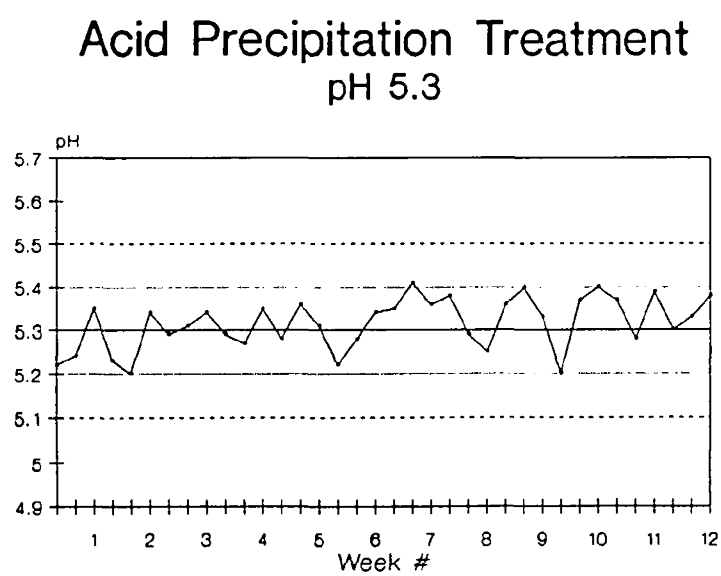
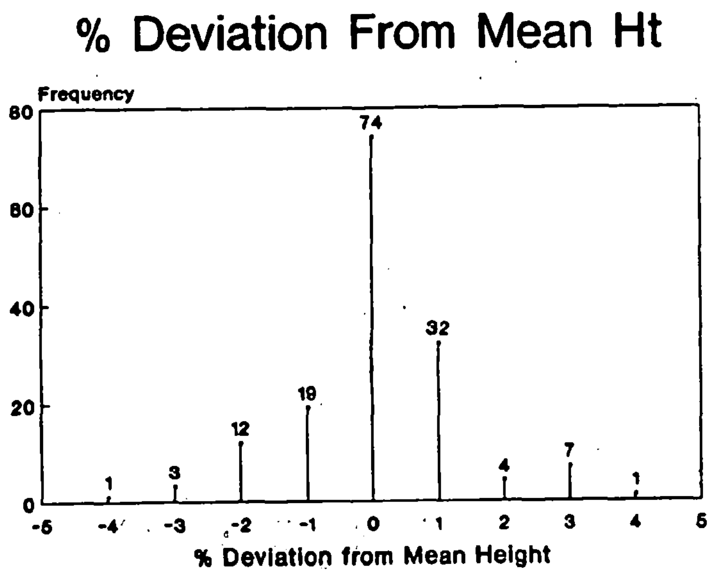


Figure 8: Variability between two sites in the accuracy of acidic precipitation treatments.



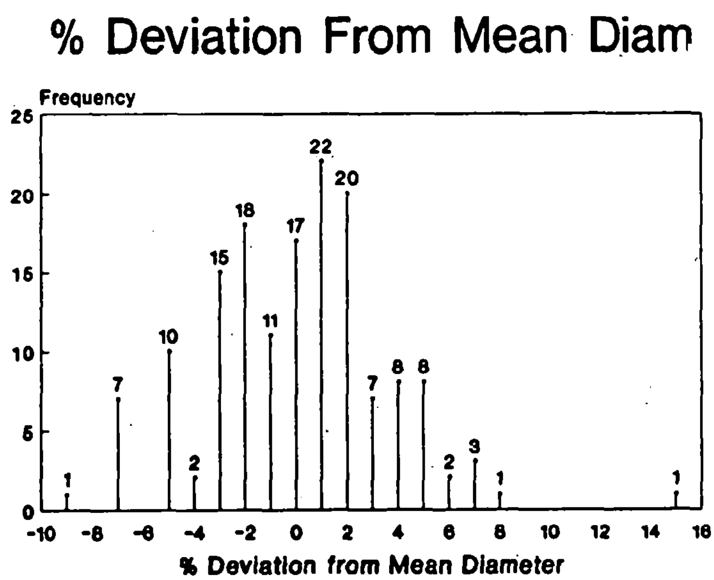


Figure 9. Variability in height and diameter variables from repeated measurement data.

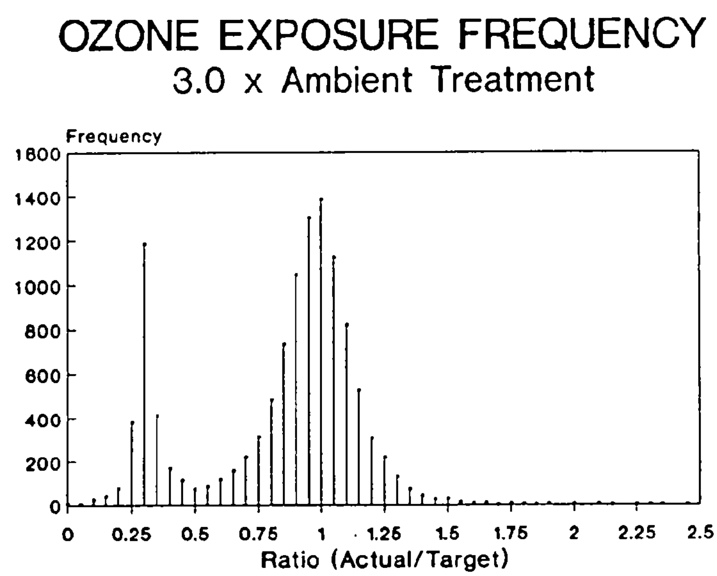


Figure 10: A bimodal histogram indicating a system failure in ozone treatment application.

ozone concentrations in a chamber targeted for 320 ppb fell below 240 and 160 ppb (other levels of ozone in the experiment). As another example, the large deviations from the ozone treatment target seen in Figure 6 signaled system defects that may have adversely affected data quality.

Measurement error: As illustrated in Figure 9, the variation in remeasurement of typical response variables such as height and diameter can affect data quality.

Shifts in environmental parameters: Figure 3 shows how environmental factors such as temperature and humidity can vary inside CSTRs throughout an experiment, which in turn can affect seedling response to treatments. These environmental parameters, as well as tree culture (seed grown or bare root nursery stock), soils, and growing medium (container or field) can contribute to variation in response.

The assessment of data quality has not been completed for all projects. When that task is accomplished, the primary use of the results will be to evaluate the relevance of the data quality objectives to see if those specifications can be realistically attained. The achievement of an average target value is relative to the accompanying fluctuations. The purpose of that information is not to reject data from a given project, but is intended to help the principal investigator monitor the system and make necessary corrections to keep the system under control. However, information such as magnitude and direction of the charted treatment fluctuations will be considered in a subjective way, when evaluating the biological data from a project. Final data quality analyses will be contained in a separate data quality document currently in preparation, and updated in MPO #4.

5 RESULTS

Results from most of the 1988 seedling experiments will not be available until later in 1989. Further analyses by the Principal Investigators are in progress, as well as review of those analyses by S&I and summaries of data quality by QA. Thus, this section contains preliminary results from two-thirds of the projects' 1986, 1987, and 1988 experiments; the amount of detail presented reflects the data summaries and interpretations provided by the investigators. Additional results will be incorporated with other FRP research in MPO #4 (due in 9/89).

Results for individual studies are presented by region (red spruce, eastern hardwoods, southern commercial pine, and western conifers). For each region, effects (visible injury and growth changes) are presented, followed by a discussion of mechanisms (foliar leaching, carbon allocation, and winter injury). Species or family sensitivities within the region are discussed where appropriate, and results within each region are summarized.

5.1 Red Spruce

Sixteen reports from seven projects comprise the seedling research on red spruce available for summary. Most studies involved exposures of seedlings to controlled levels of acid precipitation and ozone; several studies are continuing as multiple-year exposures. One project [SF13] tested Fraser fir, a species that co-exists with red spruce in the Southern Appalachians; all other projects used red spruce.

5.1.1 Visible Injury

Leith et al. [SF14] observed from 20% to 65% needle damage (browning) per seedling in red spruce after the tenth week of twice-weekly applications of the three most acidic mists (pH 2.5, 2.7, and 3.0), as shown in Figure 11. Mists of lower acidity (pH 3.5, 4.0, and 5.0) showed less than 5% damage. Primarily current-year needles were affected, particularly those on the upper surfaces and distal ends of lateral branches. There was considerable seedling-to-seedling variation; some seedlings showed no damage. In addition to the acid effect, visible damage was linearly related to accumulated nitrogen or sulfur exposures (Figure 12). Extensive needle loss occurred about 20 weeks after the first signs of discoloration. Two of the acid treatments (pH 2.5 and 3.0) also induced earlier bud flushing the following spring. There was no evidence of bud mortality due to acid mists.

Jacobson et al. [SF06(a)] performed six separate experiments during 1985 through 1987. The response of red spruce seedlings to total acidity and the ratio of sulfuric to nitric acids was examined in three field experiments. In three greenhouse experiments, the effects of acidity, anion ratio, and drying of precipitation on the foliage were evaluated. In the 1985 and 1987 field experiments, severity of injury increased in mists of pH 2.5 and 2.6 when compared with pH 3.5 or higher ($p = 0.0001$). At these low acidities, severity of injury (measured as percent of needles injured) also appeared to be a function of anion ratio; sulfate acids produced greater injury than nitrate acids (17% versus 2%; $p < 0.0002$). The pH effect was still evident the following spring after the seedlings overwintered without additional treatments, but the anion ratio effect was not. The 1985 and 1987 results were not repeated in 1986; there were no effects of acidity, anion ratio, or their combination, and severity of injury was never greater than 1%.

In Jacobson et al.'s [SF06(a)] greenhouse experiments, foliar injury of seedlings exposed to acid mists of pH 3.0 was approximately double that observed at pH 4.0 ($p = 0.001$). Needle injury at pH 3.0 averaged 33% for nitrate acids and 46% for sulfate acids. Sulfuric acids were associated

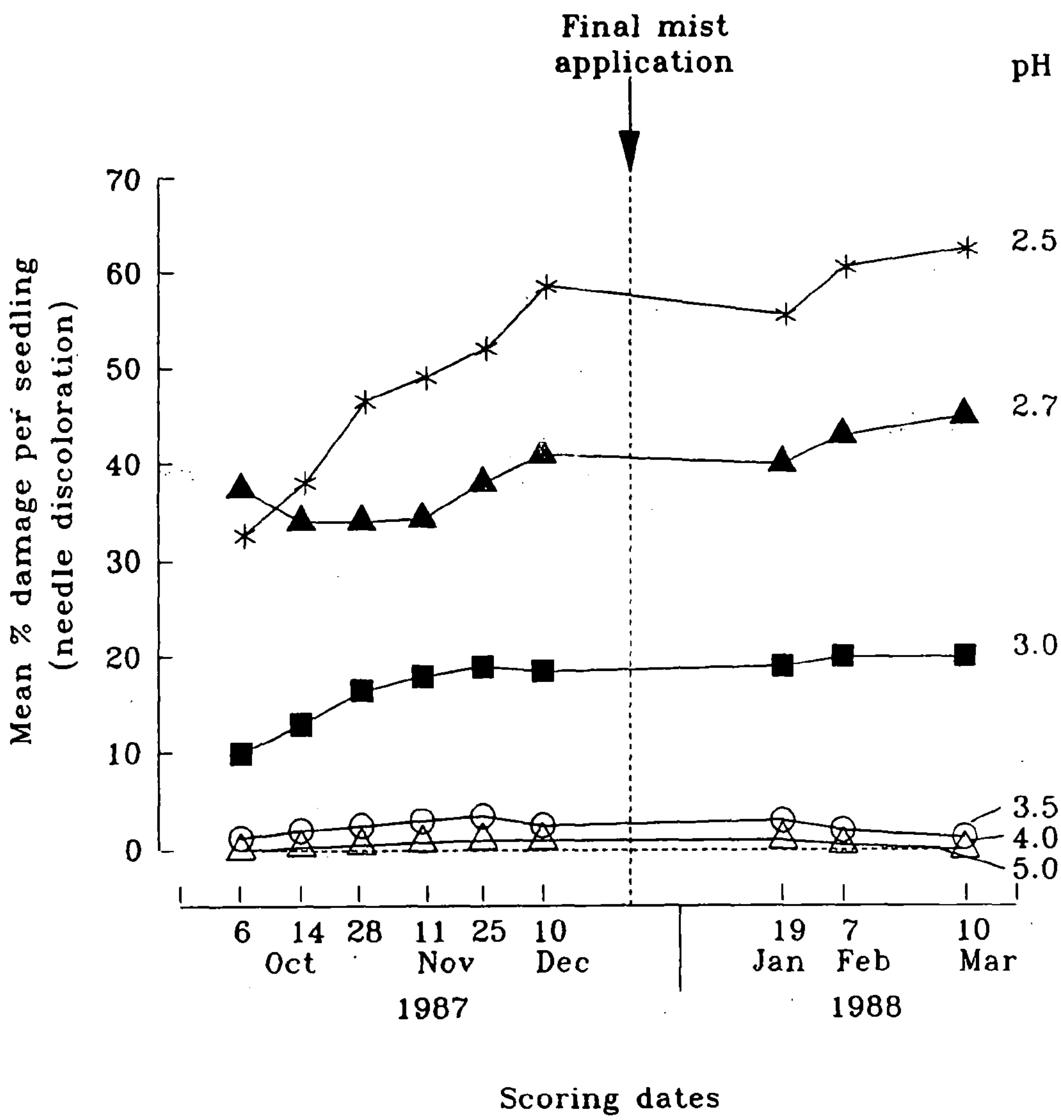


Figure 11: Visible foliar damage to red spruce seedlings exposed to twice-weekly acidic mists. Treatments began July 24; no treatment showed any damage prior to October 6. Symbols represent means; there was considerable variation across seedlings in amount of damage (from Leith et al. [SF14])

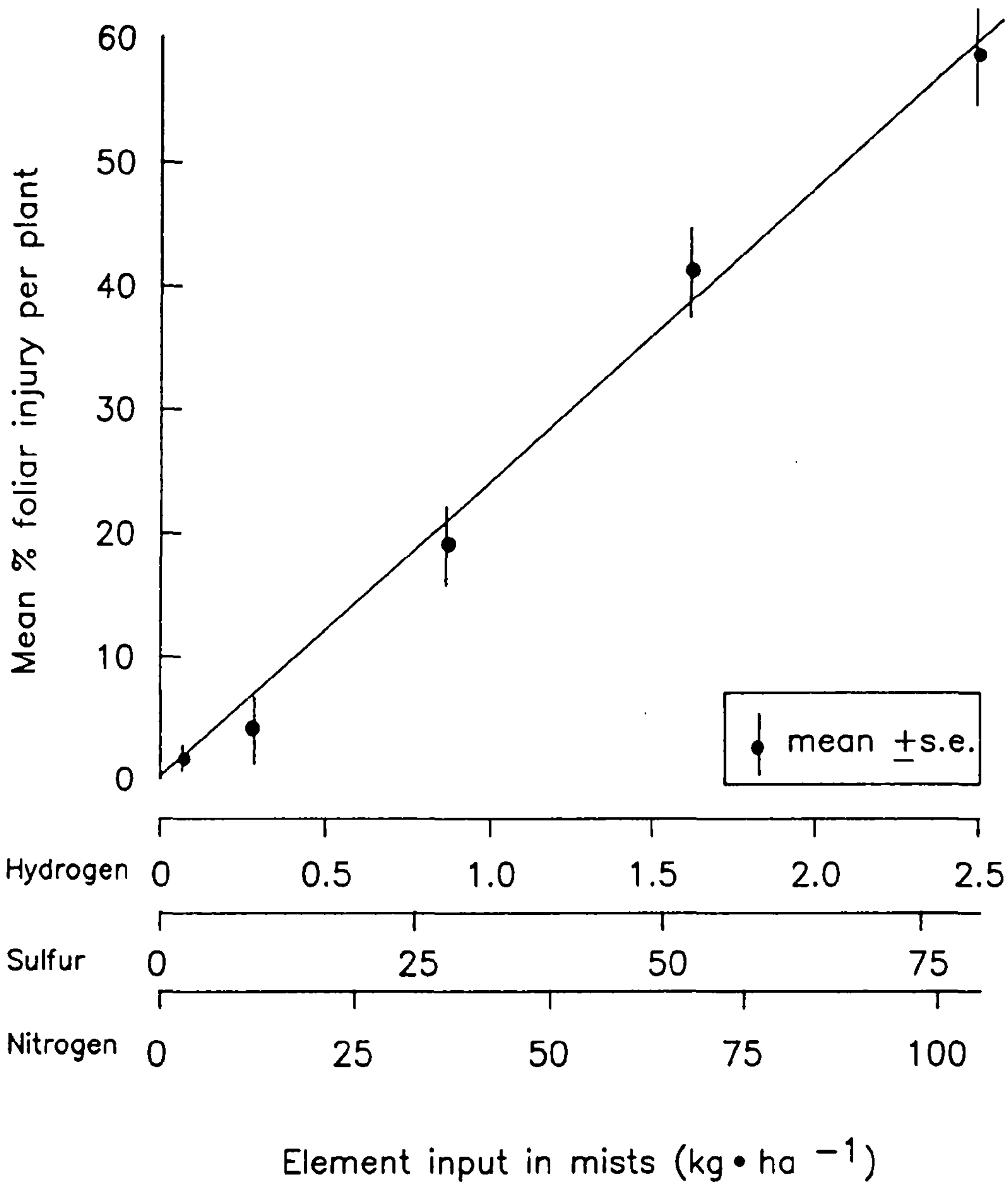


Figure 12: Linear dose-response relationships between visible foliar injury of red spruce and cumulative inputs of hydrogen as H^+, sulfur as SO_4^{2-}, or nitrogen as NO_3^- plus NH_4^+, comprising the acid mists applied to the seedlings (redrawn from Leith et al. [SF14]).

with 20% to 100% greater injury than nitrate acids at both pH levels ($p = 0.10$). In other trials, intermittent mists were as potentially harmful as continuous mists even though intermittent mists deposited less total acids on the foliage. Winds between mists enhanced foliar injury, especially with high sulfate mists. Responses of current-year needles to acidic mist depended somewhat on seed source.

Patton et al. [SF07] exposed red spruce seedlings to ozone (50, 100, 150 ppb) and acid precipitation (pH 3.0 and 4.2) for six months. They examined foliar injury (chlorosis and necrosis) under water-stressed and well-watered conditions (one versus three waterings per week). No clear effects of ozone were apparent in any treatment combination. No effects of acidity were observed under well-watered conditions; generally, more than 90% of these seedlings were classified as 0% foliar injured. Water-stressed seedlings showed more foliar injury than well-watered seedlings, and pH 3.0 treatments appeared to ameliorate the injury somewhat compared to pH 4.2. Averaged across pH, from 22% to 47% of the water-stressed seedlings were classified as 1% to 25% foliar injured. Patton et al. suggested that increased nitrogen in the pH 3.0 treatments may have reduced the foliar injury due to water stress.

To summarize, visible injury to foliage of red spruce seedlings began to occur in these studies at about pH 3.0. Injury increased with increasing acidity, with increasing acid sulfur content versus nitrogen content, and with increasing opportunity for acid precipitation to dry on the foliage. Only one study examined foliar injury due to ozone; no damage was observed.

5.1.2 Growth Changes

Laurence et al. [SF31] examined the effects of acid (pH 3.1, 4.1, and 5.1), ozone (0.5, 1.0, 1.5, and 2.0 times ambient concentrations at Ithaca, NY; ambient = 38 ppb, 12-hr average), and their combination on growth (height, roots, needles, and stems) of red spruce seedlings. During three months of exposure in 1987, few effects were found (alpha = 0.10). In some cases, interactions between main effects and time of harvests were observed. However, the response curves were relatively flat, and Laurence et al. believed such interactions were of little importance. The data showed marginal linear increases in stem dry mass (14%) and root dry mass (16%) with increasing ozone levels when averaged across the pH treatments ($p = 0.14$ and 0.11, respectively).

As part of Laurence et al.'s study, a second year of exposures of the same seedlings during 1988 is reported by Kohut et al. [SF31]. Interactions of ozone and acid were observed ($p < 0.10$). The interactions varied; they generally consisted of a decline in dry mass of needles and stems with increasing ozone in the pH 5.1 treatment, little or no effect of ozone in the pH 4.1 treatment, and an increase in dry mass with increasing ozone in the pH 3.1 treatment. An exception to this pattern was that the combination of pH 3.1 and 1.5 times ambient ozone resulted in a large depression in dry mass.

Patton and Jensen [SF07] observed few effects of simulated acid rain (pH 3.5, 4.0, and 4.5) or ozone (charcoal-filtered air, alone or with additions of ozone up to 150 ppb) on growth of red spruce seedlings after six months of exposure in 1987. Effects of pH and ozone were detected on mass of current-year stems only at an intermediate harvest ($p \leq 0.04$). The authors reported no interactions. Patton et al. [SF07] followed up on these results with a two-year study of the effects of simulated acid rain and ozone on growth. In the first year (1988), they observed many effects of acid precipitation on growth. For instance, when data were summed across ozone and water-stress treatments, pH 3.0 reduced several components of growth when compared with pH 4.2 (e.g., stem length, needle weight, root weight). These decreases averaged 33% (p typically ≤ 0.05). There were few effects of ozone reported (exceptions were roots, $p = 0.10$, and stem diameter, $p = 0.03$), and those that did occur are difficult to interpret. Treatment means for roots exposed to ozone were not reported. The two higher ozone exposures (100 and 150 ppb), averaged across the pH treatments, resulted in 11% to 18% greater stem diameters compared with the lower ozone treatment (50 ppb); however, increases were greater in the 100 ppb than in the 150 ppb exposures. There were some pH x water-stress interactions ($p \leq 0.008$). At pH 4.2, seedlings that were watered three times per week grew more than those watered once per week, but the reverse was true at pH 3.0. The reason for this effect was unclear.

Alscher et al. [SF16] and Cumming et al. [SF16] observed no effect of ozone (up to 4 times ambient concentrations at Ithaca, NY; ambient = 40 ppb, 7-hr average) on growth of red spruce seedlings after either seven months or after 16 weeks of exposure.

Jacobson et al. [SF06(a)] observed various effects of acid mists (pH 2.5 to 4.5) on red spruce seedling growth following exposures ranging from six weeks to five months. In a six-week greenhouse trial, compared with no mist, acid mists at pH 2.6 had no effect on stem diameter, reduced shoot lengths by 24% ($p = 0.004$), and reduced shoot volumes by 31% ($p = 0.03$). In another greenhouse trial, pH 3.0 and 4.0 mists led to small increases ($\leq$ 7%) on shoot length compared with no mist ($p = 0.08$). Shoot length was also affected by anion type; compared with no mist, sulfate reduced shoot length while nitrate appeared to increase shoot length ($p \leq 0.007$). Acidity or anion type had no effect on growth in field studies, however.

Seiler et al. [SF13] report that acid precipitation of pH 3.0 caused no apparent effect on either shoot or root weight when compared with pH 5.6 during 10-week exposures of four-year old Fraser fir seedlings (alpha = 0.05). In a smaller subset of the experiment, pH 3.0 treatments increased root surface area by 15% compared with pH 5.6 (not significant at alpha = 0.05) and by 24% compared with pH 4.3 ($p \leq 0.05$). Tseng et al. [SF13] observed no effects of ozone (< 20, 50, and 100 ppb) on growth of Fraser fir seedlings after 10 weeks of exposure; nor were ozone x water-stress interactions observed (alpha = 0.05). Seiler and Chevone [SF13] found no effect of ozone on needle water potential, osmotic potential, or turgor potential of these seedlings, indicating that ozone did not impair Fraser fir's ability to move water into its cells and maintain adequate turgor (alpha = 0.05).

In sum, effects of acidity and ozone on growth of red spruce and Fraser fir seedlings were variable, with most studies showing no effect. Associated with increased acidity are two examples of increased growth (roots in Seiler et al. [SF13], and stems in Jacobson et al. [SF06]), two examples of decreased growth (several measures in Patton et al. [SF07], shoots in Jacobson et al. [SF06]), and five examples of no effect (Laurence et al. [SF31], Kohut et al. [SF31], Patton and Jensen [SF07], field studies in Jacobson et al. [SF06], and Seiler et al. [SF13]). Associated with increased ozone are two examples of marginally increased growth (roots and stems in Laurence et al. [SF31], stems in Patton et al. [SF07]), and two examples of no effect (Cumming et al. and Alscher et al. [SF16], and Seiler et al. [SF13]). Work of Laurence et al. and Kohut et al. [SF31] suggested an interaction between ozone and acidity. At this point the nature of growth effects of acidity and ozone can only be described as variable.

5.1.3 Mechanism: Foliar Leaching

In five experiments performed over a three-year period, Jacobson et al. [SF06(b)] found that, compared with no mist, repeated long-term exposures of red spruce seedlings to sulfate mist increased foliar sulfur by as much as 117%, and exposure to nitrate increased foliar nitrogen content by as much as 10% when exposures were intermittent with repeated opportunities for drying of liquid on the foliage. They also found that, compared with no mists, acidic mists decreased foliar potassium, calcium, and magnesium content by 20% to 30%.

Patton et al. [SF07] found that, compared with 50 ppb ozone, 150 ppb ozone caused throughfall concentrations from red spruce seedlings to be elevated by 80% for magnesium ($p \leq 0.001$) and 20% for manganese ($p = 0.107$). No difference was observed for potassium or calcium. Increases in the micronutrient boron and the element sodium were also measured. Patton et al. suggested membrane damage as a mechanism for increased leaching of magnesium and manganese but they do not discuss the absence of increases in calcium and potassium. Throughfall concentrations of iron and manganese were higher with precipitation of pH 3.0, while highest levels of potassium and boron occurred with pH 4.2.

5.1.4 Mechanism: Carbon Allocation

Laurence et al. [SF31] report no effects of acid (pH 3.1, 4.1, and 5.1) or ozone (0.5, 1, 1.5, and 2 times ambient concentrations), alone or in combination, on photosynthesis of red spruce seedlings following three-month exposures in 1987 (alpha = 0.05). However, photosynthesis rate decreased by 11% in the two highest ozone levels compared with the two lowest ozone levels. In 1988, Kohut et al. [SF31] observed that rates of photosynthesis in the same seedlings increased 17% at pH 3.1 precipitation treatments when compared with pH 5.1 ($p \leq 0.01$) and that ozone had no effect on photosynthesis rates. The higher rates of photosynthesis at higher acidity were not reflected in greater growth, however. A third year of exposures is planned.

Patton and Jensen [SF07] found few effects of simulated acid rain (pH 3.5, 4.0, and 4.5) or ozone (charcoal-filtered air, either alone or with additions of ozone up to 150 ppb) on carbon allocation in red spruce seedlings following six-month exposures. Twelve parameters of nonstructural carbohydrates in needles and roots were examined. Ozone was associated with increases in levels of starch in the current-year needles when measured during mid-treatment ($p = 0.024$), but the effect disappeared with continued treatments ($p = 0.980$).

Thornton et al. [SF27] observed some enhancement of photosynthesis in commercially grown red spruce seedlings following either exclusion of clouds but not ambient ozone or exclusion of both clouds and ozone on top of Whitetop Mountain, VA (Figure 13). Native stock seedlings did not appear to respond in a similar way. Statistical analyses have not yet been performed.

Fincher et al. [SF16] observed effects of ozone (charcoal-filtered air and 1, 2, and 3 times ambient concentrations at Ithaca, NY) on mesophyll cells of red spruce seedlings following seven-month exposures during the summer and fall of 1987. In November and December, 1987, after the onset of frosts, mesophyll cell damage was 2.0 to 4.8 times greater in the ozone treatments compared with charcoal-filtered air as shown in Figure 14 ($p = 0.017$). High cell-to-cell variation in damage was observed, and no visible signs of foliar damage were evident at the time of data collection. There was no evidence that photosynthetic rates, chlorophyll content, or carotenoid contents were different among ozone treatments. However, by May following overwintering with no additional ozone treatments, seedlings exposed to ozone did show 5% to 50% reductions of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) when compared with charcoal-filtered air. The observations of visual damage in spring did not correlate with the prior mesophyll damage (see Fincher et al. in Section 5.1.5).

Cumming et al. [SF16], in combination with the work of Fincher et al. [SF16], reported several trends due to ozone in red spruce seedlings during exposures in 1986. Compared with charcoal-filtered air, ozone at 2 times ambient resulted in 12% decreases in total chlorophyll concentrations ($p \leq 0.05$), 20% increases in the rate of photosynthesis (expressed on per mg chlorophyll basis, $p \leq 0.05$), but no changes in the rate of respiration or electron transport (alpha = 0.05). The effects of ozone appeared to be a function of season; while there were no differences in photosynthesis in July, seedlings exposed to charcoal-filtered air showed decreased photosynthesis in October, while those exposed to all levels of ozone did not ($p \leq 0.05$). Photosynthesis normally decreases in the fall as frost hardening occurs. Therefore, ozone could have a negative effect on seedlings by causing photosynthesis to be maintained at pre-hardening levels. A

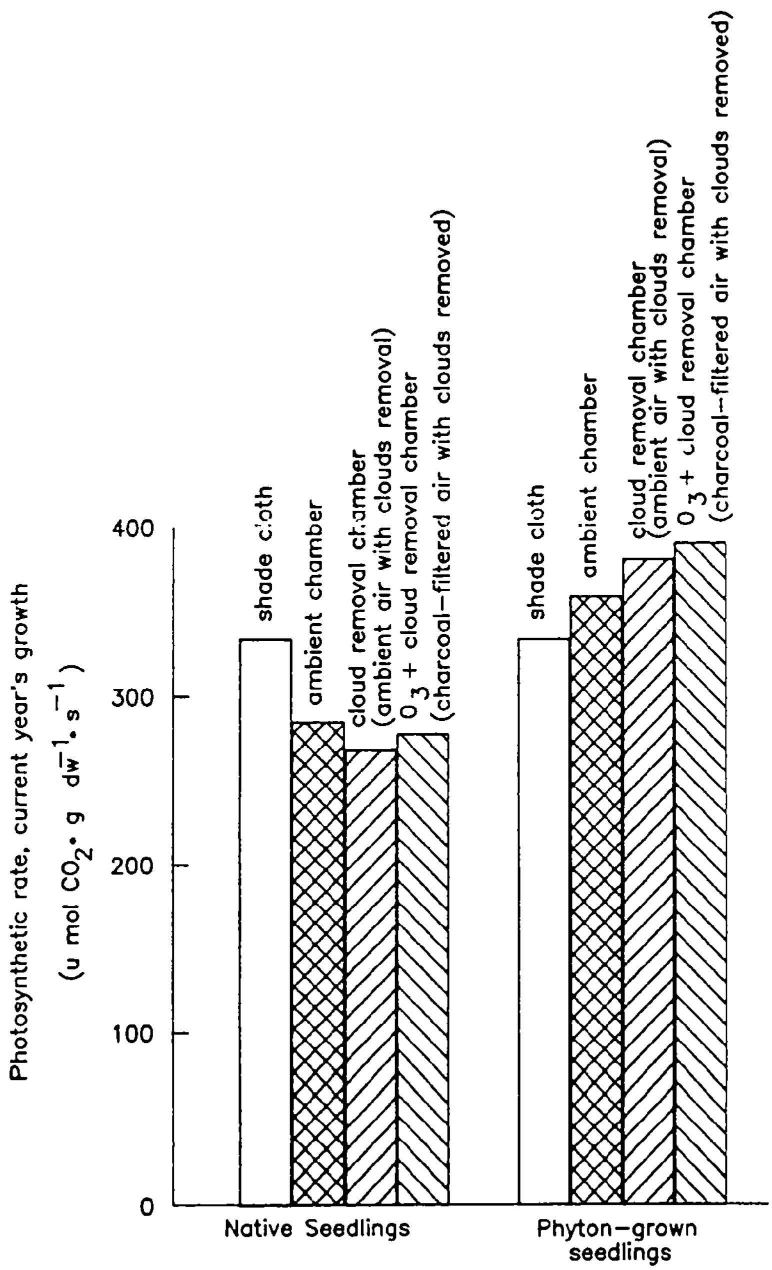


Figure 13: Effects of exclusion of ozone only or ozone and clouds on photosynthesis of red spruce seedlings after being placed in the chamber (from Thornton et al. [SF27]). Chamber effects are also shown as the difference between shade and ambient chamber.

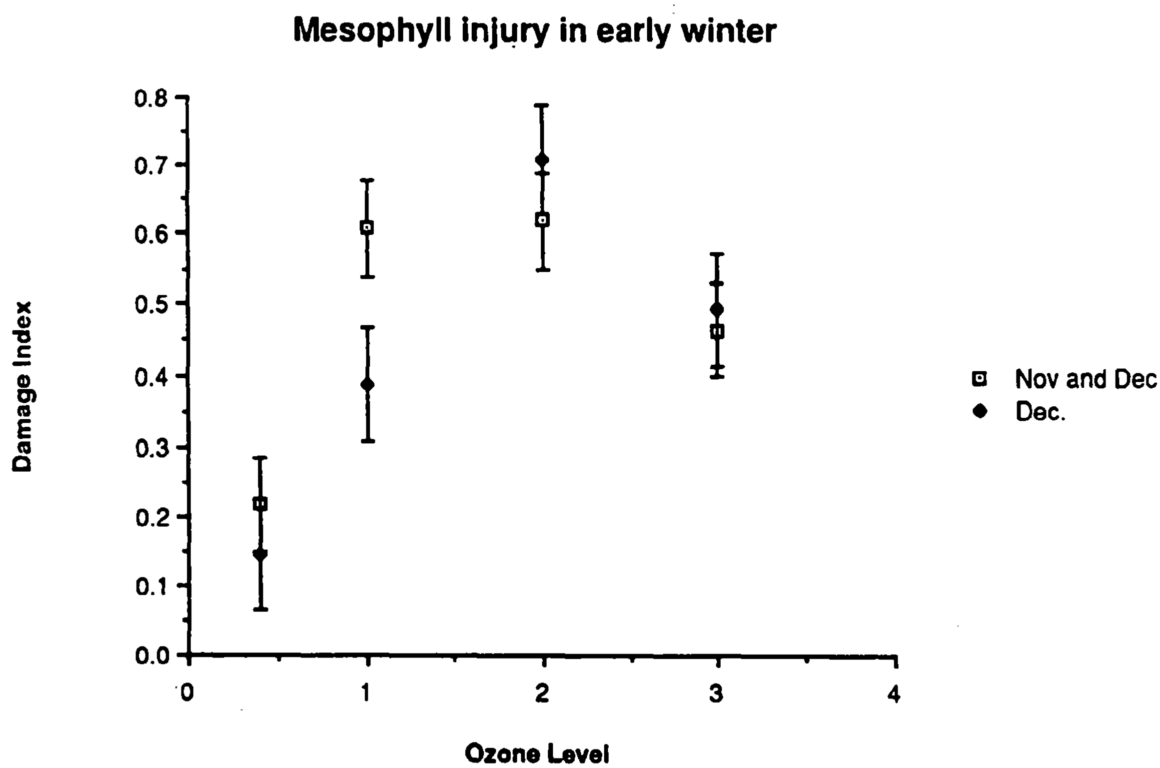


Figure 14: Mesophyll cell damage in red spruce seedlings observed in December following exposure to ozone for seven months (from Fincher et al. [SF16]).

reduction in densely-staining material was also noted in microscopic examinations of tissues from ozone treatments; this is suggestive of reduction in the rate of frost hardening.

Tseng et al. [SF13] measured no effects of ozone (< 20, 50, 100 ppb) on transpiration or needle conductance of Fraser fir seedlings (alpha = 0.05). Photosynthesis rates decreased in all treatments during the experiment's duration, possibly due to needle aging or handling. The decrease in photosynthesis was 30% to 100% greater in the highest ozone treatment at the fifth week than in the lower treatments ($p \leq 0.05$). Although the highest ozone treatment was associated with a nearly 100% greater decrease in photosynthetic rates at the tenth week, differences were not significant, implying a great amount of variability in the results. There were no significant interactions of ozone with moisture stress.

Overall, there appears to have been little effect of acidity on physiological processes associated with carbon allocation. Ozone may have caused alterations in seasonal patterns of photosynthesis (Cumming et al. [SF16]), damage to mesophyll cells (Fincher et al. [SF16]), and depressions of photosynthetic pigments (Fincher et al. [SF16], Cumming et al. [SF16]). However, there is no evidence that ozone caused changes in rates of photosynthesis (Kohut et al. [SF31], Thornton et al. [SF27], Fincher et al. [SF16], Tseng et al. [SF27]).

5.1.5 Mechanism: Winter Injury

Fincher et al. [SF16] assessed the effects of ozone expressed as winter injury. They classified seedlings for visible injury in spring after summer and fall exposures to ozone as high as three times ambient at Ithaca, NY (ambient = 40 ppb, 7-hr average) followed by exposure to winter conditions. They found no effect of ozone on either total seedling condition or the number of shoots with brown needles among 85 seedlings examined ($p = 0.93$ and 0.87, respectively). Half of the seedlings did show some browning, and among these seedlings there was an effect of ozone ($p = 0.005$). However, the effect was not consistent; compared with charcoal-filtered air, intermediate ozone levels were associated with increased browning while the highest ozone level was associated with reduced browning. It should be noted that the same pattern was observed with mesophyll damage (see Figure 14).

Murray et al. [SF14] found increases in the rate of foliar leaching from red spruce seedlings at successively colder exposures below freezing (Figure 15). Analysis of variance indicated two different leaching rates ($p \leq 0.05$). The lower leaching rate was produced by shoots exposed to higher temperatures. These shoots were regarded as still living. The higher leaching rate was produced by shoots exposed to lower temperatures. These shoots were regarded as dead. To separate live from dead tissue, the authors derived a critical leaching rate of 0.4% h^{-1} (expressed as a percent of that amount leached when the shoots were autoclaved).

Fowler et al. [SF14], building on Murray et al.'s work [SF14], also found that foliar tissues of red spruce seedlings exposed to increasingly colder temperatures resulted in increased foliar leaching rates. Leaching rate corresponded with visual damage, and a temperature that resulted in a leaching rate corresponding to more than 50% needle death was established. The temperature that produced this critical leaching rate was designated as an LT_{50}. LT_{50}s decreased over all treatments with time as the seedlings developed frost hardiness during the fall months. However, lower pH mists applied over a 22-week interval elevated the LT_{50} by as much as 12°C or, alternatively, delayed frost hardening by two to four weeks (Figure 16).

These studies indicate that acidity increased potential for winter injury, particularly during the frost hardening periods. The mechanism of action appears to have been cellular disruptions caused by freezing resulting in excess foliar leaching.

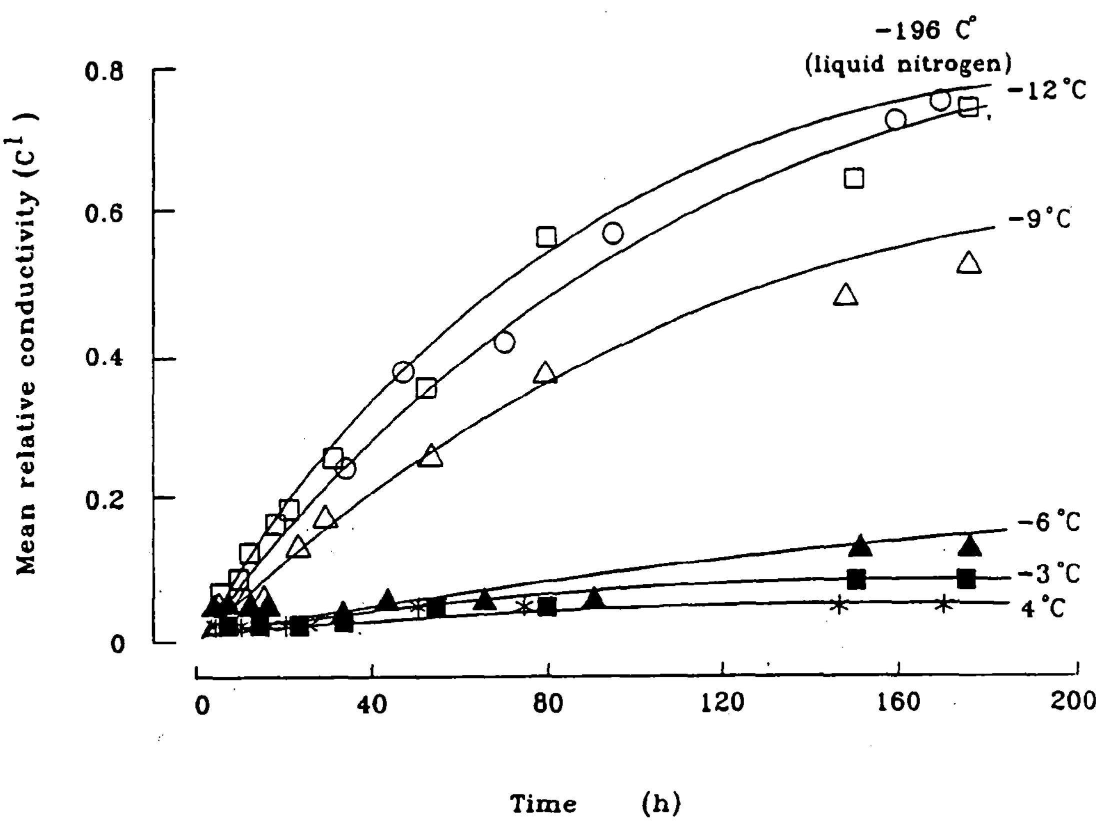


Figure 15: Increases in conductivity over time (leaching rate) of red spruce seedling foliage exposed to varying temperatures (from Murray et al. [SF14]).

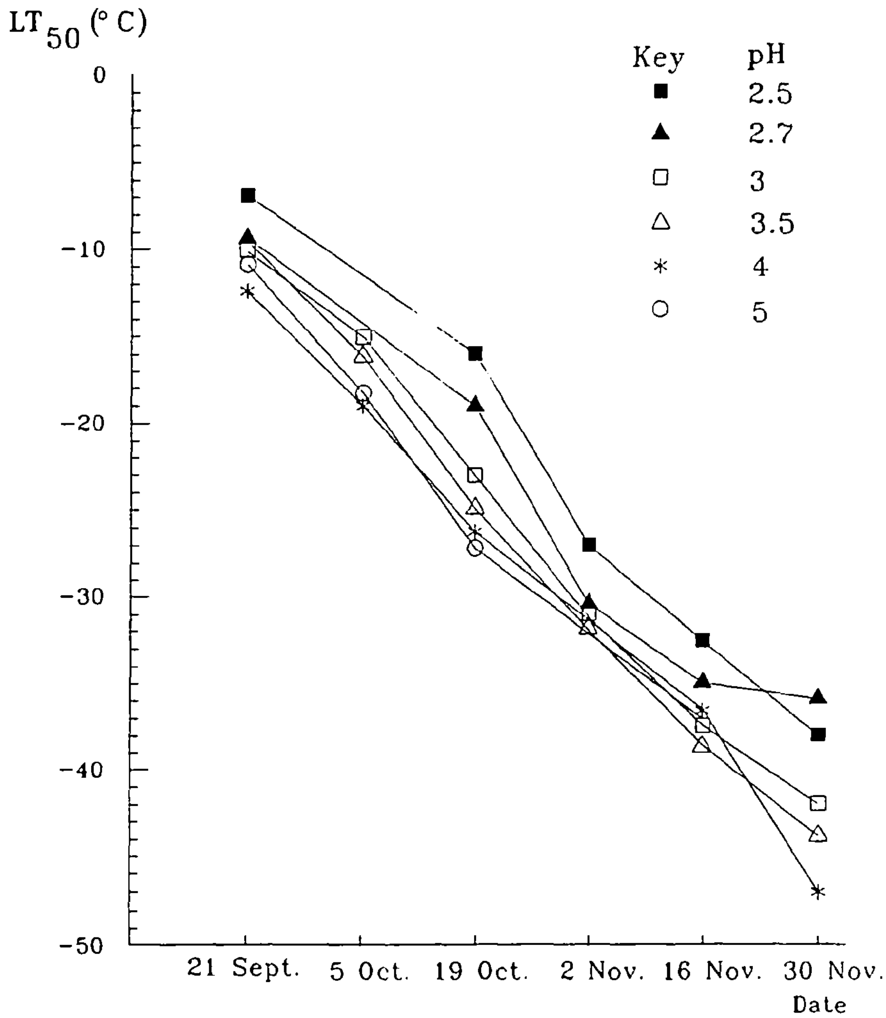


Figure 16: Elevated LT_{50}s (lethal temperature affecting 50% of the shoots tested) of red spruce seedling foliage following exposure to acid mist beginning July 24 (from Fowler et al. [SF14]).

5.1.6 Summary

While there is some evidence that acidity and ozone are deleterious to red spruce seedlings, the results are inconclusive at this time. The variations in seedling response across studies, and in some cases within studies, leads to the conclusion that there were sources of uncontrolled variation in some of the studies.

Controlled exposures of generally one-season duration did not decisively demonstrate growth depressions due to acidity and ozone. Visible injury due to acid did begin to occur at pH levels about 3.0 and lower. Acid and ozone may induce foliar leaching. There is evidence that ozone may damage foliar mesophyll cells and thus disrupt photosynthesis. However, consistent depressions in photosynthesis due to ozone were not evident across the studies. The most conclusive evidence for potential damage to red spruce is the reduction of frost hardiness due to acid precipitation.

5.2 Eastern Hardwoods

Two reports from two projects are reported here [EH01, EH06]. Twelve species of hardwoods were examined for visible injury and growth changes due to acid deposition and ozone. Although sulfur dioxide exposures were planned, experimentation problems precluded a clear study.

5.2.1 Visible Injury

Jensen and Dochinger [EH06] measured stipple injury and defoliation in response to 16-week exposures to all combinations of acid precipitation (pH 3.0, 3.5, and 4.2), ozone (0, 70, and 150 ppb), and sulfur dioxide (0 and 20 ppb). There were no effects of sulfur dioxide or acidity on stipple injury or defoliation of any of 10 hardwood species tested. No stipple injury due to ozone was observed on white oak, shagbark hickory, American beech, sugar maple, and European beech. The five remaining species (yellow-poplar, yellow birch, sweetgum, red maple, and white ash) showed an increase in stipple injury with increasing ozone. The five species also showed defoliation in response to ozone; yellow birch was the most sensitive, losing 30% of the total leaves.

Davis and Skelly [EH01] observed no consistent effects of 12 weekly applications of acid precipitation (pH 3.0 compared to 4.2) on foliar response variables of hardwood seedlings, either alone or in combination with ozone. However, ozone (75 or 150 ppb, 2 days/week) was associated with several types of foliar injury. Adaxial stipple was observed on the older leaves, and most predominantly on black cherry, followed by sweetgum, yellow-poplar, white ash, red maple and yellow birch. No stippling occurred on leaves of either red oak or white oak. Chlorosis occurred in some degree on all species, but was most common on red maple, red oak, and white oak. Necrosis was most common on black cherry and yellow-poplar, while fleck was seen on white ash.

5.2.2 Growth Changes

Jensen and Dochinger [EH06] measured height, leaf dry weight, leaf area, and new-growth dry weight at the end of 16-week exposures to the combinations of acid precipitation, ozone, and sulfur dioxide described above. Because of experimentation problems, the effect of sulfur dioxide on all species was inconclusive. No significant growth changes were observed in response to acidity, ozone, or sulfur dioxide for white oak, shagbark hickory, American beech, and European beech. For the remaining five species, (yellow birch, sweetgum, sugar maple, red maple, and white ash) varying pH had a greater effect on the growth variables than did ozone. However, depending upon the species and variable considered, the response to acidity was sometimes inhibitory and sometimes stimulatory. White ash showed decreases of 6% to 20% in the four growth measures. Red maple, yellow-poplar, sweetgum, and yellow birch showed increases in most growth measures, typically ranging from 3% to 20%, in response to increased acidity. Increased ozone was associated with decreases in some growth measures for red maple, yellow-poplar, white ash, and yellow birch.

Davis and Skelly [EH01] report that, of eight species tested, only black cherry seedlings exhibited a consistent decrease in growth variables in response to increased acidity (pH 3.0 versus pH 4.2). Ozone (75 and 150 ppb, 2 days/week) was associated with suppressed growth measures for black cherry, red maple, yellow birch, and possibly white oak seedlings compared with charcoal-filtered air. Ozone appeared to have no effect on growth measures for yellow-poplar, white ash, or red oak seedlings. Growth of sweetgum seedlings appeared to have been stimulated by ozone. Ozone and acidity did not appear to have interactive effects on growth of hardwood seedlings.

5.2.3 Species Sensitivity

Jensen and Dochinger [EH06] found white oak, shagbark hickory, American beech, and European beech to be tolerant to acid precipitation, ozone, and sulfur dioxide. Although sugar maple had decreased new-growth dry weight with increasing acidity, it exhibited no foliar injury. The species showing foliar injury and/or growth responses to acidity or ozone included the remainder: white ash, yellow birch, sweetgum, red maple, yellow-pop[illegible] [illegible]d sugar maple.

Davis and Skelly [EH01] found black cherry to be the most sensitive species to acidity based on growth reductions and the most sensitive to ozone based upon stipple response.

5.2.4 Summary

Neither acid precipitation (pH 3.0 versus 4.2) nor sulfur dioxide was associated with any visible injury on hardwood seedlings. Ozone (75 and 150 ppb) was associated with several types of foliar injury on hardwood seedlings: defoliation, adaxial stipple, chlorosis, necrosis, and fleck. The response appeared to be species-specific; black cherry was most sensitive to ozone injury.

Growth responses were varied in response to acid precipitation; as acidity increased, white ash and black cherry showed decreases in growth measures, while red maple, yellow-poplar, sweetgum, and yellow birch showed increases in growth measures. Increasing ozone was associated with decreased growth for black cherry, red maple, yellow-poplar, white ash, and yellow birch.

5.3 Southern Commercial Pines

Reports from five projects are discussed here, based on four study sites: SC05 at North Carolina State University (NCSU), SC04 at Oak Ridge National Laboratory (ORNL), SC06 at Duke Forest (DUKE), and SC02 at Texas A & M University (TAMU). An additional study (SC07) used plant material treated under project SC06. Descriptions of these projects are in Appendix A. Approximate ambient ozone levels observed at the field sites (see Table 6) were 43 ppb and 47 ppb for DUKE (1987 and 1988, respectively) and 26 ppb for ORNL in 1986.

Most of the research on southern commercial pines has focused on the response of loblolly pine seedlings, particularly the relative responses of 100 commercially important families (in this report, all family references are to open-pollinated plant material). Evaluation of responses by different families was based on quantitative measurement of stem height, rootcollar diameter, above-ground biomass, and net photosynthetic efficiency, with biomass as the major growth variable.

In addition to results from the individual studies, Shafer et al. used a subset of data from the study sites (up to eight common families of loblolly pine) to examine both inter- and intra-site continuity of growth results. For each family, relative response models were computed so both the observed and predicted values were adjusted to predicted values at control treatment levels. One of the first questions asked was whether biomass responses of eight families to ozone were consistent among the three sites with indoor facilities (CSTRs and growth chambers at SC04, SC05, and SC02) in a way that permitted pooling of data. It was determined that the responses of the eight families were too heterogeneous to pool, leading to the conclusion that exposure/response relationships on a family basis differed among sites. Examples of the results are given in Figure 17 for relative biomass changes, indicating no close patterns of family response among the sites, except for one that the authors acknowledge could have occurred by chance alone (Family C). Given the heterogeneity of responses from the eight common families, many contrasts reported by Shafer et al. in the following discussions are qualitative, and thus no probability levels are specified.

5.3.1 Visible Injury

Unlike the other regions, the motivating factor for studying effects of air pollutants in southern pines is a suspected growth decline, without visible injury observed in the field. Therefore, any visible injury reported is discussed with other effects.

5.3.2 Growth Changes

Kress et al. [SC06] noted reductions in four growth variables of three families exposed to ozone in open-top chambers when compared with charcoal-filtered (CF) air (61 ppm-hrs) after the first year of exposure (from March to December, 1987). Averaged over the three families, seedlings exposed to non-filtered air (132 ppm-hrs, considered ambient) showed reductions from 1% to 9% in height, diameter, foliage biomass, and stem biomass. Exposure to 193 ppm-hrs (1.5 times ambient, 12 hr/day) resulted in increases of 3% to 20% in these four measures. Seedlings exposed to 288 ppm-hrs (2.25 times ambient) exhibited growth reductions ranging from 9% to 37%. In all cases, the larger changes in growth were seen for biomass. Shafer et al. [SC06] produced a linear regression for the foliage biomass data, but the regression severely underestimated the biomass of the 193 ppm-hrs treatment; a reduction of 9.5% was predicted, while the data showed a 6% increase. After the first exposure season, Schoeneberger (pers. comm., SC06) found changes in tap root biomasses of +5% to -21% (0% average) and -19% to -27% (-26% average) for respective treatments of 1.5 and 3 times ambient when compared with CF.

At the end of the second year of exposure (1988), Kress et al. [SC06] found that, compared with CF (60 ppm-hrs), non-filtered air (136 ppm-hrs) led to reductions in 1988 growth measures ranging from 14% to 32%, with total above-ground biomass and total stem biomass showing larger changes than diameter and height increments. Furthermore, all growth variables exhibited reductions ranging from 30% to 80% at 298 and 396 ppm-hrs (2.25 and 3 times ambient), respectively. However, at 205 ppm-hrs (1.5 times ambient) growth stimulations of 1% to 6% occurred compared with CF air, as well as some increases compared with 136 ppm-hrs ($p = 0.05$ for stem and diameter increment; $p = 0.10$ for total tree biomass). This finding suggests a possible charcoal-filtered versus non-filtered air protocol problem (SC06 03/15/89 quarterly report).

In comparison to CF air, McLaughlin et al. [SC04] found that for 33 of the 44 families of loblolly pine seedlings tested at ORNL, 12-week exposures to ambient levels of ozone (non-filtered air) suppressed height and diameter growth (Figure 18). They also report that height growth of seedlings was stimulated by moderate levels of ozone (ambient + 40 ppb) while diameter growth of the same seedlings was suppressed (Figure 19). At higher levels of ozone (ambient + 160 ppb)

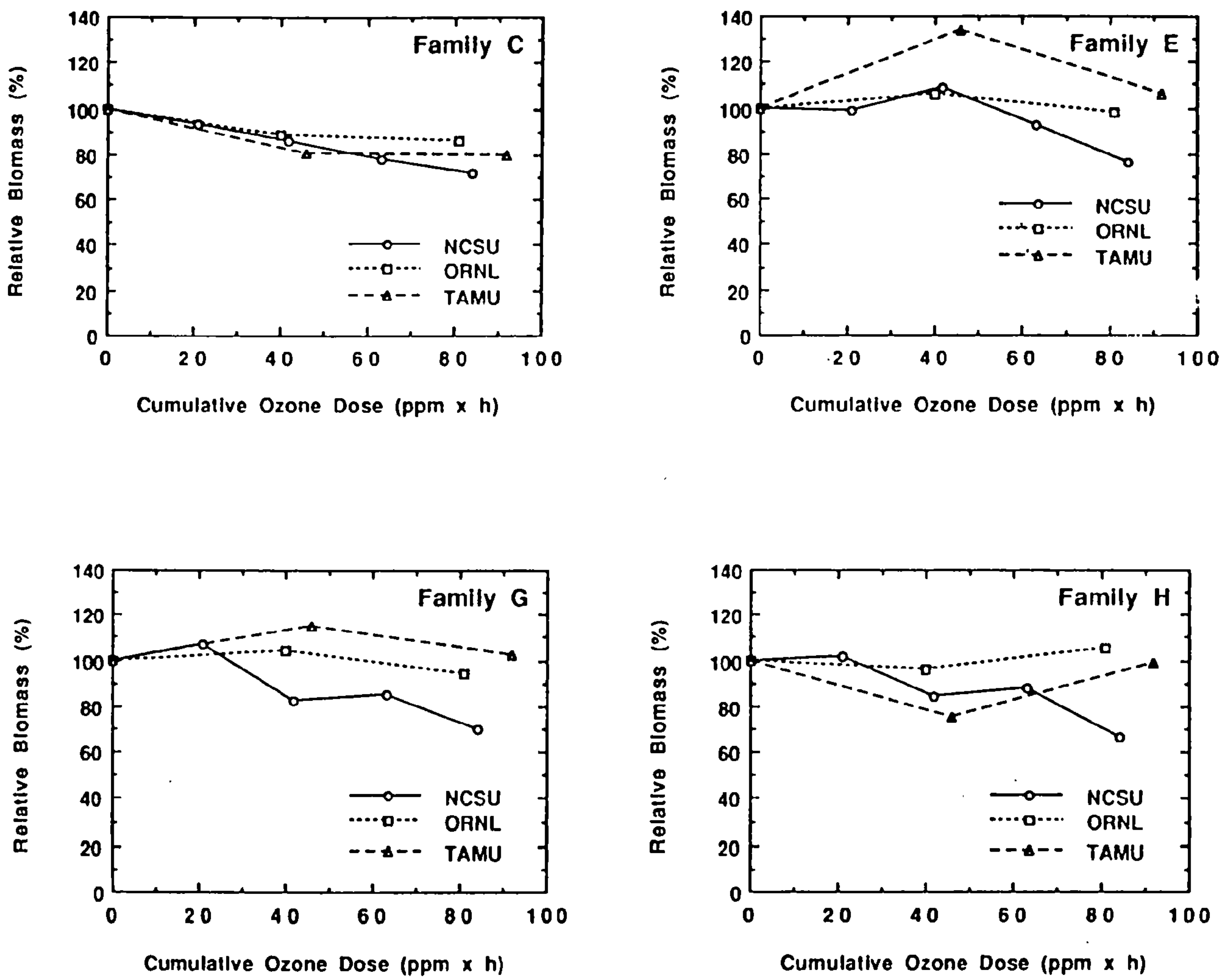


Figure 17: Relative total above-ground biomass (1986 data) of four open-pollinated families of *Pinus taeda* after exposure to ozone in controlled-environment facilities at NCSU, ORNL, or TAMU (from Shafer et al. [SC04, SC05, SC07]).

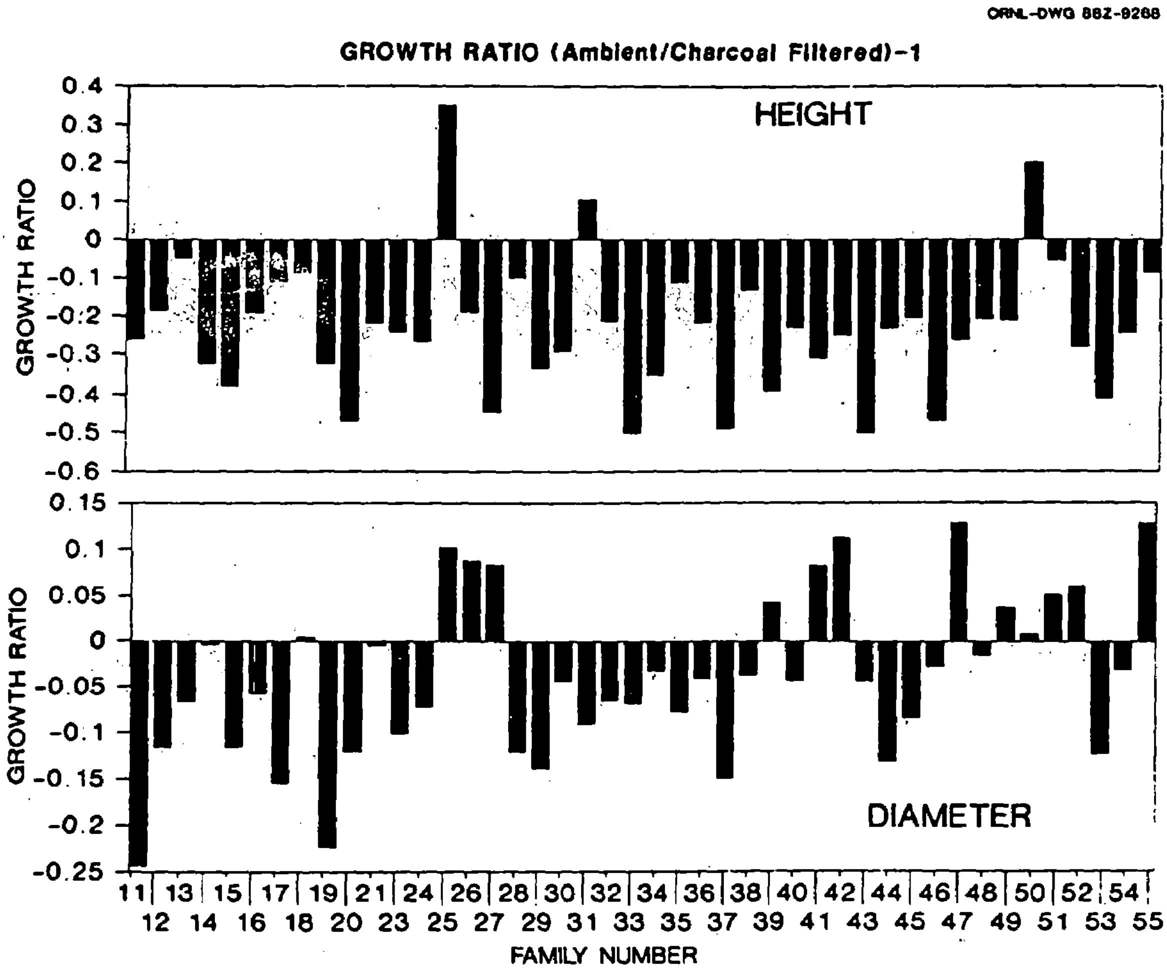


Figure 18: A comparison of 44 loblolly pine families for the ratio (minus 1.0) of their height and diameter growth in ambient ozone to that in charcoal filtered chambers (Figure 3.8 in McLaughlin et al. [SC04]).

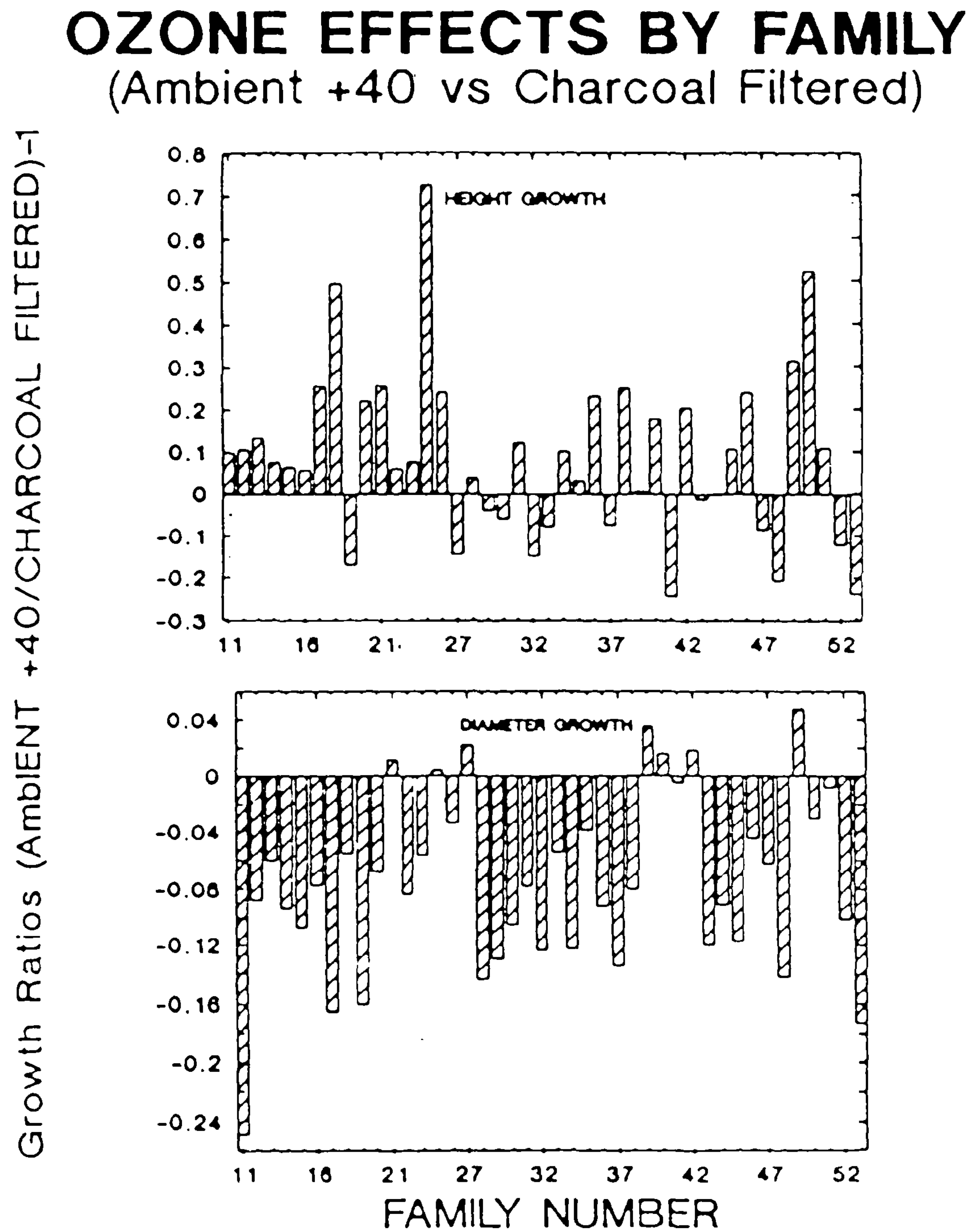


Figure 19: A comparison of 44 loblolly pine families for the ratio (minus 1.0) of their height and diameter growth in ambient +40 ppb to that in charcoal-filtered chambers (Figure 3.8 in McLaughlin et al. [SC04]).

both measures of growth were suppressed. Finally, compared to pH 5.2, height growth was stimulated at pH 4.5 and depressed at pH 3.3 (Figure 20).

Reinert et al. [SC05] report that for both the 1986 and 1987 single-season (11-week) exposures of three loblolly families to either acidity or acidity and ozone, an acidity x ozone interaction was found for diameter growth of one family. Ozone alone suppressed both height growth and diameter growth in both 1986 and 1987 ($p < 0.05$). The data means are graphically depicted in Figure 21. Shafer et al. [SC05] report that, in general, family responses at NCSU in 1986 were replicated in 1987 at the same facility. Diameter growth for 12 families was suppressed by ozone in 1986, and 11 of these 12 showed similar suppressions in 1987. In 1987, Shafer et al. [SC05] also examined repeatability of family responses to an ozone x rain acidity interaction found at NCSU in 1986. The general nature of the 1986 response curves was repeated; diameter growth was suppressed by ozone in the three families tested in both years. However, the effect of acidity varied by family. There was no effect for two families, and an altered response of the third due to a treatment interaction; in the absence of acidity, low levels of ozone produced a positive response and high levels of ozone produced a negative response. However, with increased acidity, the stimulatory effect of ozone disappeared.

Comparability of indoor and field results was examined by Shafer et al. using data from three common families. One comparison was of responses to a combination of ozone and acid precipitation for three families of loblolly pine in greenhouse conditions at NCSU [SC05] versus responses from an ORNL [SC04] field experiment. Biomass was unaffected by either ozone or acidity at ORNL, and although low levels of ozone at NCSU produced a positive response and high levels of ozone produced a negative response in the absence of acidity (i.e., a possible treatment interaction), the stimulatory effect of ozone disappeared with increased acidity. Overall, both types of experiments showed a pattern of negative biomass responses to ozone. At ORNL, only two of eight families tested showed the same linear ozone response surface when comparing field results (at pH 4.5 or 5.2) and indoor results (at pH 4.3) at the same site. For the NCSU-indoor and DUKE-field comparison of three families, the ozone exposure/response models were quadratic and acidity-dependent at NCSU, but linear and unaffected by acidity at DUKE.

Available analyses by Shafer et al. from studies in open-top chambers show differences among families in sensitivity to acid rain and ozone [SC04, SC06]. Responses to ozone were variable, with apparent stimulation of height but not diameter growth at the lowest ozone levels (no ozone in CSTRs and CF in field), and reduction in height and diameter with increasing ozone concentrations. Near-ambient levels of acidity in simulated acid rain stimulated height growth at below-ambient ozone levels. Overall sensitivity of seedling growth to ozone appears to be greater under field conditions than in associated laboratory studies. This greater sensitivity may be due to greater growth in the field or to higher cumulative ozone exposures due to ambient levels in the field environment. This difference might also be due to other factors such as variability in age of seedling material, exposure length, soils, or fertility regimes.

In general, decreased diameter growth with increased ozone was observed at ORNL [SC04] for the majority of 44 families of potted seedlings tested. Kress et al. [SC06] noted the same effects for three open-pollinated families, with reductions at approximately ambient exposures. Although both facilities noted apparent growth stimulations at moderate ozone levels compared with non-filtered air, the reason for the apparent stimulation is unclear.

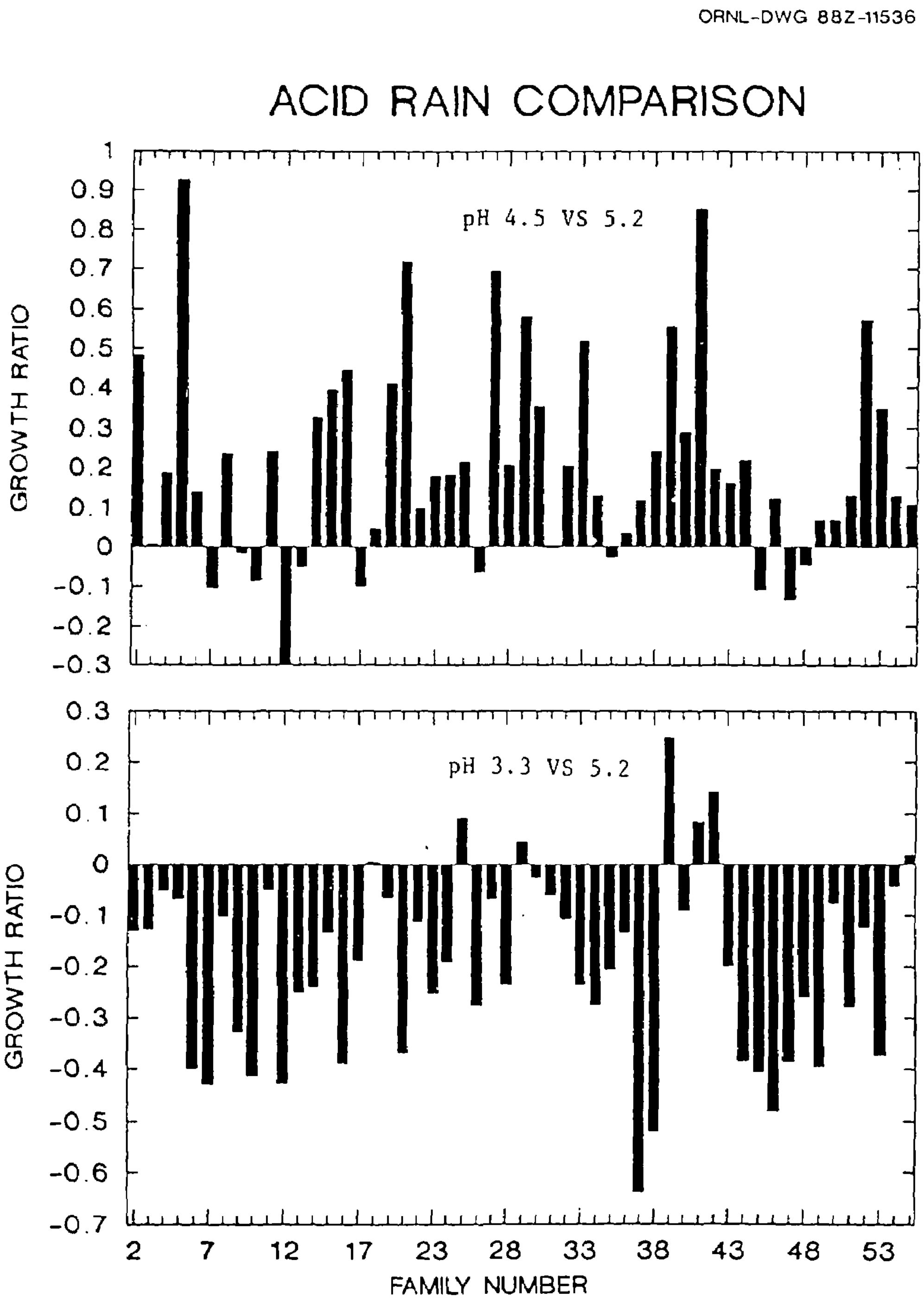


Figure 20: A comparison of 53 loblolly pine families for the ratio (minus 1.0) of their height growth at nominal pH 3.3 or pH 4.5 to that at pH 5.2. A value of 0 indicates equal growth rates, and a value of +0.20 indicates 20% better growth in the lower pH rain (Figure 3.13 in McLaughlin et al. [SC04]).

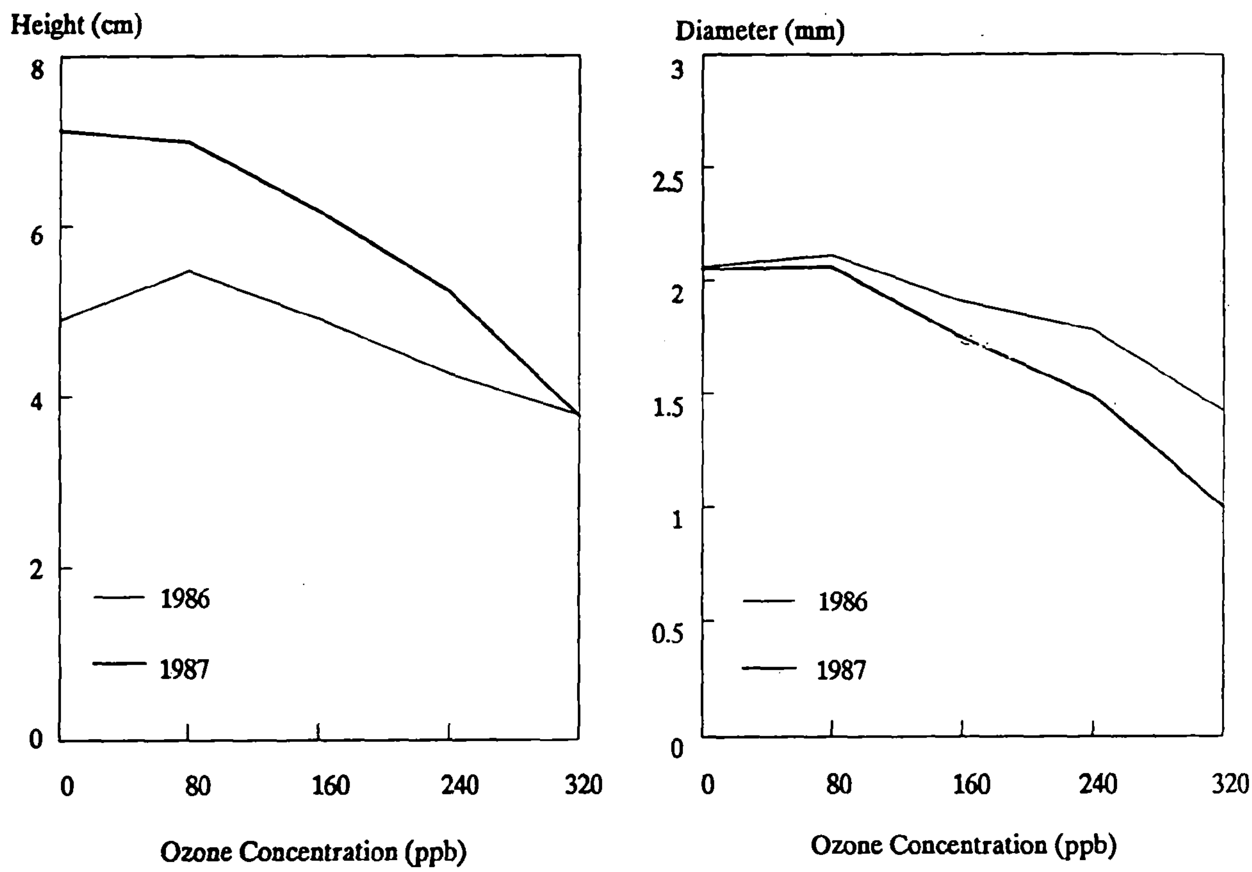


Figure 21: Final heights and diameters averaged across three loblolly pine families after 12 weeks of exposure to ozone. Two separate experiments from 1986 and 1987 are presented (from Table 4 in Reinert et al. [SC05]).

5.3.3 Family Sensitivity

McLaughlin et al. [SC04] examined variation of eight loblolly pine families in sensitivity to ozone in CSTRs and of more than 44 families to combinations of ozone and acid precipitation in open-top chambers. Ratings of sensitivity were based on changes in average height and diameter of seedlings after 12-week exposures. Responses to acid rain and ozone varied by family. Slightly more Piedmont families were responsive to acid rain treatments in both diameter growth and height growth than Coastal families (Table 7). In response to ozone, Piedmont families are also reported to show a higher percentage of changes in height growth compared to families of Coastal source (Table 7). Although these are important observations, given that height/diameter family responses were not always in the same direction (i.e., some positive and some negative), these tables require further stratification according to a known direction of response.

Reinert et al. [SC05] report on the sensitivity of 12 loblolly families exposed to ozone (ranging from 0 to 320 ppb) in two experiments conducted in 1986 and in 1987. Although the magnitude of growth changes and some rankings changed with year of exposure, the most ozone-sensitive (HS 5-56) and least ozone-sensitive (HS 1-68) families retained relative rankings. The comparison of those two families with the average of all 12 is given in Figure 22.

5.3.4 Mechanism: Carbon Allocation

Kress et al. [SC06] observed decreases in total and retained needles, and accelerated needle abscission of early cohorts of foliage on loblolly pine seedlings as levels of ozone exposure were increased from CF to 3 times ambient (Figure 23). This result suggests that accumulation of foliar injury can eventually cause premature abscission.

Richardson and Sasek [SC07] measured photosynthesis rates using an infrared gas analyzer. They found that net photosynthesis rates of loblolly pine seedlings were not affected by acid rain treatments (pH 3.5 versus 5.2). Furthermore, there were no interactive effects of acidity with ozone treatment or acidity with family. Net photosynthesis rates of loblolly pine seedlings were negatively affected, however, by cumulative ozone exposures. Compared to charcoal-filtered air, the highest ozone level (3 times ambient) was associated with 80% reductions in net photosynthetic rates after four-month exposures (Figure 24). It appears that the product of concentration and length of exposure was a good predictor of net photosynthetic response because low exposures over long periods had similar effects to high exposures over shorter periods. Thus, long-term chronic exposures to ozone appeared to have cumulative effects that resulted in reduced net photosynthetic capacity. Results further suggest that ozone decreased concentrations of chlorophyll a, chlorophyll b, and carotenoids (Richardson, pers. comm.).

McLaughlin et al. [SC04] report that more acidic treatments (i.e., pH 3.3 and 4.5 versus pH 5.2) were associated with higher photosynthesis rates (52% at 12 weeks) in loblolly pine seedlings. A fertilization effect of increasing nitrogen when increasing acidity was speculated to be a cause for the observed increases. Compared to charcoal-filtered air (14 ppb ozone), 25% reductions in photosynthetic rates were found after 12 weeks of 167-ppb ozone treatments in the field studies at ORNL. In contrast, the laboratory studies at ORNL showed no clear trends with respect to ozone treatments; ozone was associated with both increases and decreases in photosynthetic rates over time. These contrasting results between field and laboratory studies raise concern over the validity of extrapolating freely from lab studies to the field for studies on photosynthesis.

These two studies (SC04 and SC07) yielded conflicting results with respect to acid effects on photosynthesis of loblolly pine seedlings. The enhancement of photosynthesis at lower acidity (pH 3.3) observed by McLaughlin et al. [SC04] at ORNL suggests that acid treatments may stimulate growth. However, it should be noted that McLaughlin et al. [SC04] actually observed growth suppression at pH 3.3 (Figure 20). Both studies showed negative response to ozone.

Table 7. A summary of growth responses to acid rain and ozone for common loblolly pine families tested in field chambers at ORNL (from Tables 3.9 and 3.10 in McLaughlin et al. [SC04]).

		Mean growth response expressed as a percent of growth in pH 5.2 rain				Percent of families with statistically significant responses to rain pH levels	
		pH 4.5		pH 3.3		(p <= 0.10)	
Seed source	Number of families	Height	Diameter	Height	Diameter	Height	Diameter
Piedmont	25	122%	106%	79%	99%	48%	40%
Coastal	28	120	106	81	97	39	32
Common	9	120	100	77	92	33	11

		Mean growth response expressed as a percent of growth in CF air				Percent of families with statistically significant responses to ozone levels	
		Ambient		Ambient + 160 ppb		(p <= 0.10)	
Seed source	Number of families	Height	Diameter	Height	Diameter	Height	Diameter
Piedmont	25	76%	97%	78%	93%	64%	28%
Coastal	28	78	95	88	92	39	32
Common	9	-	-	93	92	11	11

Common denotes families that are also being tested at NC State and Texas A&M.

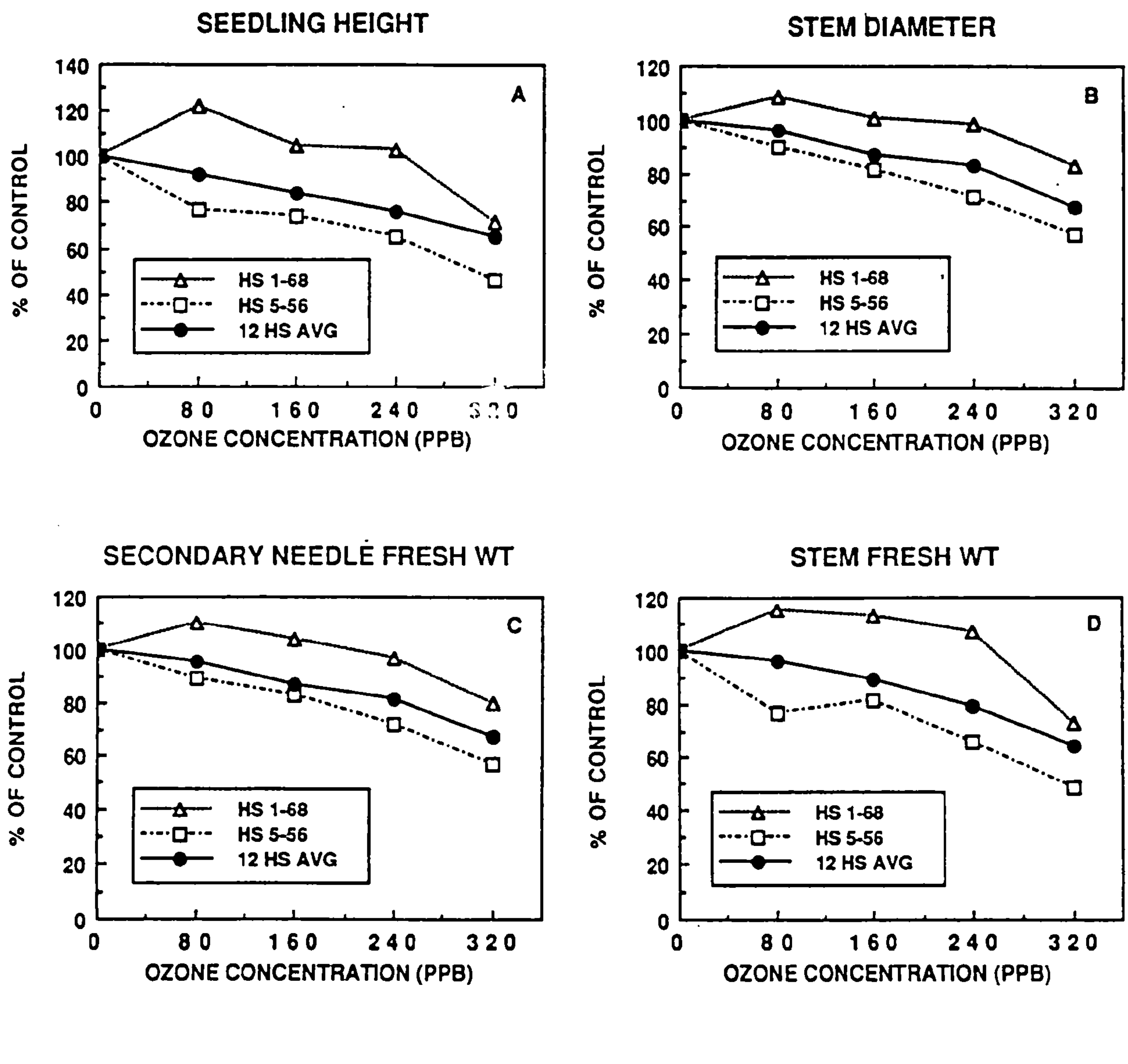


Figure 22: Average responses of loblolly pine seedlings to ozone from two separate experiments (1986 and 1987) using the same families; heavy (middle) trend in each graph represents average of 12 families. HS 1-68 and HS 5-56 were the least and most sensitive families, respectively (from Figure 1 in Reinert et al. [SC05]).

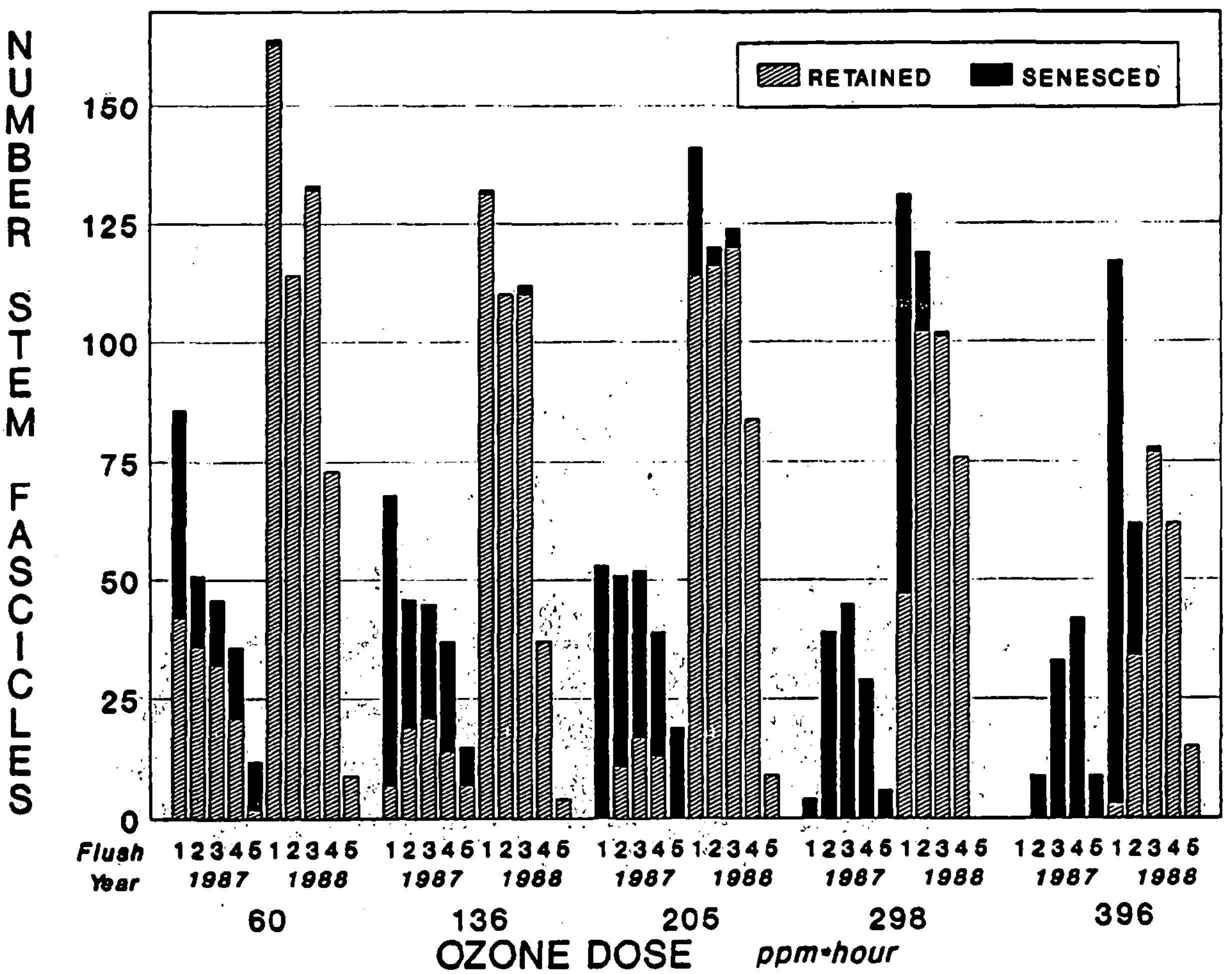


Figure 23: Needle response of loblolly pine seedlings by flush number for 1987 and 1988 as a function of ozone. Seedlings had also been exposed to similar treatments in 1987. Data for the 1987 needles reflect the status at the beginning of the 1988 exposures (from Kress, pers. comm.).

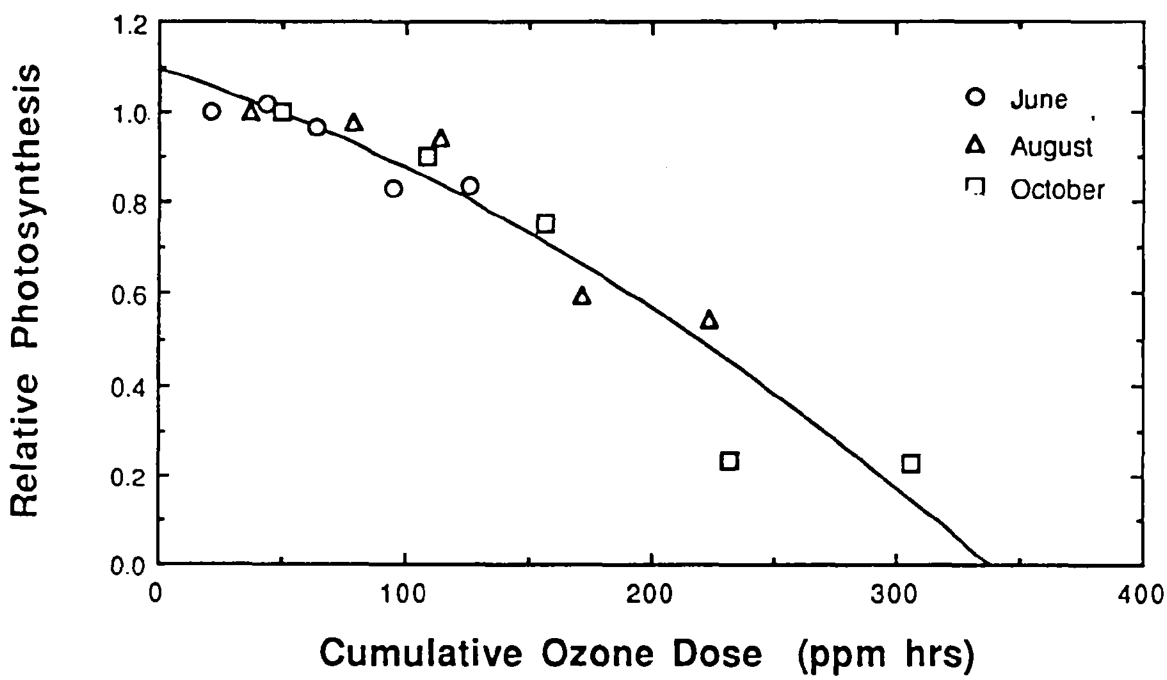


Figure 24: Photosynthesis rates relative to the charcoal-filtered (CF) treatment versus cumulative ozone dose determined at three different measurement dates during 1987. Photosynthesis is reported at each sampling date. Within a sampling date, the five data points represent (from left to right) CF, NF, 1.5, 2.25, and 3.0 x ambient ozone treatments (from Richardson and Sasek [SC07]).

ORNL [SC04] showed a positive response to acidity. DUKE [SC07] showed no response from the first year of acid treatments (1987). However, by the end of the second season of treatments, the seedlings in the pH 3.3 treatment showed greater diameter increments than those in the pH 5.3 treatment ($p = 0.05$).

In both studies, field results indicated that the highest ozone levels were associated with substantial reductions in photosynthetic rates of loblolly pine seedlings. However, short-term lab studies failed to show ozone effects. Shafer et al. [SC07] found that reductions in growth were also associated with the highest ozone levels at Richardson and Sasek's [SC07] site and with ambient levels at the McLaughlin [SC04] site (see Section 5.3.2).

5.3.5 Summary

Despite considerable differences in experimental designs and procedures, and regardless of the presence or absence of significant effects, most experiments showed patterns of either biomass or diameter growth suppressions in response to ozone treatments. For the most part, effects of acidity, effects of ozone x acidity, and shapes of exposure/response models for determining these effects differ from site to site. In general, there was no clear pattern of a direct acidity effect on either growth or photosynthesis of loblolly pine from these early short-term results.

Foliar injury from ozone, followed by premature abscission, was observed at the DUKE field site [SC07] for all ozone levels above charcoal-filtered air. Although net photosynthetic rates of loblolly seedlings were not affected by acidity, they were negatively affected by cumulative ozone exposures. The fact that height and diameter growth changes under ozone were not always in the same direction and were sometimes associated with reduced root biomass indicates that patterns of carbon allocation can change. Thus, there is the potential for long-term chronic exposures to ozone to reduce photosynthetic capacity, which, if coupled with reduced root biomass, might ultimately affect the growth and survival of seedlings.

With the exception of some preliminary information from the second year at DUKE, results on Southern pines in this report were limited to studies of one exposure season conducted in 1986 and 1987. However, several exposure studies were established in 1988 that, in addition to the ongoing work at the DUKE site, are designed to carry the same seedlings through multi-year exposures.

5.4 Western Conifers

Results from three projects are reported here [WC07, WC08, and WC09]. The latter two projects are determining the sensitivity of western conifer species to three deposition exposure scenarios likely to occur in the western United States: 1) gaseous sulfur dioxide during fall and winter; 2) acid fog during the fall and winter; and 3) acid fog during the fall and winter preceded by summer ozone exposures. In addition to immediate effects, both projects measure changes in seedling condition during the growing season following conclusion of exposure treatments (i.e., post-exposure effects).

5.4.1 Visible Injury

Miller et al. [WC09] report that Douglas-fir and Engelmann spruce did not exhibit any injury from ozone following exposures from 53 ppb to 71 ppb over three months. However, white fir, subalpine fir, and ponderosa pine showed increased injury with increased ozone levels. Visible injury on needles exposed to acid fog (pH 2.1 and 3.1 versus pH 5.6) was evident on ponderosa pine, Douglas-fir, subalpine fir, and white fir, but not Engelmann spruce.

Turner et al. [WC07] observed foliar injury (necrosis) associated with acid fog alone on western hemlock (with pH 2.1 and 3.1) and western redcedar (with pH 2.1) when compared with control fog (pH 5.6). No effects were observed in Douglas-fir or ponderosa pine. Ponderosa pine and western hemlock were the only species that showed typical chlorotic mottle and banding during the ozone exposure period. Douglas-fir and western redcedar showed no injury with ozone. In a repetition of the exposure scenario in the second year, ponderosa pine appeared again to have the greatest susceptibility to seasonal ozone and acidic fog treatments. Western redcedar appeared least susceptible to this same regime.

5.4.2 Growth Changes

Miller et al. [WC09] found that, after two months of exposure to 66 and 42 ppb of sulfur dioxide, height growth of Engelmann Spruce and white fir was increased relative to a base treatment of 21 ppb ($p < 0.006$ and $p < 0.03$, respectively). There were no effects of sulfur dioxide treatment on heights of subalpine fir, ponderosa pine, or Douglas-fir or on diameter increment for any species.

Preliminary results from ozone exposures in 1987/88 indicated that medium to high ozone concentrations (67 and 71 ppb) stimulated height growth of Douglas-fir and white fir. The height-growth stimulation from ozone continued during the acid fog treatment. With the pH 2.1 acid fog, subalpine fir also responded with increases in height growth, and diameter increases were noted for Douglas-fir, Engelmann spruce, ponderosa pine, and subalpine fir. Analysis of harvest data is in progress.

Hogsett and Tingey [WC08] examined seedling growth after one year of exposure scenarios. While all species exhibited growth over time in all measured variables, certain growth variables were altered with one or both exposure scenarios ($p < 0.10$). A repeat of the exposure scenarios in the second year (1987-1988) substantiated the previous year's pattern of species susceptibility to each of the exposure scenarios (Table 8). Results of Hogsett and Tingey's [WC08] repeated experiments are summarized below.

Under the ozone and acid fog treatment, there was a marginal reduction of diameter growth in Douglas-fir ($p = 0.08$) and ponderosa pine ($p = 0.08$). Diameter growth of ponderosa pine was progressively reduced toward the conclusion of the exposure period, resulting in a reduction the following spring. Diameter was reduced 7% and 11% from controls with the two elevated ozone regimes (67 and 71 ppb, respectively). In 1987/1988, western hemlock, lodgepole pine, and western redcedar did not show any changes in diameter or height growth rates over the course of the ozone exposures, nor did differences occur in final height measures taken at the conclusion of the second growth season. Following exposures to acid fog without ozone, final harvest showed 9% and 48% increases in stem diameter at pH 3.1 and 2.1, respectively, for Douglas-fir ($p = 0.08$), and 8% and 35% increases at pH 3.1 and 2.1, respectively, for ponderosa pine ($p = 0.07$). Stem height of ponderosa pine was increased 7% at pH 3.1 and 19% at pH 2.1 compared with the control fog of pH 5.6. Stem height of Douglas-fir was not affected.

None of the five species showed significant changes in height or diameter in response to sulfur dioxide (alpha = 0.10), although there were relatively large increases (10% to 27%) in height and diameter of western redcedar and Douglas-fir relative to the controls. The largest increase occurred at the lowest exposure level.

Bud elongation of ponderosa pine appears to have been decreased by acidic fog, depending on the level of ozone. The pH 3.1 treatment with base ozone had no effect on bud elongation, while there was a marked decrease due to pH 2.1 at the same ozone level. Both pH 2.1 and 3.1 caused a similar decrease in the rate of bud elongation with the highest ozone treatment (71 ppb, 7-hr average; 30-day sum = 34 ppm). Although there were some strong differences in bud elongation

Table 8. Summary of growth effects on western conifers exposed to three different exposure scenarios over one year (1987-88, from Table 7 in Hogsett and Tingey [WC08]).

	OZONE + ACID FOG			ACID FOG ONLY	SULFUR DIOXIDE
SPECIES	OZONE	ACID FOG	OZONE x ACID FOG		
	Final Harvest[1,2]	Final Harvest	Final Harvest	Final Harvest	Final Harvest
Ponderosa pine	sdw, rdw, 2nw, 3nw, r/s, r/n, dia	2dw, 3DW, r/s, r/n	3NW, BUD	3NW, r/s, HT, DIA	r/n
Douglas-fir	r/s, r/n, 3NW	2nw, 3NW, BUD	rdw, 3NW, r/s, r/n	SDW, 2nw, 3NW, r/n, r/s, DIA	3NW, r/s, r/n, BUD
Western hemlock	rdw	SDW, NDW, r/s, r/n	rdw, r/s, r/n, bud	NA	ndw
Western redcedar		r/s, r/n	SDW, NDW	NA	sdw, NDW, R/S
Lodgepole pine	rdw, 1nw, 2nw	SDW, 3NW, r/s, r/n	2nw, r/n	NA	rdw, 2nw

[1] Listing a variable indicates a difference between at least one of the treatment exposures and the control exposure ($p < 0.10$). Variables listed as lower case indicate that reductions were observed; variables listed in **UPPER CASE** indicate that increases were observed.

[2] bud = spring bud elongation rate or the final length of apical bud after spring elongation
dia = stem diameter
ht = stem height
ndw = needle dry weight
rdw = root dry weight
r/n = root/needle dry weight ratio
r/s = root/shoot dry weight ratio
sdw = stem dry weight
xnw = needle dry weight by age class (x=1,2 or 3)
NA = not applicable (i.e., species not included in treatment)

at final harvest, neither ozone nor acid fog effects could be detected in any species due mainly to large coefficients of variation. The largest response to any of the treatments was a 37% increase in bud elongation of western hemlock in the pH 2.1 acidic fog treatment. The largest change in bud elongation in response to ozone was a 10% increase for western hemlock; no effects of the acid fog without ozone treatment were detected for either ponderosa pine or Douglas-fir.

Douglas-fir showed respective increases in mean bud elongation of 12% and 18% at the two highest sulfur dioxide treatments (42 and 66 ppb, 60-day average; $p = 0.03$). Growth rate of western redcedar increased by more than 40% for all sulfur dioxide treatments ($p = 0.001$). With increasing sulfur dioxide, there were moderate decreases (11% to 13%) in bud elongation for Douglas-fir and lodgepole pine, and small decreases (< 10%) for ponderosa pine and western hemlock.

Compared with the other species, ponderosa pine showed the greatest vegetative biomass response to the ozone/acidic fog treatment. All tissue parts (stem, roots, and needles) were reduced. Stem dry weight was reduced by 8% to 11% with elevated ozone ($p = 0.004$). Root dry weight was reduced 5% to 26% with increasing ozone levels ($p = 0.001$). Second-year needles had 10% to 32% less biomass than controls, whereas third-year needles were increased in biomass above controls with increasing ozone.

The acidic fog effect was similar to that of ozone. Second-year needles biomass was reduced approximately 12% compared with the pH 5.6 control, while third-year needles increased 9% to 26%. Both root/shoot and root/needle ratios were reduced 10% to 24% by both ozone and acidic fog in the combined exposure scenarios. Douglas-fir, western hemlock, and lodgepole pine showed intermediate responses to this exposure scenario, while western redcedar appeared to be the least responsive species and ponderosa pine the most responsive. Both Douglas-fir and ponderosa pine showed increased biomass in response to the acidic-fog-only treatment. Stem dry weight of Douglas-fir increased 7% to 30% at pH 3.1 and pH 2.1, respectively. The current-year needles increased 50% to 117% above controls at the lower pH treatments. Root/needle and root/shoot ratios were reduced up to 27% in acid fog treatments. Ponderosa pine exhibited similar, but less dramatic, increases in growth variables. For example, needle dry weight increased 20% to 57% with exposure. Above-ground biomass appeared to increase in both species in response to the acidic fog treatments with no effect on below-ground biomass.

Most of the changes in response to sulfur dioxide were increases in above-ground biomass, although there were reductions in root biomass and root/shoot ratios for Douglas-fir, ponderosa pine and lodgepole pine. Western hemlock showed a marginal (8%) increase in needle dry weight at the second highest treatment (42 ppb, 60-day average). There were minor reductions in root dry weight of Douglas-fir (7%) as a result of exposure to sulfur dioxide. There is slight evidence that stem dry weight of western redcedar was reduced by 11% to 13% with the three sulfur dioxide treatments ($p = 0.10$).

Turner et al. [WC07] found highest foliar and root tissue dry weights with the pH 3.1 fog treatment for ponderosa pine, red cedar, and Douglas-fir. These increases were not significant, however (alpha = 0.10). Root dry weight of western hemlock was reduced at the higher acid fogs (pH 2.1 and 3.1 versus 5.6).

5.4.3 Species Sensitivity

Preliminary observations of foliar injury suggest that western hemlock and western redcedar were more sensitive to low pH fogs than were Douglas-fir and ponderosa pine (Turner et al. [WC07] and Hogsett and Tingey [WC08]). Western redcedar exhibited significant increases in many growth variables as a result of ozone/acid fog exposures, including the bud elongation the following spring. Both western hemlock and western redcedar appeared to be more responsive to acid fog at both pH 2.1 and 3.1 than any other species.

5.4.4 Mechanism: Foliar Leaching

Turner et al. [WC07] grew Douglas-fir in solution cultures whereby nutrient depletion in the solution estimated seedling uptake. They found that foliar leaching of potassium, calcium, and magnesium was greater with twice-weekly fogs of pH 3.1 than for pH 5.6. However, the rate of foliar cation leaching during applications of four-hour fogs at pH 3.1 were relatively small compared with the daily uptake rates for these seedlings. Furthermore, under conditions of low nutrient availability where growth was suppressed, low-pH fogs were not associated with reductions in foliar nutrient concentrations. Epicuticular waxes were not affected by either pH treatment.

5.4.5 Summary

The evaluation of responses of western conifer species to ozone, acid fog, and sulfur dioxide is made with one season of treatment, rather than multi-year exposures. Confidence in the conclusions is increased by one study's replication of treatments in a second year on a separate population of seedlings [WC07]. Overall, the same responses were observed in the second year.

Visible injury attributable to ozone exposure was found on ponderosa pine, white fir, subalpine fir, and western hemlock. Only western hemlock and western redcedar showed visible injury after exposure to acidic fog, although western redcedar appeared less susceptible to the ozone and acidic fog seasonal exposure.

Only Douglas-fir and ponderosa pine were exposed to acid-fog-only, and Douglas-fir appeared to be most susceptible to this treatment. Both species showed increases in above-ground biomass with no apparent change in below-ground biomass. This imbalance in biomass and the relative strengths of the source/sink functions of these tissues could cause problems in seedling establishment.

It is important to note that most growth effects due to treatment, especially changes in biomass, did not occur during the exposure period, but were observed following the spring bud elongation period. These changes in spring growth after exposure, whether increases or decreases, may indicate treatment effects on carbohydrate allocation patterns or on strengths of the carbohydrate sources for initiating spring growth. These conclusions are drawn from one year of treatment and further study is necessary to determine if multiple seasons of exposure alter the responses as the seedlings integrate their environment.

6 SUMMARY

In the previous section, results within each region were discussed by the general categories of response (i.e., visible injury and growth effects, and the mechanisms of foliar leaching, carbon allocation, and winter injury). The results pertaining to a specific response are now discussed across regions in an attempt to draw general conclusions regarding effects of acid deposition, ozone, and other pollutants on seedlings.

6.1 Visible Injury

All nine studies that examined visible injury to seedlings reported some effect of exposures to either acid precipitation or ozone. No visible injury was associated with sulfur dioxide in the two studies that examined these effects (Jensen and Dochinger [EH06], Hogsett and Tingey [WC08]).

Exposure to acid precipitation resulted in visible injury to most seedlings at or below pH 3.0. Only one of the six studies that examined acid effects did not observe some visible injury to some of the species (Davis and Skelly [EH01]). However, that study did not expose seedlings to acid below pH 3.0. Six studies reported visible injury associated with acid below pH 3.0 (Leith et al. [SF14], Jacobson and Lassoie [SF06], Jensen and Dochinger [EH06], Hogsett and Tingey [WC08], Miller et al. [WC09], and Turner et al. [WC07]). Only Engelmann spruce (Miller et al. [WC09]), Douglas-fir, and ponderosa pine (Turner et al. [WC07], Hogsett and Tingey [WC08]) showed no visible injury when exposed to acid at pH less than 3.0. It should be noted that Douglas-fir and ponderosa pine did show visible injury when exposed to acid less than 3.0 in Miller et al.'s [WC09] study.

Seedlings exhibited injury at ambient ozone concentrations (Kress et al. [SC06]) or slightly above ambient levels (Miller et al. [WC09], and Davis and Skelly [EH01]). All six studies that examined visible injury from ozone observed other effects (Fincher et al. [SF16], Jensen and Dochinger [EH06], Davis and Skelly [EH01], Kress et al. [SC06], Hogsett and Tingey [WC08], and Miller et al. [WC09]). Injury was observed at about 70 ppb (Miller et al. [WC09], and Davis and Skelly [EH01]). In addition, Kress et al. [SC06] showed a trend of increasing injury with increasing ozone concentrations. It should be noted that elevated ozone was associated with both increases and decreases in needle browning in red spruce (Fincher et al. [SF16]).

These short-term studies generally failed to show correlations of visible injury with changes in plant growth. Perhaps the seedlings have a capacity to recover from injury. Alternatively, growth responses might not be detected in the first season of measurement. The multiple-year studies in progress will help to determine whether visible injury will translate into growth effects.

6.2 Growth Effects

The effects of acidic precipitation on growth responses were highly variable among the seven studies that addressed this question. Results depended on species and pH level. Growth of most hardwood species was stimulated at pH levels of 3.0 and 3.5 versus 4.2 (Jensen and Dochinger [EH06], Davis and Skelly [EH01]). Growth of loblolly pine was stimulated at pH 4.5 versus 5.2 but depressed at 3.2 (McLaughlin [SC04]). Growth of red spruce was suppressed at pH levels below 3.0 when compared to 4.2 (Jacobson et al. [SF06(a)]). Growth of most species of western conifers was stimulated at pH levels of 2.1 (Miller et al. [WC09]). Other potential sources of variation included method of exposure; results from Jacobson et al. [SF06(a)] suggested that intermittent mists may have been more deleterious than continuous mists and sulfate acids may have been more harmful than nitrate acids.

Ozone effects on growth were most evident in the results for loblolly pine. Growth of roots and stems of loblolly pine appeared to be consistently suppressed at high concentrations of ozone (greater than 100 ppb). At intermediate levels (near 70 ppb), suppression and sometimes enhancement of growth occurred, depending on family. Neither red spruce nor the eastern hardwoods showed consistent growth effects due to ozone. Results for the western conifers indicated that some stimulation of growth occurred with medium to high levels of ozone (less than 100 ppb), except for ponderosa pine where growth suppression occurred.

Sulfur dioxide was associated with enhanced height growth of Engelmann spruce, white fir, Douglas-fir, and western redcedar in the western conifers (Miller et al. [WC09], Hogsett and Tingey [WC08]). Douglas-fir, ponderosa pine, and lodgepole pine showed reductions in root biomass and root/shoot ratios (Hogsett and Tingey [WC08]). Compared to a control, bud elongation was increased by higher sulfur dioxide levels (up to 66 ppb base level) for ponderosa pine, Douglas-fir, western hemlock, and western redcedar (Hogsett and Tingey [WC08]). No consistent effects were observed in the eastern hardwoods. Sulfur dioxide was not examined in the other regions.

An important finding in western conifers was that some effects, particularly growth changes, were not apparent until the spring after pollutant exposures were conducted. This result illustrates that seedling studies should be routinely carried over through the winter to the spring growing season following exposure.

6.3 Carbon Allocation

Most of the information on the effects of acid and ozone on carbon allocation is from research on red spruce and loblolly pine. Acid precipitation did not appear to suppress rates of photosynthesis based on the five studies reviewed (Laurence et al. [SF31], Patton and Jensen [SF07], Thornton et al. [SF27], McLaughlin et al. [SC04], and Richardson and Sasek [SC07]). Three studies found no effect of acid on photosynthesis, Thornton et al.'s [SF27] study suggested suppression of photosynthesis of red spruce seedlings with cloud exclusion, while McLaughlin et al. [SC04] found enhanced rates of photosynthesis of loblolly pine seedlings at pH 3.3 and 4.5 (possible due to nitrogen fertilization). It should be noted that none of the photosynthesis studies employed exposures below pH 3.1. Thus, it is not appropriate to contrast the results of photosynthesis studies (e.g., lack of effect above pH 3.1) with those of growth and visible injury (e.g., suppression below pH 3.0).

Although there was variability among studies, ozone at high levels (2 to 4 times ambient) appeared to disrupt photosynthesis. These concentrations reduced levels of photosynthetic pigments, and increased mesophyll cell disruptions in red spruce seedlings (Fincher et al. [SF16]). The effects on photosynthesis of red spruce seedlings are yet undetermined; three studies found no effects (Fincher et al. [SF16], Patton and Jensen [SF07], and Laurence et al. [SF31]), one study reported both enhancement and suppression depending on season (Cumming et al. [SF16]), and one study reported suppression associated with ambient ozone versus ozone removal (Thornton et al. [SF27]). The highest levels of ozone were associated with reductions in photosynthesis of loblolly pine seedlings (Richardson and Sasek [SC07]); furthermore, the effects of ozone on photosynthesis appeared to be cumulative (i.e., low concentrations for long periods also caused reductions).

6.4 Foliar Leaching

Although acid fog treatments increased foliar leaching of potassium, calcium, and magnesium from Douglas-fir seedlings, the amounts leached were small compared to uptake (Turner et al. [WC07]). Leaching of nutrients from the foliage, as a process induced by acidity, did not appear to be a problem.

6.5 Winter Injury

Acid rain reduced the ability of red spruce seedlings to withstand simulated overnight frosts during the winter hardening period. The degree of reduction in frost hardiness was proportional to acidity; compared to pH 5.0, mists of pH 2.5 raised the LT_{50}s by as much as 12°C, or, alternatively, delayed frost hardening by two to four weeks. While there was no overall effect of ozone on visible injury to red spruce seedlings following overwintering, a subset of seedlings did demonstrate some potential for visible injury.

7 CONCLUSIONS AND RECOMMENDATIONS

7.1 Principal Findings

Thus far, the objective has been to summarize results from individual seedling exposure studies. The contribution of these results toward understanding potential effects of sulfur dioxide, simulated acidic precipitation, and ozone on seedling populations is now discussed.

7.1.1 Sulfur Dioxide

Two projects examined visible effects of sulfur dioxide, and three examined growth effects. No visible injury was observed in response to concentrations as high as 66 ppb. Increased above-ground growth due to sulfur dioxide occurred for Engelmann spruce, white fir, western redcedar, and Douglas-fir, relative to a control treatment. Douglas-fir, ponderosa pine, and lodgepole pine showed reductions in root biomass and root/shoot ratios. Compared to a control, bud elongation was increased by higher sulfur dioxide levels (up to 66 ppb base level) for ponderosa pine, Douglas-fir, western hemlock, and western redcedar. The altered post-exposure growth and imbalance in above- and below-ground responses indicate that changes in carbon allocation patterns occurred. Under chronic exposure, survival or eventual tree productivity could potentially be negatively impacted. No effects of sulfur dioxide were seen for eastern hardwoods. Sulfur dioxide was not tested with red spruce or southern pines.

7.1.2 Simulated Acid Deposition

The clearest effect of simulated acid deposition was a reduction of frost hardiness in red spruce seedlings at pH 3.0 and higher rates of foliar tissue mortality during extreme cold. Most species that were tested at pH levels below 3.0 showed some visible injury. Across all species, there were no conclusive short-term effects of simulated acid deposition by itself on seedling growth. However, growth of black cherry was decreased by pH 3.0 versus 4.2. Furthermore, increased above-ground growth coupled with no apparent effects on below-ground biomass in western conifers at pH 2.1 compared with pH 5.6 indicated that changes in carbon allocation patterns occurred.

7.1.3 Ozone

The direct effect of ozone varied from physiological changes in the foliage of red spruce to suppressed growth of loblolly pine, ponderosa pine, and some hardwood species. Eastern hardwood species showed visible injury with ozone of 70 ppb or higher. Yellow-poplar, yellow birch, sweetgum, red maple, white ash, and black cherry appeared to be the most sensitive species tested. Among western conifers, white fir, subalpine fir, ponderosa pine, and western hemlock also showed visible injury in response to ozone at 70 ppb. Despite considerable differences in experimental designs and procedures, there was a pattern of root and stem growth decreases at ozone near 80 ppb or higher for loblolly pine. At intermediate levels (40 to 80 ppb) results were more variable; it was not uncommon for growth rate to be greater at intermediate levels than in charcoal-filtered air. In the West, only ponderosa pine showed consistent decreases across several growth variables due to ozone; most other species showed increased growth rates at levels less than 100 ppb. At this point in time, there are no data with which to address the anomaly of increased seedling growth at these levels (approximately 1.5 times ambient). Among the hardwoods, there was some association between ozone of 70 ppb and higher and decreased growth for black cherry, white oak, red maple, and yellow birch; yellow poplar, white ash, and red oak displayed no response at the same levels. Cumulative decreases in net photosynthetic rate in response to ozone were found for loblolly pine. Red spruce did not exhibit consistent decreases in net photosynthesis. However, damage to foliar mesophyll cells, decreased photosynthetic pigments, and seasonal changes in photosynthesis in red spruce in response to ozone at 40 ppb and higher suggest an increased potential for winter injury to red spruce.

7.1.4 Pollutant Interactions

There is preliminary evidence of some anion x pH interactions, where sulfur-based acids caused greater foliar injury on red spruce than nitrogen-based acids at the same pH. Nine projects exposed seedlings to both acidity and ozone, eight of which discussed interactions. Of these, four reported no interactions of acidity and ozone for stem growth of seedlings. Of the four projects that did observe some interactions, only two provided discussions adequate to evaluate the nature of the effect. In a second year of exposures of red spruce seedlings, an interaction was observed; elevated ozone caused reduced growth at pH 5.1 but caused increased growth at pH 3.1. A similar interaction in loblolly pine seedlings was found when 53 families were analyzed simultaneously. However, only 20% of the individual families showed a significant acidity x ozone interaction on height or diameter growth ($p \leq 0.10$). For western conifers, interactions between acid fog and ozone were common but inconclusive. In general, most projects did report some interactions, but no clear interpretations can be made from the studies at this time.

7.1.5 Summary

The studies summarized in this document provided information with respect to the mechanisms under investigation. Although acidic deposition had no effect on foliar leaching in western conifers, it led to short-term decreases in foliar nutrient concentrations in red spruce. Acid deposition also delayed frost hardening, thus increasing winter injury in red spruce. Regarding aspects of what has been collectively identified as altered carbon allocation patterns, seasonal changes in photosynthesis in response to ozone also suggested an increased potential for winter injury to red spruce. Moreover, given the disproportionate changes in below-ground and above-ground growth in response to either acid precipitation or ozone, there is a potential for altered carbon allocation patterns, which under long-term chronic exposures to either ozone or acid deposition might impact seedling survival.

Relative species sensitivity was tested only in western conifers and in eastern hardwoods. Visible injury and growth changes indicate that ponderosa pine was the most sensitive, and western redcedar the least sensitive, of species exposed to expected pollutant scenarios in the west. Results of visible injury to eastern hardwoods from one year of exposure indicate that black cherry was the most sensitive of species exposed to either acid precipitation or ozone.

There is preliminary evidence of some anion x pH interactions, where sulfur-based acids caused greater foliar injury than nitrogen-based acids at the same pH. Although, there are some indications of ozone x acid deposition interactions, the interpretations of these interactions are inconclusive at this time. Any further information that becomes available will be incorporated in MPO #4.

7.2 Recommendations for Future Research

The studies reported in this document were designed to quantify the effects of acid precipitation, ozone, and sulfur dioxide on seedlings. Building on exposure studies of crops, the experimental designs were generally randomized blocks, sometimes containing split-plots. Data were analyzed via additive linear models (i.e., analysis of variance, analysis of covariance, or regression). One product of the seedling exposure studies has been the identification of issues that qualify the above results and that should be addressed in new and continuing studies involving tree material. Although many other issues will likely surface before these studies are concluded, some of the major issues at this point are:

7.2.1 Species/Plant Variability

A major source of variation in plant response lies in the plant material itself. Virtually all the analyses demonstrated the large variation in growth that can be attributed to large differences in initial size (height and/or diameter). Careful selection and randomization of plants prior to treatment would help to alleviate this nonuniformity. The large variability and range of responses to pollutant exposures among loblolly pine families show the importance of reducing that variability if results of different experiments are to be compared.

7.2.2 Choice of Response Variables

The value of studying mechanisms for change in tree conditions is already apparent, as shown in the summary above. Choosing the appropriate response variables to correspond with the mechanism is essential. For instance, early indications from changes in rates of photosynthesis, below-ground growth changes, and above-ground growth changes in response to short-term exposures of pollutants point to altered patterns of carbon allocation. Presently, there is insufficient information from existing studies on changes in below-ground biomass to evaluate this mechanism. Therefore, future studies need measurements from the same plant on both below-ground (i.e. at least root biomass) and above-ground changes.

Before the end of NAPAP, exposure/response information for sapling-sized trees and for foliage response of mature trees will be collected. The most promising way in which seedling studies will provide insight into the response of mature trees to pollutants over time appears to be through examination of physiological processes (e.g., carbon allocation). While remaining NAPAP studies will contribute additional information, all future exposure studies involving seedlings and larger/older trees must emphasize physiological measurements.

It should also be noted that other sources of variability such as seedling genotype should be considered as a major source of variation in response. Genotypic variability currently ranges from well-documented open-pollinated families to commercial nursery stock.

7.2.3 Microclimate Characterization

Possible interactions between air pollutants and other environmental factors, such as those in the chamber microclimate, need to be characterized. In addition to allowing assessment of interactions, this information is important when replicating experiments. Differences in seedling responses among sites, or among years at a given site, cannot be correctly interpreted without knowing corresponding spatial and temporal differences in factors such as light, temperature, and humidity.

7.2.4 Duration of Experiments

Most of the initial studies were designed to look at effects over short periods of exposure (e.g., 12 to 16 weeks). While useful information can be obtained from short-term exposures, certain types of information such as growth response and winter injury can be better evaluated with multiple-year exposures. In addition, the variability of results among studies may be reduced with longer-term exposures. Variability in short-term studies is often a result of variation in initial age or size of plant material. These differences cannot always be solved through use of a covariate in the statistical analyses. Biologically, the cumulative effects or even lag in effects will remain unknown unless those changes are monitored on the same plants exposed over two to three growing seasons. An important finding from experiments on western conifers [WC08] was that some effects, particularly growth effects, were not apparent until the spring after pollutant exposures were conducted; growth differences due to treatment were reflected in bud elongation (i.e., reduced shoot growth) in the growing season following the season of treatment. There are currently ongoing multi-year exposure studies in southern pines and red spruce from which results will be forthcoming in the next two years.

7.2.5 Statistical Power

The adequacy of the sample size to determine effects due to treatments should be calculated at the conclusion of each project by computing power (i.e., the probability of detecting a consequential difference). This calculation includes measurements made from destructive sampling as well as additional sampling and design considerations as a function of measurement intervals or frequency of repeated measures. Successful planning of future experiments requires specification of the magnitude of treatment differences to be detected and knowledge of expected variation. Therefore, when possible, power should be considered when planning research as well as at the conclusion of each project. In the absence of formal significance due to low power, evidence of treatment effects may still be present in the form of trends or patterns, and should not be overlooked. However, it is important that users of the experimental results have realistic expectations of the confidence to be placed in treatment effects. Statistical power has been addressed to date for projects studying conifers in the west and loblolly pines in the southeast [WC08, WC09, SC02, SC04, SC05, and SC06]. Preliminary calculations suggest that for most growth variables of interest, these experiments have an 80% probability of detecting treatment differences of 20% and greater for a significance level of 0.05.

7.2.6 Repeatability of Experiments

Any experiment worth doing once should be considered for replication, regardless of the treatment duration (i.e., single-year versus multi-year exposures) or the statistical significance of the initial outcome. The ability to replicate an experiment in time or place is critical to confirmation of results. For example, trends in height and diameter response of loblolly pine to ozone, averaged across three families, were similar between the 1986 exposures and a replication of the experiment in 1987. Furthermore, the relative responses among the three loblolly pine families observed in 1986 were reproduced in 1987 for diameter changes, albeit not for height changes. As another example, in the west, two consecutive single-season exposure scenarios of acid fog plus ozone followed by sulfur dioxide were conducted on independent samples of Douglas-fir, western redcedar, ponderosa pine, western hemlock, and lodgepole pine. The 1986-1987 season indicated that the most sensitive species was ponderosa pine and the least affected species was redcedar. A repeat of the experiment in 1987-1988 confirmed these results. On the other hand, a pH effect on visible injury of red spruce in 1985 was confirmed from a repeated experiment in 1987, but not from a repeated experiment in 1986.

7.2.7 Repeated Measures

Although most studies have repeated measurements over time, very few projects have attempted any repeated measures analysis in the statistical treatment of the data. There is a strong effort by some statisticians within the FRP to adapt the existing literature on repeated measures analysis to use on seedling exposure research. Projects that currently have repeated measurements, yet have no repeated measures analysis, are not taking full advantage of available information.

8 ACKNOWLEDGMENTS

We would like to thank all the Principal Investigators of the seedling studies for continued cooperation in response to our requests. We are grateful to W.G. "Bill" Warren for his special efforts in helping promote and coordinate consensus-building for issues on statistical analysis, and to the numerous other statisticians involved with each individual project. The continuing support of the Co-op Directors and National Program Management is also appreciated. Finally, we would like to thank Jeff Brandt for his assistance in the revisions and Lynn Arnaut for help with editing and production.

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10 ABBREVIATIONS

ANCOVA . . . . Analysis of covariance
ANOVA . . . . Analysis of variance
CF . . . . Charcoal-filtered
CSTR . . . . Continuously-stirred tank reactor
df . . . . Degrees of freedom
DQO . . . . Data Quality Objective
DUKE . . . . Duke Forest
EPA . . . . Environmental Protection Agency
E/R . . . . Exposure/Response
FRP . . . . Forest Response Program
MS . . . . Mean sums of squares
MPO . . . . Major Program Output
NAPAP . . . . National Acid Precipitation Assessment Program
NCLAN . . . . National Crop Loss Assessment Network
NCSU . . . . North Carolina State University
NF . . . . Non-filtered
ORNL . . . . Oak Ridge National Laboratory
p . . . . Probability-value
ppb . . . . Parts per billion
QA/QC . . . . Quality Assurance/Quality Control
S&I . . . . Synthesis & Integration
SC . . . . Southern Commercial
SF . . . . Spruce-fir
SQ . . . . Scientific Question
TAMU . . . . Texas A & M University
USFS . . . . United States Forest Service
USDA . . . . United States Department of Agriculture
WC . . . . Western Conifers

11 APPENDICES

11.1 Appendix A: Project Summaries

This appendix contains summaries for the following FRP projects:

EH01 (1987) .... D.D. Davis and J. Skelly
EH01 (1988) .... D.D. Davis and J. Skelly
EH01(1988 OTC) .... J. Skelly and D.D. Davis
EH03 .... D. Karnosky and J. Witter
EH04 .... J. Skelly and D.D. Davis
EH06(1988) .... K.F. Jensen and L.S. Dochinger
EH06(1989) .... K.F. Jensen and L.S. Dochinger
SC02 .... F. Fong
SC04 .... S.B. McLaughlin
SC05 .... R.Reinert, C. Wells, & M. Schoeneberger
SC06 .... H. Allen, W. Heck, & L. Kress
SC07 .... C.J. Richardson
SC12 .... D. McGregor
SC13 .... J. Johnson
SC14 .... R.B. Flagler
SC15 .... B.G. Lockaby and A.H. Chappelka
SF06 .... J.S. Jacobson and J. Lassoie
SF07 .... K.F. Jensen and G.A. Schier
SF10 .... S.B. McLaughlin
SF11 .... C.J. Richardson
SF13 .... J. Seiler
SF14 .... M.H. Unsworth
SF16 .... L. Weinstein
SF27 .... F. Thornton
SF31 .... R. Kohut
SF32 .... J. Rebbeck, M.S. Greenwood, and K.F. Jensen
WC07 .... D. Turner and D.T. Tingey
WC08 .... W. Hogsett and D.T. Tingey
WC09 .... P. Miller

Project Number: EH01 (1987)

Principal Investigator: D.D. Davis and J. Skelly

Cooperative: Eastern Hardwoods

Title: Testing the Sensitivity of Eight Eastern Hardwood Species to Ozone and Acidic Precipitation.

Tree Species: 8 hardwood species

Objectives: The specific objective of this study is to screen seedlings of 8 eastern hardwood species for relative sensitivity to O_3, and acid precipitation.

Deliverables: Documentation of relative sensitivities of species to O_3 and acid precipitation, 12/87, 12/88, spring/89.

Summary: Conducted in close coordination with the Jensen and Dochinger project. Controlled environment chambers are used to expose seedlings to various levels of ozone, sulfur dioxide and acidic precipitation. Response variables include foliar symptoms, fresh weight of roots and stems, dry weight of roots and stems, shoot length, and number of leaves.

Project Number: EH01 (1988)
Principal Investigator: D.D. Davis and J. Skelly

Cooperative: Eastern Hardwoods

Title: Relative Sensitivity of Four Eastern Hardwood Species to Ozone, Sulfur Dioxide, and/or Acidic Precipitation.

Tree Species: 4 hardwood species

Objectives: The specific objective of this study is to screen seedlings of 4 eastern hardwood species for relative sensitivity to O_3, and acid precipitation.

Deliverables: Documentation of relative sensitivities of 4 eastern hardwood species to O_3 and acid precipitation, 12/88, 12/89, spring/90.

Summary: CSTR chambers are used to expose seedlings to various levels of ozone, sulfur dioxide and acidic precipitation. Response variables include foliar symptoms; fresh and dry weights of roots, stems and leaves; stem base diameter growth; shoot diameter growth; height; and number of leaves.

Project Number: EH01 (1988 Open-top Study)
Principal Investigator: J. Skelly and D.D. Davis

Cooperative: Eastern Hardwoods

Title: Testing the Sensitivity of Four Eastern Hardwood Species to Ambient Levels and Filtered Levels of Air Pollution along the Pennsylvania Gradient.

Tree Species: 4 hardwood species

Objectives: The specific objective of this study is to study the response of seedlings of 4 eastern hardwood species to ambient pollutants.

Deliverables: Documentation of relative sensitivities of species to ozone and acid precipitation, 12/88, 12/89, spring/90.

Summary: Open-top chambers have been located at three rural sites in northern Pennsylvania. Seedlings within the chambers are exposed to full-filtered, half-filtered, or non-filtered air. Response variables include foliar symptoms, leaf area, fresh and dry weight, height growth, and biomass at final harvest.

Project Number: EH03
Principal Investigator: D. Karnosky and J. Witter

Cooperative: Eastern Hardwoods

Title: Effect of an Air Pollution Gradient on Northern Hardwood Forests in the Northern Lakes Region: Open-Top Chamber Study.

Tree Species: 2 hardwood species

Objectives: To determine the response of trembling aspen and sugar maple to ozone along a nitrate gradient.

Deliverables: Document response of trembling aspen and sugar maple to ozone, 12/89.

Summary: Conducted as part of the Michigan gradient project. Open-top chambers are used to expose seedlings to various levels of ozone. Response variables include foliar injury, stem diameter, stem height, and stem, root, and leaf dry weight.

Project Number: EH04

Principal Investigator: J. Skelly and D.D. Davis

Cooperative: Eastern Hardwoods

Title: Foliar Sensitivity and Growth of Four Hardwood Species exposed to Ambient Concentrations of Ozone in Open-Top Chambers.

Tree Species: 4 hardwood species

Objectives: To determine the influence of ambient, half-ambient, and charcoal-filtered levels of ozone on foliar sensitivity and growth of four hardwood species.r

Deliverables: Documentation of relative sensitivities to ozone, 12/88, 12/89, and 12/90.

Summary: At three locations in rural Pennsylvania, seedlings are being exposed to ambient and partially-filtered levels of ozone. Foliar sensitivity and growth parameters are being measured.

Project Number: EH06 (1988 phase)

Principal Investigator: K.F. Jensen and L.S. Dochinger

Cooperative: Eastern Hardwoods

Title: Response of Eastern Hardwood Tree Seedlings to Atmospheric Deposition.

Tree Species: 6 hardwood species

Objectives: To evaluate the effect of atmospheric deposition on eastern hardwood seedlings and determine if drought retarded this effect.

Deliverables: Document the effect of sulfate, ozone, and drought on three oak species and dogwood, and the effect of ozone and drought on sugar and red maple, 12/88, 12/89.

Summary: Conducted in close coordination with the Ohio Corridor Gradient project. CSTRs are used to expose seedlings to various levels of ozone, sulfate, and drought. Response variables include foliar injury, leaf area, stem height, and stem, root, and leaf dry weight. Part two was conducted in close coordination with the Michigan Gradient. Open-top chambers are used to expose seedlings to various levels of ozone and drought. Response variables were the same.

Project Number: EH06 (1989 phase)
Principal Investigator: K.F. Jensen and L.S. Dochinger

Cooperative: Eastern Hardwoods

Title: Response of Eastern Hardwood Tree Seedlings to Atmospheric Deposition.

Tree Species: 2 hardwood species

Objectives: To determine the response of sugar maple and trembling aspen to ozone, nitrogen, deposition and drought.

Deliverables: Document response of sugar maple and trembling aspen to ozone, drought, and nitrate, 12/89, 9/90.

Summary: Conducted in close coordination with the Michigan Gradient project. Open-top chambers and CSTRs are used to expose seedlings to various levels of ozone, nitrate and drought. Response variables include foliar injury, leaf area, stem height, and stem, root, and leaf dry weight.

Project Number: SC02
Principal Investigator: F. Fong

Cooperative: Southern Commercial

Title: Growth Response of Loblolly Pine Seedlings to Ozone and Low-water Stress.

Tree Species: Loblolly pine

Objectives: 1) Assess responsiveness of loblolly pine as a species to ozone; quantify the genetic variation in response to ozone, and characterize; symptomatology and mechanisms (physiological responses) of ozone phytotoxicity; 2) characterize physiological responses of seedlings to ozone x low-water stress interactions.

Deliverables: Reports on responses of loblolly pine to ozone and water stress, 7/87, 12/88.

Summary: Uses fumigation chambers to quantify the genetic variation in the response of loblolly pine to ozone and to characterize the symptomatology and mechanisms of ozone phytotoxicity. Thirty half-sib families are exposed to different levels of ozone and moisture stress. Response variables include plant height, root collar diameter, total fresh and dry weight of needles, stem, and root, photosynthesis, respiration, and total non-structural carbohydrate.

Project Number: SC04
Principal Investigator: S.B. McLaughlin

Cooperative: Southern Commercial

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

Tree Species: Loblolly pine

Objectives: 1) Quantify growth and physiological responses of 53 loblolly pine genotypes to ozone and acid rain in the field and laboratory; compare lab vs. field results. 2) Develop protocols to quantify physiological and growth responses of large trees in the field.

Deliverables: Interim report during summer of 1987. Final report 12/88.

Summary: The objectives are met 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 Coop. Laboratory studies have focused on testing the physiological responses of 8 common families to ozone while using the approximate ambient rainfall pH level as a common background irrigant. Field studies have used open-top chambers to examine individual and interactive effects of ozone and simulated acid rain. Response variables include growth, photosynthesis, carbon metabolism, and mycorrhizal development.

Project Number: SC05

Principal Investigator: R. Reinert, C. Wells, & M. Schoeneberger

Cooperative: Southern Commercial

Title: Comparative Responses of Loblolly Pine Families to Ozone and Simulated Acid Rain

Tree Species: Loblolly pine

Objectives: Evaluate loblolly pine "sensitivity" to ozone and acid rain.

Deliverables: Dose-response model, 3/88; Quantification of growth and physiological responses of various half-sib families to ozone and acid rain, 12/88

Summary: This is a genotype screening study using short-term seedling exposures in the greenhouse. It seeks to determine relative responses of different half-sib families of loblolly pine. The primary response variables are needle injury, stem diameter and height, and biomass.

Project Number: SC06

Principal Investigator: H. Allen, W. Heck, & L. Kress

Cooperative: Southern Commercial

Title: Response of Loblolly Pine to Acidic Precipitation and Ozone Stress

Tree Species: Loblolly pine

Objectives: 1) Determine responses of a number of loblolly pine families to ozone exposure. 2) Estimate effects of ambient ozone concentrations on young loblolly pine. 3) Study ozone x acid rain interactions.

Deliverables: Quantification of loblolly response to ozone and acid rain. Development of 15' open-top chambers. Assessment of exposure effects on internal nutrient content and mycorrhizal development. Interim report 12/87. Final report 12/90.

Summary: The approach is to study plant responses over a range of ozone concentrations. A range of acidic precipitation treatments is used in a factorial design with the ozone concentrations. Phase I uses 10' chambers to test experimental protocols, assess physiological responses (see project SC07, PI: Richardson), and provide initial dose-response estimates. Phase II will use 15' diameter chambers to study larger trees for longer experimental periods.

Project Number: SC07

Principal Investigator: C.J. Richardson

Cooperative: Southern Commercial

Title: Effects of Gaseous Pollutants and Acid Deposition on Open-top Chambered Loblolly Pine Seedlings

Tree Species: Loblolly pine

Objectives: 1) Characterize physiological effects of loblolly genotypes exposed to ozone and acid rain. 2) Establish dose-response relationships: ozone vs. physiological and biochemical responses. 3) Develop mathematical relationships between physiology, biochemical responses, and growth across 5 levels of ozone and 2 levels of acid rain. 4) Study relationship between ozone exposure and status of the antioxidant defense system. 5) Develop diagnostic measurements for ozone exposure (e.g., light response curves).

Deliverables: Annual Reports 1/88, 12/88; Quantification of physiological and biochemical responses and relationship to growth.

Summary: Trees taken from Project SC06 (PI: Kress), uses open-top chambers, a range of ozone treatments, and two levels of simulated acid rain. Physiological measurements include survey measurements of photosynthesis, transpiration, and stomatal conductance. Monthly measurements of photosynthetic responses at both high and low irradiation levels for each treatment group will be coupled with measurements of transpiration, stomatal conductance, chlorophyll and carbohydrates. Dose-response relationships will also be developed.

Project Number: SC12
Principal Investigator: D. McGregor

Cooperative: Southern Commercial

Title: Response of Shortleaf Pine Families to Acidic Precipitation and Ozone in South Carolina.

Tree Species: Shortleaf pine

Objectives: Determine the influences of acidic precipitation and ozone on the growth, nutrition, and physiology of shortleaf pine under field conditions.

Deliverables: Quantification of Shortleaf Pine Response to Acid Precipitation and Ozone, 6/91.

Summary: One-year-old loblolly pine seedlings are grown in 15' open-top chambers. They are exposed to 3 levels of pH and 4 ozone concentrations during the growing season. Treatment effects are quantified on plant dry weight, stem diameter, cumulative height growth, fascicle length, and leaf area.

Project Number: SC13

Principal Investigator: J. Johnson

Cooperative: Southern Commercial

Title: Response of Slash Pine Families to Acidic Precipitation and Ozone in North Florida.

Tree Species: Slash pine

Objectives: Determine the influences of acidic precipitation and ozone on the growth, nutrition, and physiology of slash pine under field conditions.

Deliverables: Quantification of slash pine response to acid precipitation and ozone, 6/91.

Summary: One-year-old seedlings are grown in open-top chambers. They are exposed to 3 levels of pH and 4 ozone concentrations. Treatment effects are quantified on plant dry weight, stem diameter, total height, average fascicle length of the current flush, total leaf area, and visible damage.

Project Number: SC14

Principal Investigator: R.B. Flagler

Cooperative: Southern Commercial

Title: Response of Shortleaf Pine Families to Acidic Precipitation and Ozone in East Texas

Tree Species: Shortleaf pine

Objectives: Determine the influence of acidic precipitation and ozone on the growth, nutrition, and physiology of shortleaf pine under field conditions.

Deliverables: Quantification of shortleaf pine response to acid precipitation and ozone, 6/91.

Summary: One-year-old seedlings are grown in open-top chambers. They are exposed to 3 levels of pH and 4 ozone concentrations. Treatment effects are quantified on plant dry weight, stem diameter, total height, fascicle length, visible injury, foliar chlorophyll content, photosynthesis, and concentration of selected nutrients.

Project Number: SC15

Principal Investigator: B.G. Lockaby and A.H. Chappelka

Cooperative: Southern Commercial

Title: Response of Loblolly Pine Families to Acidic Precipitation and Ozone in Alabama.

Tree Species: Loblolly

Objectives: Determine the influences of acidic precipitation and ozone on the growth, nutrition, and physiology of loblolly pine under field conditions.

Deliverables: Quantification of loblolly pine response to acidic precipitation and ozone, 6/91.

Summary: One-year-old loblolly pine seedlings are grown in 15' open-top chambers. They are exposed to 3 levels of pH and 4 ozone concentrations during the growing season. Treatment effects are quantified on above-ground plant biomass by component part, stem diameter, cumulative height growth, and visible injury.

Project Number: SF06

Principal Investigator: J.S. Jacobson and J. Lassoie

Cooperative: Spruce-Fir

Title: Test of the Nitrogen Fertilization Hypothesis of Red Spruce Decline.

Tree Species: Red spruce

Objectives: Determine if combinations of sulfate and nitrate acidic mist alter the growth, development, cold tolerance, or water relations of red spruce seedlings in ways that might contribute to premature decline.

Deliverables: Dose-response relationship of acid mist vs. seedling growth, development, phenology, water relations, nutrient, balance, and biochemistry, 3/89.

Summary: Red spruce seedlings are exposed repeatedly, for extended durations, to simulated acidic mist at levels of acidity, sulfate, and nitrate concentrations that range from above to below those found in ambient wet deposition at high elevations in spruce-fir forests. Cold tolerance tests are performed in the fall as seedlings enter dormancy and in the early spring as they break dormancy. Needles and roots are analyzed for total nitrogen and sulfur concentration. Seasonal measurements are taken of needle water potential. Other measurements include needle diffusive conductance and transpiration, total chlorophyll, and cuticular wax content.

In six different experiments, Jacobson et al. [8] exposed seedlings of varying ages (one to three years) obtained from varying locations (from New York to Nova Scotia) to acid mists ranging in pH between 2.5 and 4.5 over varying time intervals (41 to 116 days). Their objective was to determine the effects of continuous and intermittent exposures to sulfate, nitrate, and combined sulfate-nitrate acidic mists on seedlings. Seedlings were scored visually in a variety of ways in an attempt to quantify the foliar damage (needle discoloration per seedling, the percent area of discoloration per affected needle, and needle abscission). Stem diameters, shoot lengths, shoot displacement volumes, and root displacement volumes were measured on seedlings over the course of the treatments. Sample sizes were not given.

Project Number: SF07
Principal Investigator: K.F. Jensen and G.A. Schier

Cooperative: Spruce-Fir

Title: Impact of Ozone and Acid Deposition on Foliar Leaching and Growth of Red Spruce Seedlings

Tree Species: Red spruce

Objectives: 1) Determine effects of acid rain and ozone on photosynthesis and water relations of spruce seedlings. 2) Determine if acid rain and ozone reduce carbohydrate concentrations of red spruce roots.

Deliverables: Dose-response of acid rain and ozone on red spruce needle leachate, 3/88. Dose-response of ozone and acid rain on red spruce needle development and physiology, 9/88. Effect of ozone and acid rain on spruce budworm development, 9/88.

Summary: Red spruce seedlings are exposed to ozone and acid rain treatments in CSTRs. Photosynthesis is measured periodically and the composition of foliar leachate is analyzed. At four periodical harvests, needle, stem, and root dry weights, total starch, and sugar contents are measured. Supplemental tests will involve infestation of treated and untreated seedlings with spruce budworm larvae.

Patton and Jensen [2] exposed one-year-old seedlings (from a common seed source) to three levels of ozone exposure (charcoal-filtered air, either alone or with additions of ozone for varying time periods) and three levels of simulated acid rain (pH 3.5, 4.0, and 4.5) over a six-month interval (first week in May - first week in November, 1988). Seedlings were harvested over the course of the treatments; heights, diameters, and dry masses of components (current-year needles, old needles, current year stems, old stems, and roots) were measured and 12 parameters of nonstructural carbohydrates in needles and roots were examined. Sample size was three seedlings per ozone-acid treatment for each of five harvests.

Project Number: SF10

Principal Investigator: S.B. McLaughlin

Cooperative: Spruce-Fir

Title: Interactive Effects of Natural and Anthropogenic Factors on Growth and Physiology of Red Spruce

Tree Species: Red spruce

Objectives: 1) Determine dose/response of HNO_3, H_2O_2, Al, and Mn on spruce seedlings. 2) Characterize differences in gas exchange, carbon allocation, and growth patterns across a "gradient" in decline and presumed deposition.

Deliverables: 1) Characterization of physiological changes associated with declining red spruce. 2) Determination of effects of nitric acid vapor, hydrogen peroxide, Al, and Mn, 10/88.

Summary: This project includes both field and laboratory components. The field study is a comparison of one high and one low elevation site in the Great Smoky Mountain National Park. Growth is estimated and monitored on canopy and sapling trees at each site. Photosynthetic capacity, respiration, and water relations are estimated on saplings. Carbon metabolism studies are also conducted using C^{14} techniques.

In the laboratory, red spruce seedlings are exposed to various levels of H_2O_2 and NO_2. Height, diameter, p[illegible]thesis, and nitrogen reductase activity are all measured. Screening techniques are being developed for examining the toxicity of red spruce to individual and combined trace metals.

Project Number: SF11
Principal Investigator: C.J. Richardson

Cooperative: Spruce-Fir

Title: Effects of Atmospheric Deposition on Red Spruce: A Free Radical Based Approach

Tree Species: Red spruce

Objectives: To determine if the key mechanism for atmospheric imposed stress in forest vegetation is the generation of oxygen-based free radicals by photochemical oxidants in the tissues of affected plants.

Deliverables: Evaluation of radical formation in leaf tissue of red spruce, 11/87.

Summary: Seedlings from the Weinstein project that have previously been exposed to different ozone treatments are used. Analyses include activities of superoxide dismutase and peroxidase, and concentrations of glutathione and malondialdehyde.

Project Number: SF13

Principal Investigator: Seiler

Cooperative: Spruce-Fir

Title:

Tree Species:

Objectives:

Deliverables:

Summary:

Tseng et al. [12] exposed 3-year-old Fraser fir seedlings (obtained from a commercial seed source in North Carolina) to three levels of ozone (< 20, 50, 100 ppb) and three levels of moisture stress over a 10-week interval. This study differed from most in that shorter exposures were employed (4 hrs/day, 3 days/week). Dry mass of shoots, dry mass of roots, root-to-shoot ratios, and stem diameters were measured on the seedlings at the end of the treatment interval. Photosynthesis, transpiration, and needle conductance (using a LI-COR 6000 portable photosynthesis system) were measured on the seedlings, after overnight rehydration, throughout the ozone and moisture stress treatments. Sample size for physiological measures was six seedlings per ozone-moisture treatment for each of three measurement dates.

Project Number: SF14

Principal Investigator: M.H. Unsworth

Cooperative: Spruce-Fir

Tree Species: Red spruce

Title: Frost Hardiness of Red Spruce in Relation to Forest Decline and Effects of Winter Exposure to SO_2 and NO_2.

Objectives: Determine if SO_2, NO_2, SO_4 acidic mist and O_3: i) alter frost hardening and ii) result in winter accumulation of phytotoxic substances that alter metabolism and growth of red spruce.

Deliverables: Evaluation of risk of frost injury based on weather data and field observations. Experimental evaluation of relationship of S, N, and acidity to physiology and biochemistry of frost hardiness. Model relating deposition to risk of frost injury, 1/89.

Summary: Shoots excised from red spruce growing at Whiteface Mt., NY, were tested in Scotland for frost hardiness. Results indicate that susceptible trees were likely to be damaged in 6 of the last 7 years at Newfound Gap and in 4 of the last 7 years at Whiteface Mt. An exposure facility for simulated acid mist has been established. Filtered and unfiltered chambers will be used to test for interactions with air quality. Preliminary results indicate that shoots exposed to SO_2 + NO_2 were more damaged than controls after freezing to -4 and -7°C. Ion chromatographic analyses of xylem sap from red spruce shows increased nitrate, nitrite, sulfite, and sulfate in sap from trees exposed to SO_2 + NO_2. The study has also developed NMR techniques to measure intracellular pH in spruce needles.

Murray et al. [10] developed a method whereby frost damage to foliar tissues may be assessed. In August of 1987, they cut shoots from one-year-old red spruce seedlings and exposed sections of each shoot to a range of temperatures (unfrozen control, -3° C, -6°, -9°, -12° C, and immersion in liquid nitrogen). After the treatment, the rate of leaching of electrolytes from the tissues into deionized water was measured.

Fowler et al. [9] exposed two-year-old seedlings (grown from seeds from New Hampshire) to six levels of acid mists (pH 2.5, 2.7, 3.0, 3.5, 4.0, and 5.0) over a 22-week interval (July 24 - December 18, 1987). Sections of shoots were cut from seedlings over the course of the treatments and further exposed to six levels of simulated overnight frosts (the range of temperatures was changed in order to reflect the developing frost hardiness of the seedlings). After the frost treatments, foliar cell damage was assessed by placing the shoot sections in deionized water whereby conductivity measurements were used to derive an index of leaching rate. Sample size was 20 shoot sections per acid-temperature treatment for each of six measurement dates. Leaching rate in unfrozen control shoots was two-fold higher in pH 2.5 versus pH 4.0 or 5.0.

Project Number: SF16
Principal Investigator: L. Weinstein

Cooperative: Spruce-Fir

Title: Effect of Ozone and Soil Nutrient Status on the Physiology of Photosynthesis, Carbohydrate Allocation, Nutrition, and Winter Hardiness in Red Spruce

Tree Species: Red spruce

Objectives: Evaluate effects of ozone on the physiology, growth, and development of spruce seedlings using open-top chambers. Study effects on chloroplast function, respiration, carbon assimilation and allocation.

Deliverables: Dose-response relationships between ozone exposure and various physiological response parameters, 1/88.

Summary: Red spruce seedlings are exposed to various levels of ozone in open-top chambers. Variables measured include those related to photosynthesis, carbohydrate production and translocation, and shoot and root growth.

Alscher et al. [5] exposed two-year-old seedlings (grown from seeds from Nova Scotia) to levels of ozone (1, 2, and 3 times ambient concentrations, and charcoal-filtered air) over a seven-month interval (May 30 - December 15, 1987). Seedlings were harvested over the course of the treatments, and dry masses of components (roots, current-year stems, [illegible] stems) were measured. Sample size was three seedlings per ozone level for each of four bimonthly harvests.

Cumming et al. [7] exposed four-year-old seedlings (grown from seed from Vermont) to three levels of ozone (1 and 2 times ambient concentrations, and charcoal-filtered) over a 16-week interval (beginning in late June, 1986). They also exposed three-year-old seedlings (grown from seed from Nova Scotia) to five levels of ozone (1, 2, 3 and 4 times ambient concentrations, and charcoal-filtered) over a 16-week interval (beginning June 1, 1987). Growth measures were not described. Sample size in 1986 was six seedlings per ozone level for each of four monthly harvests; sample size in 1987 was three seedlings per ozone level for each of 12 biweekly harvests. Statistical analyses were not discussed.

Fincher et al. [4] exposed two-year-old seedlings (grown from seeds from Nova Scotia) to five levels of ozone (1, 2, 3, and 4 times ambient concentrations, and charcoal-filtered air) over a seven-month interval (May 30 - December 20, 1987). Photosynthetic rates on seedlings (LiCor 6000 photosynthesis unit) were measured throughout the growing season. Needles were examined microscopically for cell damage and analyzed for photosynthetic pigments. Sample size was nine seedlings per each of four ozone levels (0.4, 1, 2, 3 times ambient) for microscopy and pigment analyses. (Sample size was not given for photosynthetic measures).

Project Number: SF27

Principal Investigator: F. Thornton

Cooperative: Spruce-Fir

Title: A Field Chamber Study of the Response of Red Spruce to Cloud Interception and Ozone

Tree Species: Red spruce

Objectives: Determine the effects of acidic cloud water and O_3, alone and in combination, on red spruce seedling root and shoot growth, photosynthetic rates and tissue nutrient concentrations.

Deliverables: Evaluation of use of chambers for cloud exclusion studies, 11/87. Results of exclusion experiments, 1/88, 1/89.

Summary: Uses open-top chambers on Whitetop Mt., VA. The chambers are used to exclude ambient ozone, clouds, or both. Throughout the growing season periodic evaluation of physiological response is determined by measuring photosynthetic rate, stomatal conductance, and needle water potential. Periodic event sampling of foliar interception of cloud water is also conducted. Root and shoot biomass and tissue nutrient concentrations are determined from a subset of destructively sampled seedlings.

Thornton and Peir [3] exposed two seedling types of unspecified age (native stock from the study site and phyton-grown from a seed source in the Great Smoky Mountains National Park) to varying conditions on top of Whitetop Mountain, VA (1680 m) during 1988. This study is unique in that effects were removed by chambers instead of added. Exposures in chambers were ambient air, ambient air with clouds removed, and charcoal-filtered air with clouds removed. A fourth treatment (shade cloth) was conducted to assess the effect of the chamber. Photosynthesis and respiration measurements (LI-COR LI-6200 Portable Photosynthesis System) were taken over the course of the growing season. Sample size was generally nine per treatment-seedling type for each measurement date.

Project Number: SF31
Principal Investigator: R. Kohut

Cooperative: Spruce-Fir

Title: Comparison of the Responses of Seedling and Sapling Red Spruce Exposed to Ozone and Acidic Precipitation Under Field Conditions.

Tree Species: Red spruce

Objectives: 1) Assess effects of acid precipitation and ozone on photosynthesis and growth of red spruce seedlings. 2) Produce dose/response functions using measures of photosynthesis and growth as response variables for seedlings and saplings. 3) Develop quantitative assessments of the effects of previous exposure and tree age on the dose/response function.

Deliverables: Comparison of dose/response relationships of 1- to 3-year old spruce seedlings and saplings for acid and ozone treatments, 9/88.

Summary: Red spruce seedlings and saplings are exposed to ozone and acidic deposition in open-top chambers over a three year period. Response variables include foliar and root pathology, net photosynthesis, stomatal conductance, growth, carbon allocation, projected leaf area, leaf dry weight per age class, and chlorophyll content.

Laurence et al. [1] exposed one-year-old seedlings (grown from seeds from Maine) to four levels of ozone (0.5, 1, 1.5, and 2.0 times ambient concentrations) and three levels of simulated acid rain (pH 3.1, 4.1, and 5.1) over a three-month interval (June 26 - September 30). Seedlings were harvested over the course of the treatments, and heights and dry masses of components (roots, needles, and stems) were measured. Rates of photosynthesis were measured on the seedlings (using an ADC portable photosynthesis unit) over the course of the treatments. Sample size was three seedlings per ozone-acid treatment for each of three harvests.

<u>Project Number: SF32</u>
Principal Investigator: J. Rebbeck, M.S. Greenwood, and K.F. Jensen

Cooperative: Spruce-Fir

Title: Evaluation of the Impact of Ozone on Seedling and Grafted, Mature Red Spruce Using Open-Top Chambers

Tree Species: Red spruce, balsam fir

Objectives: 1) Determine the influence of tissue maturation on red spruce response to ozone; 2) Determine, under controlled field conditions, the effects of ozone on the growth and morphology of red spruce and balsam fir seedlings, via the mechanism of altered carbon allocation and physiological processes.

Deliverables: Dose-response of ozone on red spruce as a function of tissue maturation, 12/88,2/90. Response of red spruce and balsam fir seedling growth and physiology to ambient ozone at a low-elevation site, 12/88, 2/90.

Summary: Open-top chambers are used to expose mature and juvenile grafted red spruce scions to various levels of ozone. In a second study, balsam fir and red spruce seedlings are being grown in open-top chambers to assess the impact of ambient ozone at the Howland, Maine, low-elevation forest. Height, diameter, foliar injury, photosynthetic rate, and stomatal conductance will be measured. Seedlings and grafts will periodically be destructively sampled and analyzed for root and shoot (scion and rootstock) biomass, chlorophyll, epicuticular wax, and leaf area.

Project Number: WC07
Principal Investigator: D.T. Tingey and D. Turner

Cooperative: Western Conifers

Title: The Effect of Acid Precipitation on Seedling Growth as a Function of Foliar Leaching, Foliar Nitrate Uptake, and Cation Availability

Tree Species: Douglas-fir, Engelmann spruce

Objectives: To evaluate the role of foliar cation leaching and cation availability to roots of Douglas-fir and Engelmann spruce in response to acidified mist.

Deliverables: Report on first-year effects of simulated rainfall chemistry on throughfall chemistry, and seedling biomass and nutrient content, 10/87. Final report, 10/88.

Summary: *Results of a pilot study on Douglas-fir include:*

- Biomass per plant increased at the end of the 12-week experiment in response to higher levels of nutrient availability.

- Content of Ca, Mg, and K in second-year foliage increased in response to higher levels of nutrient availability, but did not show a response to fog pH.

- Leaching of Ca, Mg, and K from foliage was considerably higher with fog of pH 3.1 than with fog at pH 5.6, with K being most susceptible to leaching. However, the amounts of nutrients removed at pH 3.1 are small relative to the observed uptake rates of these trees.

- Epicuticular waxes were not affected by either treatment.

Further studies will focus on foliar leaching in Engelmann spruce and on the cation exchange capacity and buffering capacity of conifer needles.

Project Number: WC08

Principal Investigator: W. Hogsett and D.T. Tingey

Cooperative: Western Conifers

Title: Sensitivity of Important Western Conifer Species to SO_2 and Seasonal Interaction of Acid Fog and Ozone

Tree Species: Douglas-fir, ponderosa pine, lodgepole pine, western hemlock, western redcedar

Objectives: Assess relative sensitivity of western species to: 1) a seasonal pattern of acid fog and ozone, and 2) SO_2 exposure during fall and winter.

Deliverables: Interim sensitivity ranking, 10/87. Final sensitivity rankings, 10/88.

Summary: The screening of various species for sensitivity in growth and visible needle injury is accomplished with two deposition exposure scenarios: 1) acid fog (winter)/ozone (summer) as a seasonal combination of pollutants, and 2) gaseous SO_2 deposition (winter). Seedling sensitivities are being assessed as a growth response over two growth periods with year-round exposures. The fumigation regimes reflect the seasonality of deposition duration, frequency of events, fog chemistry, and the seasonality of the frequency and distribution parameters of ozone and SO_2 characteristic of selected regions of the west. A range of treatment concentrations is employed for each pollutant which is representative of the possible air quality conditions of the west. The fumigation periods are those months when these deposition patterns occur. Seasonal interaction of the pollutants, rather than concurrent pollutant combinations, represent a realistic exposure scenario for much of the climatic conditions of the coastal western U.S. and Cascade and Sierra foothills.

Project Number: WC09
Principal Investigator: P. Miller

Cooperative: Western Conifers

Title: Testing the Sensitivity of Five Western Conifer Species to SO_2 Alone, and Ozone Followed by Acidic Fog

Tree Species: Douglas-fir, ponderosa pine, white fir, subalpine fir, Engelmann spruce

Objectives: Using open-top chambers, determine the sensitivity of the five conifer species to SO_2 alone, and acid fog and O_3 in combination.

Deliverables: Report of preliminary rankings, 10/87. Report of final rankings, 10/88.

Summary: This project is conducted in coordination with the Hogsett and Tingey project. Exposures are conducted year-round in modified open-top chambers under natural environmental conditions. Simulated ambient exposure profiles for acidic for, SO_2, and ozone are employed. Exposure regimes for the gaseous pollutants were developed by averaging air quality characteristics from a number of sites across a region. These average values were then used to construct a 30- or 60- day hourly concentration regime that reflects the ambient air quality characteristics of the region of interest. A range of treatments for each pollutant is created by building additional hourly concentration profiles within 1-2 standard deviations of the base profile. Development of fog regimes follows a similar methodology reflecting the frequency, chemistry, and deposition volume of selected regions.

11.2 Appendix B: Example of Power Curve

The following figure illustrates a power curve computed by Project WC08 for ponderosa pine seedlings. Power was computed for treatments of acid fog (AF), ozone (OZ), and their interaction (AFxOZ), with number of chambers and plots fixed (i.e., held constant). As might be expected, there is a higher probability ($p > 0.70$) of detecting a treatment effect when the differences are quite large (>40%). Conversely, the power of the experiment to detect differences of less than 20% is very weak.

Suppose, for example, the goal is to detect a 30% difference due to treatment, at an alpha level of 0.05. The probability of detecting that effect from AF is about 0.40, whereas the probability of detecting the same change from OZ is about 0.70. Both the alpha level and power desired for the test can be set, and the sample size varied, as in Table B1. From these computations, the number of plants needed per treatment chamber to detect differences as low as 10%, with a high level of probability (power = 0.90), can be determined.

Note that the example in Table 1 is specific not only to the species, but to the response variable under consideration (in this case, biomass). Additionally, these are sample sizes for a given power and alpha level, which assume a fixed or limited number of chambers. Similar tables could be arrayed by number of plants per harvest, relative differences to detect, and the estimated population coefficient of variation, in order to determine the number of chambers needed at a specified level of power. This would require compiling such information for each response variable used in both repeated measures and final biomass component data for the species or family of interest.

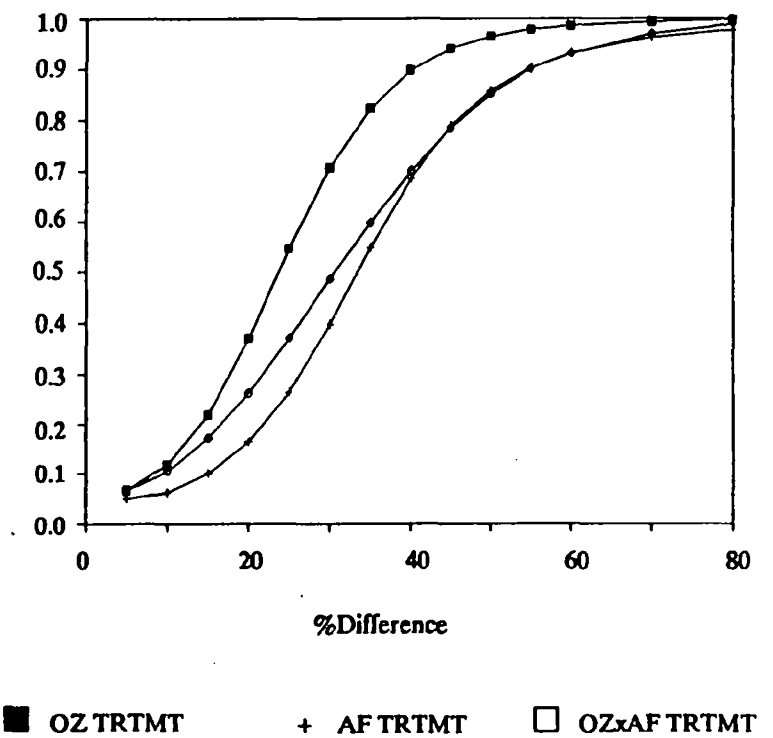

Figure B1. Power curve constructed from ponderosa pine seedling data of project WC08, where OZ = ozone, AF = acid fog, and OZxAF is the interaction.

Table B1. Example of sample size determination for a seedling study (project WC08) in the Forest Response Program.

Ponderosa pine sample size calculations for biomass dry weight.

LN transformation used; power=.90; 2-sided t-test; a=.05.
6 error df for the OZ main effect test; 3 OZ trt; 3 Ch/trt; MSE=.17.
3 error df for the AF main effect test; 3 AF trt; 2 Ch/trt; MSE=.18.
36 error df for the OZxAF test; 9 OZxAF trt; 6 Ch combo/trt; MSE=.13.

% Difference	# Plts per OZ Chamber	# Plts per AF Chamber	# Plts per OZxAF trtmt
10	188	460	318
15	88	214	74
20	52	126	43
25	34	84	29
30	25	61	21
35	19	46	16
40	15	37	13
45	12	30	10
50	10	25	9
55	9	22	8
60	8	19	7
65	7	17	6
70	6	15	5
75	5	13	5

$$n = \{[(T(.025)-T(.90))^2]*[2*MSE/(DIFFLN^2)]\} / \#Ch$$

Where: # Plts = number of seedling plants.
OZ = ozone
AF = acid fog
Ch = chamber
n = sample size
T = Student's t statistic
MSE = mean squared error
DIFFLN = natural log of treatment difference