

**GROWTH RESPONSE AND DROUGHT SUSCEPTIBILITY
OF FOREST TREES EXPOSED TO
SIMULATED ACIDIC RAIN AND OZONE**

by

Woong S. Lee

Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Plant Physiology

APPROVED:

—
B. I. Chevone, Chairman


J. L. Hess

L. D. Moore

D. M. Orcutt

—
J. R. Seiler

May, 1988

Blacksburg, Virginia

**GROWTH RESPONSE AND DROUGHT SUSCEPTIBILITY
OF FOREST TREES EXPOSED TO
SIMULATED ACIDIC RAIN AND OZONE**

by

Woong S. Lee

(ABSTRACT)

One-year-old seedlings of red spruce, loblolly pine, yellow-poplar, and sweetgum were exposed to ozone (0.0 or 0.1 ppm, 4 hr d⁻¹, 3 d wk⁻¹) in combination with simulated acidic rain (pH 5.6 or 3.0, 1 h d⁻¹, 2 d wk⁻¹, 0.75 cm hr⁻¹) for ten weeks. After the ten-week treatment with ozone and simulated acidic rain, the seedlings were submitted to two drought cycles, and water potential, net photosynthesis (Pn), stomatal conductance (Cs), and transpiration (Ts) were measured.

Whole-plant fresh weight increment (FWT) and dry weight were significantly reduced in red spruce seedlings after the ten-week treatment with ozone. Ozone also significantly reduced shoot height growth (SHG) and increased the apparent plastochron duration (APD) of sweetgum seedlings. Treatment with simulated rain at pH 3.0 significantly increased FWT and SHG of red spruce compared to pH 5.6 as well as FWT and relative growth rate (RGR) of loblolly pine. The APD was significantly reduced in sweetgum

seedlings exposed to simulated acid rain at pH 3.0 compared to 5.6. Significant interactions between ozone and simulated acidic rain occurred in all species except red spruce, and they were additive. The SHG was significantly lower in loblolly seedlings exposed to 0.1 ppm ozone + pH 5.6 than in seedlings exposed to either 0.1 ppm ozone + pH 3.0 or 0.0 ppm ozone + pH 5.6. The APD was significantly increased in yellow-poplar and sweetgum seedlings exposed to 0.1 ppm ozone + pH 5.6 compared to any other treatment.

Visible symptoms appeared on the adaxial leaf surface of yellow-poplar seedlings submitted to acid rain at pH 3.0 regardless of ozone treatment. Visible foliar symptoms were also observed on the adaxial surface of sweetgum seedlings exposed to 0.1 ppm ozone. The symptoms were characterized by premature red pigmentation with small brown necrotic lesions.

Foliar concentrations of P and S were significantly increased in all seedlings exposed to simulated rain at pH 3.0 compared with pH 5.6 except for red spruce which exhibited the increases of K and S. In general, there were neither significant effects of ozone nor interactions between ozone and rain pH on foliar nutrient concentrations.

There were no significant effects of ozone on Pn, Cs, Ts, or water-use efficiency (WUE) prior to the drought cycles for all species. However, after the first drought

cycle, Pn and Cs were significantly changed in loblolly pine and sweetgum pre-exposed to 0.1 ppm ozone compared with controls. The ten-week treatment of simulated acidic rain at pH 3.0 significantly increased Pn and Ts of loblolly pine and reduced Cs of yellow-poplar. After the first drought cycle, yellow-poplar treated with pH 3.0 rain showed lower Pn and WUE than seedlings exposed to pH 5.6 solution.

A 0.5 MPa shift in the response of net photosynthesis (Pn) to decreasing water potential occurred in red spruce seedlings across all air pollutant treatments after the drought cycles indicating photosynthetic acclimation to drought stress. During the second drought cycle, Pn was more sensitive to water potential in red spruce and loblolly pine seedlings exposed to 0.10 ppm ozone + pH 3.0 solution compared with seedlings exposed to 0.0 ppm ozone + pH 5.6 solution. This increased sensitivity of Pn to water potential might be explained through a change in root hydraulic conductivity (Lp).

In general, Lp showed significant effects of ozone and simulated acidic rain after moisture stress. After the first drought cycle, Lp was decreased in yellow-poplar and sweetgum seedlings exposed to 0.10 ppm ozone. After the second drought cycle, Lp was decreased in yellow-poplar and red spruce seedlings exposed to 0.1 ppm ozone. Significant interactions between ozone and simulated acidic rain

occurred in the Lp of red spruce and yellow-poplar seedlings after the first drought cycle.

The results from this dissertation indicate that ozone and/or simulated acidic rain can alter the growth and drought susceptibility of forest tree species under laboratory conditions. Forest trees which are periodically exposed to ambient ozone and acidic rain in natural forest ecosystems probably have a response similar to the results from this study. Additional research is necessary to determine if such effects are occurring in natural forest ecosystems.

ACKNOWLEDGEMENTS

The author would like to thank the author's advisor, Dr. B. I. Chevone for his support throughout the experimentation and preparation of this manuscript. The author thanks his committee members; Dr. J. L. Hess, Dr. L. D. Moore, Dr. D. M. Orcutt, and Dr. J. R. Seiler. The author is grateful to all his fellow of the Air Pollution Laboratory;

The author's wife, , deserves special thanks for her help in typing this manuscript. Final thanks goes to my parents and all members of Korean Baptist church of Blacksburg and Korean Baptist Church of Roanoke for their constant prayer and support.

Table of Contents

Abstract	ii
Acknowledgements	vi
Table of Contents	vii
List of Tables	ix
List of Figures	xii
Introduction	1
Literature review	
Introduction	5
Effects of ozone and simulated acidic rain on growth of red spruce and loblolly pine seedlings	7
Effects of ozone and simulated acidic rain on growth of yellow-poplar and sweetgum seedlings	14
Effects of acidic rain on growth of forest tree species	18
Combined effects of simulated acidic rain and ozone on growth of forest tree species	25
Combined effects of ozone and water stress on plants.....	28
Effects of air pollutants on gas exchange of plants.....	33
Effects of ozone on photosynthate partitioning in forest tree species	41
Effects of air pollutants on foliar nutrient concentrations	46
Factors affecting root hydraulic conductivity in plants	50
Literature Cited	60
Chapter I GROWTH RESPONSE AND DROUGHT SUSCEPTIBILITY OF RED SPRUCE SEEDLINGS EXPOSED TO SIMULATED ACIDIC RAIN AND OZONE	

Introduction	74
Material and Methods	75
Results	81
Discussion	94
Literature Cited	101
Chapter II GROWTH RESPONSES OF THREE SOUTHEASTERN COMMERCIAL TREE SPECIES EXPOSED TO SIMULATED ACIDIC RAIN AND OZONE	
Introduction	106
Materials and Methods	108
Results	113
Discussion	126
Literature Cited	131
Chapter III WATER RELATIONS AND PHYSIOLOGY OF TREE SPECIES AS INFLUENCED BY DROUGHT AND AIR POLLUTANTS	
Introduction	136
Materials and Methods	139
Results	144
Discussion	161
Literature Cited	168
Summary and Conclusions	175
Appendix	182
Vita	192

LIST OF TABLES

Table 1. Changes in mean fresh weight increment (FWT), total dry weight (DWT), root to shoot ratio (RSR), and relative growth rate (RGR) of one-year-old red spruce after 10 wks of treatment with ozone and simulated acidic.....	82
Table 2. Summary of results of foliar analysis of one-year-old red spruce seedlings after 10 weeks of treatment with ozone and simulated acid rain.....	85
Table 3. Changes in mean root hydraulic conductivity of one-year old red spruce seedlings during and after 10 wks of pollutant treatment with ozone and simulated acid rain. All seedlings were submitted to two drought cycles after 10 wks of treatment.....	87
Table 4. Changes in net photosynthesis (Pn), transpiration (Ts), and water-use efficiency (WUE) of one-year-old red spruce seedlings after 10 weeks of treatment with ozone and simulated acidic rain.....	88
Table 5. Regression models and coefficients of determination for net photosynthesis (Pn) of one-year-old red spruce seedlings during two drought cycles following exposure to ozone and simulated acidic rain.....	90
Table 6. Regression models and coefficients of determination for transpiration (Ts) of one-year-old red spruce seedling during two drought cycles following exposure to ozone and simulated acidic rain.....	93
Table 7. Changes in fresh weight increase (FWT), total dry weight (DWT), root to shoot ratio (RSR), and relative growth rate (RGR) of one-year-old loblolly pine after 10 wks of exposure to ozone and simulated rain.....	114
Table 8. Changes in mean fresh weight increment (FWT), total dry weight (DWT), root to shoot ratio (RSR), and relative growth rate (RGR) of one-year-old yellow-poplar after 10 wks of treatment with ozone and simulated rain.....	116

Table 9. Apparent plastochron duration (days) of one-year-old sweetgum (SG) and yellow-poplar (YP) seedlings during 10 wks of treatment with ozone and simulated acid rain.....	118
Table 10. Changes in mean fresh weight increment (FWT), total dry weight (DWT), root to shoot ratio (RSR), and relative growth rate (RGR) of one-year-old sweetgum after 10 wks of treatment.....	121
Table 11. Summary of results of needle analysis of one-year-old loblolly pine after 10 weeks of treatment with ozone and simulated acid rain. Values (% per gram needle dry weight) are means of five samples.....	123
Table 12. Summary of results of foliar analysis of yellow-poplar after 10 weeks of treatment with ozone and simulated acid rain. Values (% per gram leaf dry weight) are means of five samples.....	124
Table 13. Summary of results of foliar analysis of one-year-old sweetgum after 10 weeks of treatment with ozone and simulated acid rain. Values (% per gram leaf dry weight) are means of five samples.....	125
Table 14. Changes in net photosynthesis (Pn), stomatal conductance (Cs), transpiration (Ts), and water-use efficiency (WUE) after 10 weeks of ozone fumigation (OF), and the first drought cycle (DC).....	145
Table 15. Changes in net photosynthesis (Pn), stomatal conductance (Cs), transpiration (Ts), and water-use efficiency (WUE) after 10 weeks of acid rain treatment (AT) and the first drought cycle (DC).....	146
Table 16. Regression models and coefficients of determination for net photosynthesis (Pn) and transpiration (Ts) of one-year-old pine seedlings during two drought cycles following exposure to ozone and simulated acidic rain.....	149
Table 17. Regression models and coefficients of determination for net photosynthesis (Pn)	

	of one-year-old yellow-poplar seedlings during two drought cycles following exposure to ozone and simulated acidic rain.....	154
Table 18.	Regression models and coefficients of determination for stomatal conductance (Cs) and transpiration (Ts) of one-year-old yellow-poplar seedlings during two drought cycles following exposure to ozone and simulated acidic rain.....	156
Table 19.	Regression models and coefficients of determination for net photosynthesis (Pn), stomatal conductance (Cs), and transpiration (Ts) of one-year-old sweetgum seedlings during two drought cycles following exposure to ozone and simulated acidic rain.....	157
Table 20.	Changes in mean root hydraulic conductivity of one-year-old loblolly pine seedlings during and after 10 wks of treatment with simulated acid rain. All seedlings were submitted to two drought cycle after 10 wks of treatment.....	158
Table 21.	Changes in mean root hydraulic conductivity of one-year-old yellow poplar seedlings during and after 10 wks of treatment with ozone and simulated acid rain. All seedlings were submitted to two drought cycles.....	159
Table 22.	Changes in mean root hydraulic conductivity of one-year-old sweetgum seedlings during and after 10 wks of treatment with ozone and simulated acid rain. All seedlings were submitted to two drought cycles.....	160
Table A1.	Major ion chemistry of simulated rain.....	183

LIST OF FIGURES

Figure 1. Changes in cumulative shoot height growth (cm) of one-year-old red spruce seedlings during 10 wks of treatment with simulated rain.....84

Figure 2. Predicted regression response of net photosynthesis (Pn) in one-year-old red spruce seedlings across all air pollutant treatments during the first and second drought cycle.....91

Figure 3. Predicted regression response of net photosynthesis (Pn) in one-year-old red spruce seedlings as affected by the 10-wk exposure to ozone and simulated rain during the second drought cycle.....92

Figure 4. Changes in cumulative shoot height growth (cm) of one-year-old loblolly pine seedlings during 10 wks of treatment with ozone and simulated rain.....115

Figure 5. Changes in cumulative shoot height growth (cm) of one-year-old yellow-poplar seedlings during 10 wks of treatment with ozone and simulated rain.....117

Figure 6. Changes in cumulative shoot height growth (cm) of one-year-old sweetgum seedlings during 10 wks of treatment with ozone.....120

Figure 7. Predicted regression response of net photosynthesis (Pn) in one-year-old loblolly pine seedlings as affected by the 10-wk exposure to ozone and simulated rain during the first drought.....150

Figure 8. Predicted regression response of net photosynthesis (Pn) in one-year-old loblolly pine seedlings as affected by the 10-wk exposure to ozone and simulated rain during the second drought cycle.....151

Figure 9. Predicted regression response of transpiration (Ts) in one-year-old loblolly pine seedlings as affected by the 10-wk exposure to ozone and simulated rain during the first drought cycle.....152

Figure 10. Predicted regression response of transpiration (Ts) in one-year-old loblolly pine seedlings as affected by the 10-wk exposure to ozone and simulated rain during the second drought cycle.....	153
Figure A1. Photosynthesis and regression response of one-year-old red spruce seedlings affected by ozone and simulated acidic rain during the first drought cycle.....	184
Figure A2. Photosynthesis and regression response of one-year-old red spruce seedlings affected by ozone and simulated acidic rain during the second drought cycle.....	185
Figure A3. Transpiration and regression response of one-year-old red spruce seedlings affected by ozone and simulated acidic rain during the first drought cycle.....	186
Figure A4. Transpiration and regression response of one-year-old red spruce seedlings affected by ozone and simulated acidic rain during the second drought cycle.....	187
Figure A5. Photosynthesis and regression response of one-year-old loblolly pine seedlings affected by ozone and simulated acidic rain during the first drought cycle.....	188
Figure A6. Photosynthesis and regression response of one-year-old loblolly pine seedlings affected by ozone and simulated acidic rain during the second drought cycle.....	189
Figure A7. Transpiration and regression response of one-year-old loblolly pine seedlings affected by ozone and simulated acidic rain during the first drought cycle.....	190
Figure A8. Transpiration and regression response of one-year-old red spruce seedlings affected by ozone and simulated acidic rain during the second drought cycle.....	191

INTRODUCTION

Ozone is the most prevalent photochemical oxidant in the atmosphere and is known to have the greatest harmful effects on plants among gaseous pollutants. Ozone is formed in the atmosphere under the effect of solar radiation from anthropogenic sources of precursors, mainly NO_x . The harmful effect of ozone on plants has been reported in many species. Ozone is known to damage leaf ultrastructure and leaf surface characteristics, and induce leaching of nutrients from leaves. Ozone is also known to change photosynthate allocation, which can result in an alteration of plant growth pattern and yield.

Acid rain is considered a major environmental concern throughout the northeastern United States. The recent decrease in pH is known to be largely the result of combustion of fossil fuels leading to increased emission of sulphur and nitrogen oxides. The acidity of precipitation is continuing to increase, with monitoring stations commonly reporting an annual average pH of 4.2 to 4.3.

The presence of strong acids in rain may affect the growth of individual forest plants, and subsequently alter forest ecosystems. Although a recently accelerated reduction of red spruce growth has been reported in the Appalachian Mountains, and many possible causes of this decline have been suggested, none have been established conclu-

sively at present. However, the decline of mature trees in the northeast, beginning in the early 1960's, appeared to have been initiated by a severe drought. The similar coincidence between the initiation of growth decline and drought was reported in mid-Appalachian forests. For the recent extensive decline of red spruce in the northeastern United States, the hypothesis has been suggested that acid deposition, heavy metal inputs, and possibly ozone stress, can impair root growth and root physiology to such an extent that affected trees become more highly drought susceptible. Although effects of ozone and water stress in combination have been studied in forest plants, the effect of drought stress on plants already subjected to ozone and acid rain has not been investigated.

Water stress is known to affect many physiological processes and morphological characteristics of plants. Water stress in plants occurs whenever transpirational demand for water vapor in the atmosphere exceeds water supply to the root system. Water supply is not only a function of the available water in the soil, but it is also a function of the ability of the roots of a plant to take up water from the soil or the root hydraulic conductance (L_p). Root hydraulic conductivity is known to have a major role in controlling water relations in plants, and can be affected by many factors including root age, temperature, degree of suberization, hormone concentration, disease,

vesicular-arbuscular mycorrhiza, and nutrients. However, the effects of ozone and acid rain on the root hydraulic conductivity of plants have not been investigated.

Four forest tree species, two conifers and two hardwoods, were used in this research to examine the relative sensitivity of representative deciduous and conifer species to acid rain and ozone. Loblolly pine (*Pinus taeda* L.) is economically the most important species in the southeastern United States. This species has been used as pulp, plywood, and lumber materials and is known to exhibit considerable intraspecific variation in sensitivity to ozone. Red spruce (*Picea rubens* Sarg.) is a long-lived, shade-tolerant tree that is commonly present in the cool, moist climates at high elevations of the Appalachian Mountains. A serious decline of red spruce is now occurring in the northeastern U. S. and the etiology of this decline is unknown. Recent studies have suggested that acidic inputs, in conjunction with severe drought, may be contributing factors to the demise of this species in the northeast. Yellow-poplar (*Liriodendron tulipifera* L.) is a common and widespread species in southeastern deciduous forests and is becoming an important timber source for forestry production. The species is considered to be moderately sensitive to ozone. Sweetgum (*Liquidambar styraciflua* L.) is an important pulp wood species also common to the southeast. It is known that ambient concentrations of ozone result in reduced annual

growth in this species.

The principal objective of this study was to investigate the response of forest seedlings to simulated acidic rain and ozone, particularly as these pollutant stresses influence root and shoot growth, foliar nutrient status, drought susceptibility, and plant water relations.

Specific objectives were:

1. To determine root and shoot growth response of loblolly pine, red spruce, yellow-poplar, and sweetgum seedlings to simulated acidic rain and ozone, singly and in combination.

2. To determine the effect of these pollutants on foliar concentrations of N, P, K, Ca, Mg, Al, and S.

3. To determine the effect of these pollutants on seedling water status, drought susceptibility, and foliar gas exchange by measuring: a) needle (loblolly pine), branch (red spruce), or leaf (yellow-poplar and sweetgum) water potential of seedlings during drought stress, b) root hydraulic conductivity before and after drought stress, and c) net photosynthesis, stomatal conductance, and transpiration of seedlings during drought stress.

LITERATURE REVIEW

INTRODUCTION

Ozone and acidic precipitation are the most widespread airborne pollutants impacting forest ecosystems in the eastern United States (Cogbill and Likens, 1974; Lioy and Samson, 1979; Skelly et al., 1982). Ozone was first shown to injure vegetation in the late 1950's (Heggestad and Middleton, 1959; Richards et al., 1958). Since that time, the detrimental effects of ozone on the growth and yield of sensitive plants have been reported for many species (Heagle et al., 1974; Howell et al., 1979; Sprugel et al., 1980; Reich et al., 1982).

The decline of ponderosa pine in the San Bernadino Mountains in California, resulting from oxidant pollution generated in the Los Angeles Basin, is the classical example of the impact of ozone on forest vegetation (Miller et al., 1972). In the eastern United States, several tree species have shown reduced growth under ambient ozone concentrations (Benoit et al., 1982; Duchelle et al., 1982, 1983; Stone and Skelly, 1974; Skelly et al., 1982). Sensitive species include eastern white pine, Virginia pine, loblolly pine, tulip poplar, sweet gum, milkweed, blackberry, and clematis.

The mechanism of ozone toxicity to plants has not been clearly established; however, inhibition of photosynthesis (Yang et al., 1982, 1983a, b) and changes in photosynthate allocation (McLaughlin and McConathy, 1983; McLaughlin et al., 1982) have resulted in reduced growth of roots compared to shoots.

The pH of precipitation preserved in glaciers or continental ice sheets prior to the industrial revolution was generally found to be greater than 5.0 (Mateev, 1970; Langway et al., 1965). Recent data from Greenland and Antarctica showed that precipitation falling in these remote areas had a pH of about 5.5 (Hammer, 1977; Delmas et al., 1980). However, as early as the 1950's, Pennsylvania, West Virginia and much of New England were already receiving rainfall as acidic as pH 4.52 (Junge and Werby, 1958), ten years later, the area affected by acid rain had expanded to include many north, central and southeastern states (Cogbill and Likens, 1974; Likens and Butler, 1981). The acidity of precipitation in areas reporting acid rain in 1956 had increased with stations commonly reporting an average pH of 4.2 to 4.3 (Cogbill and Likens, 1974). Data from 1975 to 1976 compiled by Likens and Butler (1981) indicated a continuing increase in areas subjected to acidic precipitation, with further increases in the acidity of rain in areas already affected by acid rain.

Acid rain is known to have direct and indirect effects

on vegetation (Evans, 1984a, b). Simulated acidic rain has been shown to induce visible injury as lesions on plants (Wood and Bormann, 1974, 1975; Evans et al., 1978, 1982), as well as increase leaching of metabolites (Fairfax and Lepp, 1975; Scherbatskoy and Klein, 1983; Blanpied, 1979; Proctor, 1983). Acid rain is also known to affect the rate of gas exchange through stomata (Tamm and Cowling, 1977; Evans et al., 1982; Seiler and Paganelli, 1987), seed germination (Lee and Weber, 1979; Baldwin, 1934; McColl and Johnson, 1983; Raynal et al., 1982a), and seedling growth (Wood and Bormann, 1974, 1977; Matziris and Nakos, 1977; Lee and Weber, 1979; Dochinger, 1976; Raynal et al., 1982a; McColl and Johnson, 1983; Neufeld et al., 1985; Reich et al., 1987). Indirect effects of acid rain on vegetation through the soil system have been extensively studied and currently reviewed (Johnson et al., 1982; McFee, 1983).

This literature review will present the state of current knowledge, focusing on the growth and physiological impacts of air pollutants, especially, ozone and acid rain, on forest tree species.

EFFECTS OF OZONE AND SIMULATED ACIDIC RAIN ON GROWTH OF RED SPRUCE AND LOBLOLLY PINE SEEDLINGS

Red spruce (Picea rubens Sarg.) is one of the major softwood species of northern forests, whose distribution

includes 14 northeastern states (Fowells, 1965). Many researchers have reported a decline of red spruce in the Appalachians (Siccama et al., 1982; Johnson and Siccama, 1983, 1984; Scott et al., 1984; Vogelmann et al., 1985; Adams et al., 1985). Though many possible causes of this decline have been suggested (Johnson et al., 1981; Puckett, 1982; Johnson, 1983; Johnson and Siccama, 1983, 1984; Carey et al., 1984; Mclaughlin, 1985; Woodman and Cowling, 1987), none have been established conclusively at present. However, there is evidence that air pollution may have contributed to the recent decline of red spruce in high elevation forest of the eastern United States.

Dendroecological studies showed abnormal decline of red spruce increment cores in a relatively short period of time (Johnson and Siccama, 1983; Bruck, 1984) with no consistent association with local stresses, such as insects or pathogens. Foliar sulphur accumulation was observed in trees with visible decline symptoms as compared with asymptomatic trees.

Friedland et al. (1984a, b) found heavy metal accumulations in forest soils in high-elevation, spruce-fir ecosystems. Increased lead concentrations were distributed throughout the high elevations of the Appalachian mountains (Friedland et al., 1984a, b; Bruck, 1984) with these receiving more total deposition, and more frequent cloud impaction than lower elevations. Ozone has been reported to

increase foliar leaching, especially in combination with acid rain. Bosch et al (1986) reported acid mist and ozone treatments significantly increased the leaching of Ca, Mg, Fe, and Mn from shoots of Norway spruce (Picea abies [L.] Karst) trees. However, foliar deficiency of Mg and Ca was promoted only in soils poor in exchangeable Mg and Ca.

Weiss and Agerer (1986) investigated effects of mineral nutrients, ozone, and acidic mist on growth of Norway spruce. The development of the root system was reduced due to the lack of Ca and Mg nutrients, which resulted in a lower biomass of fine roots. However, there was no significant effect of ozone and acid mist on development of the root system, even though ozone slightly reduced development and resulted in a slightly lower fine-root biomass.

Meyer (1987) examined the root system of declining and healthy spruce stands. There were no significant differences in the number of root tips and fine root length with a diameter of 3 mm or less between declining and healthy stands during the spring and fall, however, differences were significant during the summer. Declining stands showed smaller numbers of roots tips and longer fine roots compared with healthy stands. The ratio of numbers of root tips to length of fine roots in declining stands was approximately one half of that in healthy stands.

Most of the experiments on spruce decline were conducted in the field, with little information available on

the effects of air pollutants on red spruce seedlings under controlled laboratory conditions. Taylor et al (1986) fumigated one-year-old red spruce seedlings with ozone at 0.0 or 0.12 ppm, four hours a day, twice weekly for four months. There was no significant effect of ozone on above ground relative growth rate, biomass, accumulation and rates of photosynthesis and transpiration. A concentration of 0.12 ppm ozone significantly increased root dry weight compared to the control.

Seiler and Paganelli (1987) studied the effects of simulated acid rain on photosynthesis and growth of red spruce and loblolly pine seedlings. The rain solutions contained ionic components similar to that of average ambient rainfall in southwestern Virginia. The pH of the rain solution was adjusted to 4.3 or 3.0 by addition of a mixture of 1 M H_2SO_4 and 0.5 M HNO_3 (1:1). Six-month-old red spruce and six-week-old loblolly pine seedlings were exposed to simulated acid rain by direct application to the soil. Acidity of simulated acid rain did not alter height growth, root and shoot dry weight, or root to shoot ratio of either species.

Loblolly pine (Pinus taeda L.) is the principal commercial pine species in the southeastern United States, and grows in the Coastal Plain and Piedmont from Delaware and central Maryland south to central Florida and west to eastern Texas (Fowells, 1965). Effects of ozone and simu-

lated acidic rain on growth of loblolly pine seedlings will be reviewed in the following section.

Berry (1974) examined the sensitivity of loblolly pine seedlings according to primary needle age. Two, four, six, eight, and ten-week-old loblolly pine seedlings were exposed to ozone at 0.25 ppm for two hours. Loblolly pine seedlings were most sensitive to ozone at two weeks of age based on foliar symptoms.

Kress (1978) conducted screening tests for 23 full-sib families of loblolly pine. Three to four and fourteen to sixteen-week-old pine seedlings were exposed to ozone at 0.10, 0.15, 0.20, 0.25 or 0.30 ppm for eight hours. Family 6-13 x 2-8 showed the most severe foliar symptoms across all ozone concentrations with the exception of 0.1 ppm ozone. No foliar symptoms were found on seedlings for any family following exposure to 0.1 ppm ozone. Family 540 x 504 was most tolerant to ozone. The author concluded there were many variations among families of loblolly pine that might be useful for breeding programs. Kress (1978) also examined the long-term effect of ozone on growth of loblolly pine seedlings. One to two-week-old seedlings were exposed to 0.05 ppm ozone, six hours a day for 28 or 56 consecutive days. Height growth was significantly suppressed in the sensitive family.

Ward (1980) screened 33 half-sib families of loblolly pine for sensitivity to ozone. The four to five-week-old

seedlings were exposed to 0.4 or 0.45 ppm ozone for eight hours. Families 2-8 and 506 were most sensitive and families 523 and 503 were most tolerant to ozone based on foliar symptoms. On the basis of these results, the author studied the growth response of loblolly pine seedlings exposed to ozone. Seedlings of six half-sib families (two sensitive, two intermediate, and two tolerant families) were exposed to ozone at 0.0, 0.05, or 0.10 ppm, eight hours a day, for 21 or 42 consecutive days. The concentration of 0.1 ppm ozone significantly decreased height growth and biomass compared with the control. However, the magnitude of the reduction in height growth and biomass did not correlate with ozone sensitivity as determined by the screening test.

Kress and Skelly (1982) examined the growth response of ten tree species including a wild type and a 6-13 x 2.3 full-sib family of loblolly pine. Four-week-old seedlings were exposed to ozone at 0.0, 0.05, 0.10, or 0.15 ppm, six hours a day, for 28 consecutive days. Only the wild type loblolly pine seedlings showed a significant reduction in height growth with the 0.05 and 0.10 ppm ozone treatment. However, the 0.15 ppm ozone treatment significantly reduced height growth in both the wild type and full-sib family. Shoot and total plant dry weight of loblolly pine seedlings were not significantly changed by any treatments of ozone. In the second experiment, the researchers selected seven of

the ten species used in the first study, utilizing the same treatments as the first study. Only the 6-13 x 2-8 family of loblolly pine seedlings showed a significant reduction in height growth with the 0.1 ppm ozone treatment.

Winner et al. (1987) screened loblolly pine seedlings differing in resistance to fusiform rust disease for responses to ozone and SO₂. Twelve families of four to six-week-old loblolly pine seedlings were exposed to 0.07 ppm ozone and 0.06 ppm SO₂ alone or in combination for five hours a day for 40 consecutive days. Ozone alone reduced shoot dry weight in family 29 R (rust resistant). Root dry weight and total dry weight were decreased in family 11-20 (rust resistant). However, root to shoot ratio was increased in family 11-20 due to 0.07 ppm ozone. There was no clear relationship between fusiform rust resistance and changes in growth due to air pollutants.

Shafer et al. (1987) examined the effects of chronic exposure to ozone on growth of loblolly pine in the field. Seedlings of four full-sib families of loblolly pine were exposed to ozone in open-top chambers. Treatments consisted of charcoal-filtered air, ambient air, and ozone concentrations ranging from 1.25 to 2.0 times that of ambient air. After five months of treatment, seedlings of each family showed foliar symptoms typical of ozone injury. Seedlings of three families showed significant linear relationships between ozone concentration and shoot height, ozone concen-

tration and dry weight of needles as well as total dry weight, and ozone concentration and stem diameter. However, one family did not exhibit a significant ozone dose-plant growth response relationship.

EFFECTS OF OZONE AND SIMULATED ACIDIC RAIN ON GROWTH OF
YELLOW-POPLAR AND SWEETGUM SEEDLINGS

Yellow-poplar (Liriodendron tulipifera L.) is an important commercial hardwood species in the eastern United States (Beck and Della-Bianca, 1981), with a distribution ranging from southern New England west to Michigan and south to central Florida and Louisiana (Fowells, 1965; Beck and Della-Bianca, 1981). Sweetgum (Liquidambar styraciflua L.) commonly grows from Connecticut southward throughout the East to central Florida and eastern Texas. It is also found as far west as Missouri, Arkansas, and Oklahoma and north to southern Illinois (Fowells, 1965). In this section, effects of ozone and simulated acidic rain on growth of yellow-poplar and sweetgum will be reviewed.

In studies on the effects of ozone on growth and leaf drop, Jensen (1973) fumigated nine deciduous tree species, including one-year-old yellow-poplar, with 0.30 ppm ozone, eight hours per day, five days per week for five months. Ozone did not significantly change the height growth of yellow-poplar seedlings, however, premature foliage loss

was increased by 60% in seedlings exposed to 0.30 ppm ozone compared with the control.

Kress and Skelly (1982) exposed two to four-week-old seedlings of ten eastern forest species, including yellow-poplar, to 0, 0.05, 0.10, or 0.15 ppm ozone, six hours per day, for 28 days. The concentration of 0.05 ppm ozone significantly increased height growth and top dry weight of yellow-poplar by 60% and 47%, respectively, compared with the control. However, there were no significant effects of ozone on height growth and dry weight at 0.1 or 0.15 ppm.

Mahoney et al. (1984) fumigated six-week-old yellow-poplar seedlings with 0.07 ppm ozone, 0.06 ppm SO₂, and 0.10 ppm NO₂ in various combinations, and examined the impact of ozone on shoot growth. Ozone alone did not change total shoot growth and seedling dry weight. However, total shoot growth and seedling dry weight were significantly reduced by the combinations of ozone + SO₂, SO₂ + NO₂, or ozone + SO₂ + NO₂.

Chappelka et al. (1985) exposed nine-week-old, half-sib, yellow-poplar to 0.1 ppm ozone 0.08 ppm SO₂ or 0.10 ppm ozone + 0.08 ppm SO₂, four hours a day, five days a week, for six weeks in combination with simulated rain (pH 3.0, 4.3, or 5.6, 1 hr d⁻¹, 2d wk⁻¹ at 0.75 cm hr⁻¹). Ozone at 0.1 ppm did not change height growth and dry weight of yellow-poplar seedlings. However, height growth and dry weight were significantly reduced by 26 and 34%,

respectively, in seedlings exposed to 0.1 ppm ozone + 0.08 ppm SO₂, and the combined effect was additive. They also observed visible symptoms on the adaxial leaf surface of 14% of the plants exposed to 0.1 ppm ozone.

Jensen (1985) used growth analysis techniques to evaluate the effects of ozone on growth of yellow poplar seedlings. One-year-old seedlings were exposed to 0.10 ppm ozone for 12 hours, one or two days a week for up to 20 weeks. Ozone decreased height, leaf area, leaf dry weight, and total dry weight compared to the control. Relative growth rate (RGR) and net assimilation rate (NAR) were significantly reduced by ozone fumigation.

Previous studies (Jensen, 1973; Kress and Skelly, 1982); Mahoney et al., 1984; Chappelka et al., 1985) in the laboratory indicate that ozone treatment ranging from 0.07 to 0.3 ppm may not alter the growth of yellow-poplar seedlings. Field studies on the growth response of yellow poplar have received less attention.

Duchelle et al. (1982) studied the effects of ambient concentrations of ozone on eight forest tree species, including yellow-poplar, in the Appalachian Mountains of Virginia where the monthly average concentration of ozone was approximately 0.05 ppm with several periods averaging 0.08 ppm. The height growth of yellow-poplar was reduced by 44% in seedlings grown in non-filtered chambers compared with charcoal-filtered chambers. They also observed purple

stippling on the adaxial leaf surface of yellow-poplar seedlings grown in ambient air. At the present time, more research is needed in the area of growth response of yellow-poplar to ozone in the field.

Only two studies have been conducted on the growth response of sweetgum exposed to ozone. One was done under controlled environment conditions, and the other in the field. For the laboratory study Kress and Skelly (1982) fumigated two to four-week-old seedlings of ten eastern forest species, including sweetgum, with 0.00, 0.05, 0.10, or 0.15 ppm ozone, six hours a day, for 28 consecutive days. Concentrations of 0.1 ppm and 0.15 ppm ozone significantly decreased height growth of sweetgum seedlings by 29% and 45%, respectively. Dry weight was also reduced by 42% in seedlings exposed to 0.15 ppm of ozone.

Duchelle et al. (1982) examined the effects of ambient concentrations of ozone on eight forest tree species, including sweetgum, in the Appalachian Mountains of Virginia where the monthly average concentration of ozone was approximately 0.05 ppm and several periods averaged 0.08 ppm. They observed premature red pigmentation of sweetgum leaves as a result of exposure to ambient ozone. Unfortunately, no accurate data were available because of terminal bud die-back during the winter.

EFFECTS OF ACIDIC RAIN ON GROWTH OF FOREST TREE SPECIES

Within the last ten years, acid rain has received considerable attention, and many contradictory reports have appeared. Some of them (Matziris and Nakos, 1977; Dochinger, 1976; Raynal et al., 1982b) reported that acid rain inhibited growth of various tree species and others showed that acid rain stimulated growth (Wood and Bormann, 1974, 1977; Lee and Weber, 1979; Reich et al., 1987) or had no effect (McColl and Johnson, 1983; Abouguendia and Baschak, 1987). In this section, the various effects of acid rain on growth of forest tree species are presented.

Wood and Bormann (1974) examined the effect of simulated acid mists on two and six-week-old yellow birch (Betula alleghaniensis Britt.) seedlings. The mist pH was adjusted to 5.5 (control), 4.0, 3.3, 3.0 and 2.3 by adding H_2SO_4 to distilled water. Mist was applied with impingement-type fog nozzles at rainfall intensity of 0.5 cm a week for 11 to 15 weeks. Total plant weight, total leaf weight, and stem length of seedlings exposed to pHs of 4.0, 3.3, or 3.0 were not significantly different from control seedlings. However, all variables were significantly less in seedlings exposed to mist at pH 2.3, and Wood and Bormann (1974) concluded that the growth of yellow birch seedlings were not significantly affected by the acid rain until the pH of the mist reached 3.0. They also observed

foliar tissue damage in seedlings exposed to mist at pH 3.0 and 2.3.

Matziris and Nakos (1977) examined the effect of simulated acid rain on growth of half-sib families of Aleppo pine (Pinus halepensis Mill). One-year-old seedlings were irrigated with simulated acid solution at pH 3.1, 3.5, or 5.1. Height growth was decreased by 8.2% in seedlings irrigated with solution at pH 3.1 compared to pH 5.6. The authors also found that solution acidity increased mortality and negatively influenced the formation of terminal buds.

Effects of simulated acidic rain on the growth and nutrient relations of eastern white pine (Pinus strobus, L.) were studied by Wood and Bormann (1977). Seedlings were exposed to simulated acid rain with each pot receiving approximately 4.2 cm wk⁻¹ for 20 weeks. Simulated acid rain consisted of H₂SO₄, HNO₃ and HCl mixed in distilled water and was adjusted to pH 5.6, 4.0, 3.3, 3.0, or 2.3. Total plant and needle weight were significantly reduced in seedlings exposed to rain at pH 5.6 compared with all other pHs. In spite of foliar damage, application of acid rain at pH 2.3 significantly increase total plant weight, total needle weight, and needle length compared with all other pHs.

Lee and Weber (1979) studied effects of simulated acidic rain on seedling emergence and growth of eleven

woody plants. Simulated acid rain consisted of ionic constituents based on a seven-year average concentration at Hubbard Brook, New Hampshire. The pHs of simulated acid rain were adjusted to 5.6, 4.0, 3.5 or 3.0 by adding H_2SO_4 . A nozzle system was used to apply simulated acid rain at the rate of 0.25 cm hr^{-1} , three hours a day, three days a week. Germination of staghorn sumac was inhibited by simulated acid rain. In contrast, simulated acid rain stimulated germination of eastern white pine, eastern red cedar, and yellow birch compared with the control (pH 5.6). The top growth of Douglas-fir and shagbark hickory was significantly increased with increasing rain acidity. Sugar maple showed a stimulation effect of acid rain on top growth at pH 3.5 and 4.0, and yellow-poplar showed the same effect at pH 4.0. Simulated acid rain did not change the growth of roots in any species except staghorn sumac which showed a significant reduction.

Dochinger(1976) examined effects of acid rain on growth and survival of several forest tree species. One-year-old seedlings, potted in river sand or peat soil, were exposed to simulated acidic rain at pHs of 3, 5, or 7 for 15 weeks. Growth and survival were retarded in red maple (Acer rubrum), white ash (Fraxinus americana), sweetgum (Liquidambar styraciflua), yellow-poplar (Liriodendron tulpifera), sycamore (Platanus occidentalis), cotton wood (Populus deltoides), and American elm (Ulmus americana) due

to solution acidity. Shoot height growth and seedling survival were higher in seedlings potted in peat soil and those receiving higher pH solution. Seedling growth and survival were lower in seedlings potted in river sand and receiving acid rain at pH 3.0. The author concluded that acid rain may influence forest tree seedlings in soils which have insufficient buffering capacity to neutralize acidic compounds of acid precipitation.

Raynal et al. (1982b) exposed sugar maple seedlings to simulated acidified canopy throughfall and found a reduction of radicle growth at pH 3.0 and below. The survival of seedlings was also decreased with increasing acidity of simulated canopy throughfall. However, extension and weight growth of leaves in seedlings exposed to simulated acidic throughfall depended on the soil nutrient supplying capacity. Under nutrient-limited conditions, seedling growth was promoted by throughfall at pH 3.0, with some foliar damage, whereas under high-nutrient conditions, growth was suppressed only at pH 2.0.

Raynal et al. (1982a) observed the effect of acidity on seed germination of five tree species. Acidity was adjusted with H_2SO_4 to pHs of 5.6, 4.0, or 3.0. Germination of red maple seeds was inhibited at pHs 4.0 and 3.0, while germination of yellow birch seeds was reduced at pH 3.0 compared with pH 5.6. In contrast, germination of eastern white pine was increased by increasing solution aci-

dity. Silver maple and eastern hemlock showed no effects of solution pH.

McColl and Johnson (1983) examined effects of simulated acid rain on germination of Douglas-fir and growth of two-year-old Douglas-fir (Pseudotsuga menziesii) and ponderosa pine (Pinus ponderosa) subjected to simulated acid rain. A mixture of H_2SO_4 and HNO_3 (2:3) was added to produce acid rain at pHs of 5.6, 4.0, 3.0 and 2.0. Germination of Douglas-fir was significantly inhibited by 30% in seeds treated with acid rain at pH 2.0. The total length of the new apical growth and dry weight of all new needles were measured for Douglas-fir, and average of the lengths of the three largest new needles and dry weight of all new needles were measured for ponderosa pine. In spite of the needle damage at pH 2.0, all growth parameters were not significantly different among treatments in both species.

Neufeld et al. (1985) examined the effects of foliar applications of simulated acid rain on growth and photosynthesis of four deciduous tree species, yellow-poplar (Liriodendron tulipifera L.), American sycamore (Platanus occidentalis L.), black locust (Robinia pseudo-acacia L.), and sweetgum (Liquidambar styraciflua L.). Simulated acid rain contained deionized water with salts and acids in $mg\ l^{-1}$: Ca 0.23; Na 0.17; K 0.08; Mg 0.05; NH_4-N 0.02; PO_4 0.007, with pH adjusted to 5.6, 4.0, 3.0, and 2.0 by addition of a 10:7:1 mixture of $SO_4:NO_3:Cl$. Simulated acid

rain was applied at a rainfall intensity of 4.75 cm h^{-1} for 20 minutes a day, every three days, for 48 days. Height growth was significantly reduced in seedlings exposed to pH 2.0 for American sycamore, black locust, and sweetgum. Total dry weight was significantly decreased at pH 2.0 for American sycamore and sweetgum. They also found a reduction in the photosynthetic rate of American sycamore seedlings exposed to acid rain at pH 2.0, and concluded that the observed reductions in growth might be due to the reduction in photosynthetic rate.

Abouguendia and Baschak (1987) examined the response of jack pine (*Picea glauca* (Moench) Voss) to simulated acid rain. Rain solution was composed of similar ion concentrations as that found in precipitation at the Cree Lake CAN-SAP (Canadian Network for Sampling Precipitation) station, located 440 Km north of Prince Albert. The pHs of the final rain solutions were adjusted by adding appropriate amounts of H_2SO_4 and HNO_3 . Two-year-old jack pine and white spruce seedlings were exposed to 2.2 cm simulated acid rain for 25 minutes a week for a period of seven weeks. No significant differences were found in height growth for either species with any of the treatments. Jack pine needles revealed no macroscopic symptoms of acid rain injury at the pHs tested. In contrast, white spruce needles showed chlorotic and/or necrotic lesions with all treatments, and foliar damage increased with elevated solution acidity over time.

Later, Reich et al. (1987) tested the combined effects of ozone and simulated acid rain on growth and photosynthesis of white pine seedlings grown in five soils. Seedlings were exposed to ozone at 0.02, 0.06, 0.10, or 0.14 ppm in combination with simulated acid rain at pHs of 5.6, 4.0, 3.5, or 3.0 for four months. Solution consisted of sulphuric and nitric acid at a weight ratio of 2:1. Simulated acid rain, in general, significantly increased plant growth and photosynthesis. There was a strong interaction between acid rain and soil type. The amount of growth increase was consistent with needle N concentrations, soil N concentrations, and foliar P:N ratios. Seedlings showed the greatest growth increase in the soil with the lowest needle N concentration and the highest foliar P:N ratio. From these results, they concluded that the main positive effect of acid rain on growth was due to nitrogen in the acid rain. Acid rain, however, will not always have a positive effect on growth and net photosynthesis. At high levels over long periods acid rain may lead to N becoming excessive to plants, leading to winter injury or a disruption in allocation patterns. Reich et al. (1987) suggested that N availability of soil, the rate of N deposition, and the internal N cycling of the ecosystem needs to be considered to predict the effects of acid rain on plants.

COMBINED EFFECTS OF SIMULATED ACIDIC RAIN AND OZONE ON
GROWTH OF FOREST TREE SPECIES

The reports on the combined effect of simulated acidic rain and ozone on growth of tree seedlings have received attention only after investigation on effects of acid rain alone on plants failed to reach a conclusion. In this section, the reports which examined the interaction between simulated acid rain and ozone will be reviewed.

Reich and Amundson (1985) reported no interaction between ozone and acid rain on photosynthesis and growth of four tree species (Sugar maple, eastern white pine, hybrid poplar, and northern red oak), although a linear reduction of net photosynthesis was correlated with a reduction in growth.

Reich et al. (1986a) examined the impact of ozone and simulated acid rain on photosynthesis, chlorophyll concentration, and growth in sugar maple and northern red oak seedlings. Two-year-old seedlings of both species were exposed to ozone (0.06, 0.09 or 0.12 ppm, 7 hr d⁻¹, 5 d wk⁻¹) and simulated acid rain (pHs of 5.6, 4.0, or 3.0, 1.25 hr wk⁻¹, 2.5 cm wk⁻¹) for six to ten weeks in all possible combinations. Sugar maple seedlings showed quadratic and linear reductions in height and diameter, respectively, with increasing ozone concentration. However, there was no significant effect of acid rain alone or

in combination with ozone on growth variables. Northern oak seedlings were not affected by any treatment except soil type.

In a study conducted on white pine seedlings, Reich et al. (1987) found a minimal interaction between ozone and acid rain, although there were significant interactions between acid rain and soil type with significant single effects of pH and ozone on growth observed. Treatments consisted of 0.02, 0.06, 0.10 and 0.14 ppm ozone in combination with rain pHs of 5.6, 4.0, 3.5, and 3.0.

Chappelka et al. (1985) found the effects of ozone and simulated acid rain more detrimental in combination than any single exposure on growth of yellow-poplar (Liriodendron tulipifera L.) seedlings. Nine-week-old half-sib seedlings were exposed to ozone (0.01, 0.10 ppm, 4 hr d⁻¹, 5 d wk⁻¹) in combination with simulated rain (pH 3.0, 4.3, or 5.6, 1 hr d⁻¹, 2 d wk⁻¹) for six weeks. Rain solutions contained ionic concentrations similar to the average ambient rainfall in southwestern Virginia, and the pHs were adjusted by adding 1M H₂SO₄ or 1M NaOH. Solution was applied to the seedlings at the rate of 0.75 cm hr⁻¹ before or after fumigation. Increasing acidity caused a linear decrease in mean root dry weight in seedlings exposed to 0.1 ppm ozone. Stem and root dry weight, shoot growth, relative growth rate, and leaf area were exhibited 10-15% in seedlings exposed to rain prior to fumigation compared

to those treated with rain after fumigation.

Similar interactions between ozone and acid rain were found in white ash seedlings (Chappelka and Chevone, 1986). Five-week-old white ash (Fraxinus americana L.) were exposed to ozone (0.00, 0.05, 0.10, or 0.15 ppm, 4 hr d⁻¹, 5 d wk⁻¹) in combination with acid rain (pH 3.0, 4.3 or 5.6, 1 hr d⁻¹, 2 d wk⁻¹, at 0.75 cm hr⁻¹) for five weeks. Biomass, root to shoot ratio (RSR), and relative growth rate (RGR) were inhibited linearly with increasing ozone concentration. Increasing rain acidity also induced a linear reduction in root dry weight, and the root to shoot ratio. Ozone and pH interactions were significant and caused a reduction in root dry weight, relative growth rate of roots, and root to shoot ratio at 0.05 ppm ozone. There was also a reduction in relative growth rate of roots and the root to shoot ratio at 0.10 ppm ozone with increasing acidity.

The combined effects of ozone and acid mist was observed by Skeffington and Roberts (1985) for Scots pine saplings. Three-year-old trees were exposed to fluctuating concentrations of ozone (0.00, 0.05, 0.1, or 0.15 ppm) and/or acid-mist (pH 2.0, twice daily, 5 d wk⁻¹) for 56 days. Fine root dry weight was significantly decreased in seedlings exposed to 0.1 ppm ozone. Acid mist alone reduced dry weight of the youngest needles on the basis of stem length. However, no significant interaction between ozone

and acid mist was observed for biomass.

Elliott et al. (1987) studied the impact of ambient ozone and simulated acid rain on growth and chlorophyll concentration of green and white ash seedlings. Two and three-year-old seedlings were exposed to ambient conditions in open-top chambers. Average pH of the rainfall was 4.1 and the ozone concentration exceeded 0.12 ppm for 78 hours during the three-year experiment period. There were no significant effects on shoot height growth and chlorophyll concentration in either species.

COMBINED EFFECTS OF OZONE AND WATER STRESS ON PLANTS

The combined effect of ozone and soil moisture was extensively studied after Heggstad and Middleton (1959) identified ozone as the causal agent of weather fleck on tobacco leaves. Tobacco weather fleck was found to occur simultaneously with high ozone concentrations and high soil moisture (Dean and Davis, 1967).

Dean and Davis (1967) found high soil moisture through irrigation increased the damage from weather fleck. They identified a protective effect of water stress through stomatal closure. Water stress may induce partial stomatal closure and reduce ozone uptake.

Later, Rosen et al. (1978) found that ozone induced stomatal closure and leaf injury were correlated with the

extent of stomatal closure in grapevines. Butler and Tibbitts (1979) also found a similar correlation between ozone damage and stomatal closure in beans (Phaseolus vulgaris L.).

Khatamian et al. (1973) found a protective effect of water stress from ozone injury in tomato (Lycopersicon esculentum Mill.) plants. They fumigated plants with ozone at 0.5 or 1.0 ppm for one hour. At the three-leaf stage, total dry matter was decreased in well watered plants by ozone treatment, but not in water stressed plants.

Olszyk and Tibbitts (1981) examined the effects of water stress and ozone exposure on stomatal response and leaf injury of peas (Pisum sativum L.). Water-stressed plants showed greater stomatal closure on the abaxial surface relative to the controls, and less ozone damage after ozone fumigation at 0.23 ppm for two hours.

Harkov and Brennan (1980) also found water stress reduced ozone damage in hybrid poplar trees (Populus maximowiczii x P. trichocarpa). Water was withheld for four days (mild stress) or nine days (severe stress) and trees were exposed to ozone at 0.1 ppm for six hours. Ozone injury was significantly lower in mild and severely water-stressed plants than in controls. Leaf resistance significantly increased in severely stressed plants before and after ozone fumigation. However, in mildly stressed plants, leaf resistance did not differ from the controls

until after fumigation with ozone. They concluded the high resistance in the severely stressed plants was the main contributor to protection from ozone damage.

Tingey et al. (1982) studied the effect of water stress on the susceptibility of plants to ozone. Beans (Phaseolus vulgaris L.) were subjected to various water stress regimes using polyethylene glycol for up to ten days and exposed to ozone at 0 - 1.0 ppm for two hours. The response of plants to ozone was measured by stress ethylene production. Stress ethylene production increased in non-droughted bean plants with increasing ozone concentration, but not in droughted plants. In the -300 KPa solution, complete protection was achieved after one day which was attributed to a reduction in leaf conductance, whereas a similar level of protection and leaf conductance was attained after three days in -80 KPa solution. From these results they concluded the reduced ozone sensitivity after water stress was clearly understood through reduction of leaf conductance.

It was observed that stomatal closure by ozone was affected not only by soil moisture but also by relative humidity (Rich and Turner, 1972). Stomatal resistance rapidly increased in water stressed bean plants after ozone exposure at 0.2-0.25 ppm for ten minutes. In moist atmosphere (RH=73), stomatal resistance of well-watered bean plants was not changed by ozone fumigation for 30 minutes.

In contrast, in dry atmosphere (RH=37), stomatal resistance increased by 81%. In a third experiment with tobacco plants, ozone-resistant cultivars closed stomata more rapidly than the ozone-susceptible in a dry atmosphere during fumigation with ozone for 60 minutes. On the other hand, in moist atmosphere, both cultivars showed a similar slow stomatal closure.

Tseng et al. (1988) studied the effects of ozone and water stress on three-year-old Fraser fir (Abies fraseri (Pursh) Poir.) seedlings. Seedlings were exposed to ozone at 0.02, 0.05, or 0.10 ppm (4 hr d⁻¹, 3 d wk⁻¹) in combination with three levels of moisture for ten weeks. Water stress significantly decreased biomass, transpiration and needle conductance, and increased water-use efficiency. However, ozone did not change biomass of Fraser fir seedlings, whereas photosynthesis significantly decreased in seedlings exposed to ozone at 0.10 ppm for five weeks. No significant interaction of ozone and water stress was found in any observations.

Tingey and Hogsett (1985) further studied the mechanism of reduction in ozone sensitivity by water stress. Beans (Phaseolus vulgaris L. cv 'Pinto III') were germinated and grown for 17 days and transferred to a water-stress system of -35 KPa (control) or -80 KPa. After four days of a water-stress system, some of the plants were sprayed with fusicoccin to induce stomatal opening, and on

the fifth day, the other plants were sprayed with ABA to induce stomatal closure. They used stress ethylene production and foliar chlorophyll concentration to monitor the effects of ozone on the plants. Water stress and ABA application increased leaf resistance which reduced ozone injury. Fusicoccin application significantly increased ozone injury in water-stressed plants. Therefore, they concluded water stress protects plants from ozone injury mainly through stomatal closure. However, these results do not imply that water stress does not affect the physiological process, but that stomatal closure by water stress has a more significant effect on the reduction of ozone injury.

Results from these studies (Rosen et al., 1978; Butler and Tibbitts, 1979; Khatamian et al., 1973; Olszyk and Tibbitts, 1981; Tingey et al., 1982; Rich and Turner, 1972; Tingey and Hogsett, 1985) indicate that water stress induces stomatal closure which lessens the amount of ozone taken up by plants, thus causing less ozone damage.

The effects of water stress on plants previously exposed to ozone was not studied until Heggstad et al. (1985) examined the combined effect of ozone and soil moisture stress (SMS) on soybean (Glycine max L.). They used five levels of ozone (charcoal-filtered, non-filtered, 0.03, 0.06, and 0.09 ppm) and two soil moisture stress regimes (averaging -0.05 MPa and -0.40 MPa at depths of 0.25 and 0.45 m). Bean yields were significantly reduced

by both ozone and SMS. The effect of ozone on yield was due to a decrease in seed size, whereas that of SMS was a reduction in seed number. The combination of ozone and SMS reduced yield, stomatal conductance, leaf size, leaf weight in a more than additive effect. They also found that plants seemed to acclimate to SMS in charcoal-filtered air but not in nonfiltered air. Although Cornic (1987) reported the detrimental effects of dehydration on five-year-old spruce (Picea abies L.) plants exposed to SO₂, investigations on the effects of water stress on plants previously exposed to ozone are lacking.

EFFECTS OF AIR POLLUTANTS ON GAS EXCHANGE OF PLANTS

It is well known that air pollutants affect plants without any visible injury. Ozone can affect biochemical and physiological progresses in plants. Therefore, studying the biochemical and physiological impact of air pollutants is essential to understand the mechanisms of the harmful effects of air pollution. In the following section, effects of air pollutants on gas exchange in plants will be reviewed.

Miller et al. (1969) fumigated three-year-old ponderosa pines (Pinus ponderosa Laws) with ozone at 0.15, 0.30, or 0.45 ppm for nine hours a day. Photosynthesis declined after 20 days exposure to 0.15 ppm ozone, whereas 0.30 ppm

ozone caused a significant reduction in only ten days. At the end of 30 days, photosynthesis rates were reduced by 10, 70, and 85% with exposure of 0.15, 0.30, or 0.45 ppm ozone, respectively.

Barnes (1972b) studied the effects of ozone on photosynthesis and respiration of slash pine (*Pinus elliotii*), pond pine (*P. serotina*), white pine (*P. strobus*), and loblolly pine (*P. taeda*). Seedlings were exposed to ozone at 0.05 or 0.15 ppm for five to eighteen weeks. Respiration was significantly stimulated in two-year-old eastern white pine, and eight-month-old slash, and loblolly pine seedlings exposed to 0.15 ppm ozone for 36 days. Net photosynthesis declined in all species with ozone exposure, but was not significant.

The effects of ozone on respiration, photosynthesis, ATP, and total adenylate concentration were examined in pinto bean (*Phaseolus vulgaris* L. var. Pinto) by Pell and Brennan (1973). Two-week-old bean seedlings were exposed to 0.25-0.30 ppm ozone for three hours. Net photosynthesis was reduced initially, but restored to the control level within 24 hours; whereas respiration significantly increased within 24 hours. ATP and total adenylate concentrations were immediately stimulated by ozone exposure. The authors concluded that the changes in photosynthesis and adenylate concentration were the initial effects of ozone injury which resulted in foliar symptoms and

increases in respiration.

Yang et al. (1983b) found similar effects of ozone on net photosynthesis and dark respiration of eastern white pine. Three eastern white pine clones differing in sensitivity to ozone were fumigated with 0.10, 0.20, or 0.30 ppm ozone for four hours. Net photosynthesis was significantly reduced by 7%, 14%, and 19% in all three clones exposed to ozone at 0.1, 0.20, and 0.30 ppm, respectively. Dark respiration significantly increased and light transpiration decreased in ozone-sensitive clone after ozone exposure.

In a study of effects of long-term exposure to ozone on photosynthesis and dark respiration of eastern white pine, Yang et al. (1983a) found contradictory results regarding dark respiration. In the ozone-sensitive clone, dark respiration was significantly declined and, in the ozone-insensitive and intermediate clone, dark respiration remained unchanged after 50 days exposure (4 hr d⁻¹) with ozone at 0.10, 0.20, or 0.30 ppm. Net photosynthesis was significantly reduced by ozone exposure at all concentrations in the sensitive clone.

Respiration results of Yang et al. (1983a) support the findings of MacDowell (1965), who found two stages of respirational response in tobacco leaves after ozone fumigation at 0.7 ppm for one hour. Respiration initially decreased, then increased within five hours, accompanied by visible symptoms of ozone damage.

Reich et al. (1986b) studied the effects of low concentrations of ozone on net photosynthesis and chlorophyll concentration of soybean. They fumigated soybean plants with 0.05, 0.09, 0.13 ppm ozone for six or eight hours every day for eight weeks. Net photosynthesis was significantly reduced and linearly correlated with ozone concentration for both individual leaves and for whole plants compared with the control (0.01 ppm ozone). Chlorophyll concentration also declined due to ozone treatments and was correlated with net photosynthesis for both individual leaves and for whole plants. However, there was no significant change in dark respiration.

Reich (1983) examined the effects of low concentrations of ozone on net photosynthesis, dark respiration, and chlorophyll concentrations of hybrid poplar (Populus deltoides x trichocarpa) plants. Plants were exposed to ozone at 0.04, 0.08, or 0.12 ppm, 5.5 hours a day, for 62 days. There was no immediate effect on photosynthesis regardless of ozone concentration (0.01-0.20 ppm), leaf age, and duration of ozone exposure. However, chronic exposure to ozone decreased net photosynthesis and leaf chlorophyll concentration, and increased dark respiration. The reduction of net photosynthesis was, in part, due to accelerated leaf aging.

Accelerated leaf aging with low concentration of ozone was found in poplar (Populus euramericana cv 'Dorskamp')

and 'Zeeland') by Mooi (1980). Seedlings were exposed to ozone at 0.04 ppm for 12 hours everyday for 23 weeks. Premature defoliation occurred in about 60% of stems.

Jensen and Roberts (1986) studied the effect of ozone on stomatal resistance in yellow poplar (Liriodendron tulipifera L.) seedlings at two humidity levels. One-year-old yellow poplar seedlings were exposed to ozone at 0.00 or 0.15 ppm in combination with 40% or 80% humidity for five hours for five consecutive days. No effect of ozone on leaf diffusive resistance was found at 40% humidity. However, at 80% humidity, leaf diffusive resistance was significantly increased by ozone exposure.

The impact of ozone on net photosynthesis in oat (Avena sativa) and duckweed (Lemna gibba) was examined by Forberg et al. (1987). Plants were exposed to ozone at 0.07, 0.18, or 0.25 ppm for two hours. Net photosynthesis was significantly reduced in oat seedlings exposed to concentrations above 0.07 ppm ozone and reduced in duckweed exposed to concentrations above 0.18 ppm ozone.

Roberts (1987) examined the effects of the antioxidant chemical N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N'-phenylurea (ethylenediurea, EDU) on photosynthesis of yellow-poplar seedlings with ozone treatment. After seven days of EDU treatment, two-year-old yellow-poplar seedlings were exposed to ozone at 0.35 or 0.95 ppm for three hours, and net photosynthesis was measured after two days. Ozone sig-

nificantly reduced net photosynthesis in yellow-poplar leaves without EDU, whereas the reduction of net photosynthesis was significantly lessened in seedlings treated with EDU. From the similar trends in stomatal conductance, the author proposed that the EDU effect on photosynthesis might be due to indirect stomatal response.

Reich and Amundson (1985) examined the impact of ozone and simulated acid rain in four tree species. Sugar maple (Acer saccharum), eastern white pine (Pinus strobus), hybrid poplar (Populus deltoides x trichocarpa), and northern red oak (Quercus rubra) seedlings were exposed to ozone at the range of 0.02 to 0.14 ppm (maximum doses were less than 30 ppm hour) in combination with simulated acid rain (pH 5.6, 4.0, or 3.0). Ozone induced a linear reduction in net photosynthesis in all species in relation to ozone concentration. However, simulated acid rain have neither significant single effect nor interaction with ozone on net photosynthesis for all species.

Reich et al. (1986a) examined the impact of ozone and simulated acid rain on photosynthesis, chlorophyll concentration, and growth in sugar maple and northern red oak seedlings. Two-year-old seedlings were exposed to ozone (0.06, 0.09 or 0.12 ppm, 7 hr d⁻¹, 5 d wk⁻¹) and simulated acid rain (pHs of 5.6, 4.0, or 3.0, 1.25 h wk⁻¹, 2.5 cm wk⁻¹) for six to ten weeks in all possible combinations. Net photosynthesis significantly decreased with increasing

ozone concentration compared with control (0.03 ppm ozone) seedlings. Chlorophyll concentration was increased in sugar maple leaves exposed to 0.09 and 0.12 ppm ozone. However, there was no significant effect of simulated acid rain on net photosynthesis and chlorophyll concentration and no interactions between acid rain and ozone for both species.

Reich et al. (1987) submitted white pine seedlings to ozone at 0.02, 0.06, 0.10, and 0.14 ppm in combination with acid rain at pHs of 5.6, 4.0, 3.5, and 3.0. for four months. In general, net photosynthesis significantly increased with increasing rain acidity, whereas the ozone treatment induced a linear reduction in net photosynthesis. The interaction between ozone and rain pH was minimal.

Effects of acid rain, alone, on gas exchange has received less attention. Ferenbaugh (1976) examined the effect of acid rain on O₂ release in beans. Bean (Phaseolus vulgaris L.) plants were exposed to sulfuric acid solutions with pHs of 5.5, 4.5, 3.5, 3.0, 2.5, 2.0 and 1.5 using hand-held atomizers. The rates of respiration and photosynthesis significantly increased with increasing rain acidity.

Neufeld et al. (1985) examined the effects of foliar applications of simulated acid rain on growth and photosynthesis of four deciduous tree species; yellow poplar (Liliodendron tulipifera L.), American sycamore (Platanus occidentalis L.), black locust (Robinia pseudo-acacia L.),

and sweetgum (Liquidambar styraciflua L.). Simulated acid rain contained deionized water with salts and acids in mg l^{-1} : Ca 0.23; Na 0.17; K 0.08; Mg 0.05; $\text{NH}_4\text{-N}$ 0.02; PO_4 0.07, and pH was adjusted to 5.6, 4.0, 3.0, and 2.0 by adding a 10:7:1 mixture of $\text{SO}_4\text{:NO}_3\text{:Cl}$. Simulated acid rain was applied for 20 minutes a day, every three days, for 48 days at a rainfall intensity of 4.75 cm hr^{-1} through nozzles. Photosynthetic rate declined in American sycamore seedlings exposed to rain of pH 2.0, but stomatal conductance did not change; whereas stomatal conductance of sweetgum seedlings was significantly reduced by acid rain of pH 2.0. This was the first report to link a reduction of biomass by acid rain exposure to photosynthetic change.

Seiler and Paganelli (1987) studied the effects of simulated acid rain on photosynthesis and growth of red spruce and loblolly pine. The rain solutions contained ionic components similar to average ambient rainfall in southwestern Virginia. The pH of the rain solution was adjusted to 4.3 or 3.0 by addition of a mixture of 1M H_2SO_4 and 0.5 M HNO_3 (1:1). Six-month-old red spruce and six-week-old loblolly pine seedlings were exposed to simulated acid rain by direct application to the soil. Photosynthesis was increased by 35% in red spruce seedlings exposed to pH 3.0 solution compared with pH 4.3. However, loblolly pine seedlings did not show any change in photosynthesis by solution pH.

EFFECTS OF OZONE ON PHOTOSYNTHATE PARTITIONING
IN FOREST TREE SPECIES

Extensive research on the impact of air pollutants on assimilate partitioning has been done with crop species which has clearly demonstrated that ozone alters the partitioning of photosynthate (Tingey et al. 1971, 1973; Ogata and Mass, 1973; Bennett and Oshima, 1976; Oshima et al., 1978; Bennett et al., 1979; Heagle et al., 1983; Foster et al., 1983). The effects of ozone on partitioning of assimilate varies with the plant species, because the demand by sinks differs from one plant species to another (Jacobson, 1982; Cooley and Manning, 1987). The various effects of ozone on photosynthate partitioning in forest species will be reviewed in this section.

Constantinidou and Kozlowski (1979) examined the effects of ozone on metabolite concentrations in elm (Ulmus americana). Four-month-old elm seedlings were exposed to ozone at 0.9 ppm for five hours. Ozone significantly reduced total nonstructural carbohydrate and protein of all plant parts within 24 hours after fumigation. At five weeks after fumigation, carbohydrate and protein concentrations had recovered to control levels in new leaves (<1 cm long at the time of fumigation), however, older leaves (>1 cm long) maintained a lower level of metabolites

than control seedlings throughout the five-week period.

Barnes (1972a) examined the impact of ozone on soluble sugar and ascorbate concentrations of pine seedlings. Four to six-week-old eastern white pine (Pinus strobus L.), short-leaf pine (P. echinata Mill.), slash pine (P. elliotii Englm.), pond pine (P. serotina Michx.), and loblolly pine (P. taeda L.) seedlings were fumigated with ozone at 0.05 or 0.15 ppm for up to 22 weeks. Total soluble carbohydrates, reducing sugars, and ascorbic acid were significantly increased in all species by ozone exposure at 0.05 ppm. Ozone at 0.15 ppm did not change ascorbic acid concentration, but increased total soluble carbohydrates and reducing sugars.

Jensen (1981a) fumigated one-year-old green ash seedlings with 0.5 ppm ozone, eight hours a day, five days a week, for up to six weeks, and measured leaf and stem dry weight and the concentration of starch, and reducing sugar and sucrose in the roots. Stem and leaf dry weight were significantly reduced in seedlings exposed to ozone compared with the control. Starch, reducing sugar and sucrose concentrations in the roots were significantly less in fumigated plants compared with non-fumigated plants.

Jensen (1981b) used a growth analysis technique to evaluate the effects of ozone on the partitioning of photosynthate in hybrid poplar cuttings. Hybrid poplar (Populus deltoides Bartr. x P. trichocarpa Torr. and Gray)

cuttings were exposed to ozone at 0.15 ppm for 12 hours everyday for seven weeks. Ozone significantly reduced relative growth rate (RGR), relative leaf-area growth rate (RLAGR), relative leaf-weight growth rate (RLWGR), and net assimilation rate (NAR). Reduction of RGR due to ozone treatment was linearly correlated with NAR, therefore, ozone might affect photosynthesis resulting in change of growth. RGR and RLWGR were also linearly related, which indicates the allocation pattern of the photosynthate was not directly affected by the pollutants.

Similar reductions of RGR, RLAGR, RLWGR, and NAR were observed by Jensen (1983) in silver maple seedlings exposed to ozone at 0.2 ppm for 12 hours daily up to 60 days. RGR and NAR were reduced by 80% and 74%, respectively between the 20th and 50th day of fumigation.

Jensen (1985) also reported RGR and NAR of one-year-old yellow poplar seedlings were reduced by ozone exposure at 0.1 ppm for 12 hours, one or two days a week for up to 20 weeks.

Tingey et al. (1976) studied the impact of ozone on metabolite pools in ponderosa pine (Pinus ponderosa Laws) seedlings. Ozone fumigation was initiated one week after seeding and maintained at 0 or 0.1 ppm, six hours a day, seven days a week for up to 20 weeks. In the tops of the ozone-treated plants, concentrations of soluble sugars, starch, and phenols increased but levels of nitrogen and

amino acids were not altered compared with the controls. In the roots, ozone reduced soluble sugars, starch, and phenol levels, and increased concentrations of nitrogen and amino acids. They proposed that the increase of sugars and phenols in the tops of the ozone-treated plants might be due to the reduced translocation of photosynthate.

To determine the effects of chronic air pollution on partitioning, photosynthesis, and growth of white pine trees, McLaughlin et al. used $^{14}\text{CO}_2$ (1982). They selected nine 25-year-old trees of differing sensitivities (three sensitive, intermediate and tolerant) based on needle color, length, and duration of retention. Even though, net photosynthesis of sensitive trees was not different from tolerant trees, the ratio of dark respiration to net photosynthesis in sensitive trees was significantly higher than in tolerant trees. The retention of ^{14}C -photosynthate was significantly higher in foliage and branches of sensitive trees. The authors proposed such factors as reductions in needle longevity and size, increase of respiration, and change of allocation due to air pollutants may be involved in white pine decline.

Reich and Lassoie (1985) studied the effect of ozone on growth, partitioning, and leaf senescence in hybrid poplar (Populus deltoides x trichocarpa) plants. Plants were exposed to ozone at 0.03, 0.06, or 0.09 ppm, five and a half hours a day, seven days a week, for ten weeks. Ozone

significantly decreased height and diameter growth, number of leaves per plant, and dry weight of shoots and roots. However, partitioning of dry matter was not changed by ozone treatment.

Chappelka et al. (1985) fumigated nine-week-old, half-sib, yellow-poplar with 0.1 ppm ozone, 0.08 ppm SO₂ or 0.10 ppm ozone + 0.08 ppm SO₂, four hours a day, five days a week, for six weeks in combination with simulated rain (pH 3.0, 4.3, or 5.6, 1 hr d⁻¹, 2 d wk⁻¹ at 0.75 cm hr⁻¹). Ozone did not affect root to shoot ratio (RSR) and leaf weight ratio (LWR, leaf weight/total weight), while the combination of ozone and SO₂ significantly reduced RSR and increased LWR. No effects of rain pH or the pollutant x rain pH interaction were observed for RSR and LWR.

Chappelka and Chevone (1986) examined the impact of ozone and acid rain on white ash (Fraxinus americana L.) seedling growth. Five-week-old seedlings were exposed to ozone (0.00, 0.05, 0.10, or, 0.15 ppm, 4 hr d⁻¹, 5 d wk⁻¹) in combination with simulated rain (pH 3.0, 4.3, or 5.6, 1 hr d⁻¹, 2 d wk⁻¹, at 0.75 cm hr⁻¹) for five weeks. Root to shoot ratio (RSR) was significantly declined as ozone concentration and rain acidity. Significant combined effects of rain pH and ozone were observed. At 0.05 ppm and 0.10 ppm ozone, RSRs significantly declined by 20% and 23%, respectively, in seedlings exposed to pH 3.0 rain compared with pH 5.6.

EFFECTS OF AIR POLLUTANTS ON FOLIAR NUTRIENT CONCENTRATION

Acid rain affects plants not only by increased acidity but also by added nutrients, usually nitrate and sulphate, which may result in altered nutrient assimilation and foliar nutrient concentration. Other pollutants, especially ozone and SO_2 , also are known to alter foliar nutrient concentrations (Skeffington and Robert, 1985; Pratt et al., 1983a, b). Changes in nutrient concentration induced by acid rain may result in altered growth and productivity of plants. These matters will be presented in this section.

Wood and Bormann (1977) examined the effect of simulated acidic rain on nutrient relations in eastern white pine. Simulated acid rain consisted of H_2SO_4 , HNO_3 , and HCl added to distilled water and adjusted to pH 5.6, 4.0, 3.3, 3.0, or 2.3. Seedlings were exposed to simulated acid rain at a rate of 4.2 cm wk^{-1} for 20 weeks. Organic N levels in the plant tissue were correlated with nitrate levels in simulated acid rain, being lowest at pH 5.6 and highest at pH 2.3, which reflected the fertilization effect of nitrate on seedlings exposed to acid rain. Foliar concentrations of K, Mg, and Ca declined at pH 3.0 and below. These decreases were thought to be a result of low levels of available cations in soils or by an increase in foliar leaching

expected at low pH levels.

Tveite (1980) examined the effects of acid rain on foliar nutrient concentrations of three species of conifers in field experiments. After three to six years of treatment with groundwater acidified with sulphuric acid, concentrations of P, Mn, and Fe were increased in current years needles of lodgepole pine saplings exposed to pH 3.0 compared to pH 5.6. Previous years needles showed increased concentrations of P, K, Mn, Fe, Al, and S at pH 3.0 compared with pH 5.6. Norway spruce saplings showed increased concentrations of S and sulphate with pHs of 3.0 and 2.5 for both current and previous years needles. However, Mg concentration was decreased in current years needles of Norway spruce at pH 2.5. Scotch pine saplings showed increased S and sulphate concentrations at pH 2.0 for both needle ages. Increased sulphate and decreased Mg concentrations were observed in current years needles of scotch pine at pH 2.5.

Proctor (1983) studied the effects of simulated acid rain on foliage, yield, and foliar nutrients of mature bearing apple trees at pHs of 5.6, 4.0, 3.0, 2.0, and 1.5. Solutions were prepared by mixing H_2SO_4 with distilled water, and then sprayed on limbs of trees. Foliar damage due to acid rain appeared at pH 3.0 and foliar lesions increased in size with increasing acidity of rain solution after ten applications of acid rain, once per week. The

concentration of N was less in leaves which received acid rain at pHs of 1.5 and 2.0 compared with other treatments after 12 applications. However, concentrations of K^+ , Ca^{2+} , Mg^{2+} were not changed.

Abouguendia and Baschak (1987) examined the response of jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss) to simulated acid rain. The rain solution consisted of similar ion concentrations as that of precipitation at the Cree Lake CANSAP (Canadian Network for Sampling Precipitation) station, located 440 km north of Prince Albert. The pH of the final rain solutions was adjusted by adding appropriate amounts of H_2SO_4 and HNO_3 . Two-year-old jack pine and white spruce seedlings were exposed to simulated acid rain through a nozzle at 2.2 cm for 25 min, once per week, for seven weeks. No significant changes were observed in foliar nutrients of both species except S concentration of white spruce. Needle S concentration significantly increased in white spruce exposed to acid rain at pH 2.6 compared with all other treatments. The concentration of chlorophyll b significantly decreased in white spruce exposed to simulated acidic rain at pH 2.6. No other treatments changed the concentrations of chlorophyll a and b in either species.

The combined effects of ozone and acid mist on nutrient concentrations of Scots pine needles were examined by Skelfington and Roberts (1985). Three-year-old trees were

exposed to fluctuating concentrations of ozone (0.00, 0.05, 0.1, or 0.15 ppm) and/or acid-mist (pH 2.99, twice per day, 5 d wk⁻¹) for 56 days. Ozone significantly increased the concentrations of Mg, K and P of the needles. However, acid mist showed neither significant effect nor an interaction with ozone. Ozone also significantly reduced the foliar uptake of nitrate from the acid mist, although nitrogen concentrations of the needles were not measured.

Tingey et al. (1976) reported that ozone treatment (0.1 ppm, 6 hr d⁻¹, 7 d wk⁻¹, for up to 20 wks) did not change the N concentration in the tops but increased the level in the roots of one-week-old ponderosa pine seedlings.

Pratt et al. (1983b) examined the effects of ozone and SO₂ on foliage and S and chlorophyll concentrations in soybean (Glycine max L.). Twenty-five-day-old soybean seedlings were exposed to ozone at 0.10 ppm and/or SO₂ at 0.40 ppm, two hours or four hours a day, for up to five consecutive days. No significant effect of SO₂ was observed on foliage and chlorophyll concentration. However, S concentration in the first trifoliolates significantly increased by 50.3% after a single four-hour fumigation and by 94% after five days of fumigation (2 hr d⁻¹) with 0.4 ppm SO₂. Ozone alone caused foliar injury after four days of fumigation (2 hr d⁻¹) and reductions of chlorophyll concentration of the first trifoliolates after five days of fumigation (2 hr d⁻¹). However, the sulphur concen-

tration of the first trifoliolates was not changed compared with controls. Combined effects of ozone and SO_2 were greater than additive for foliar injury and chlorophyll concentration. However, foliar sulphur accumulation was less with the combined fumigation of SO_2 and ozone compared with ozone or SO_2 alone.

Baes and McLaughlin (1984) examined trace elements in tree rings of short-leaf pine (*Pinus echinata*) in East Tennessee. Annual growth was suppressed and Fe concentration increased between 1863 and 1912, when SO_2 was being released from copper ore smelting. A similar decline of annual ring growth and increase of trace metals were observed in rings formed in the past 20 to 25 years, when SO_2 emission increased in twofold after reconstruction. From these results, the authors suggested that the recent increase of many trace metals in xylem might be due to the increased deposition of fossil-fuel combustion products.

FACTORS AFFECTING ROOT HYDRAULIC CONDUCTIVITY IN PLANTS

Water is one of the most important substances for the existence of life. It is essential for plants as a constituent of organisms, solvent, reactant in metabolic processes, and maintainer of turgidity (Kramer, 1983). However, water is often depleted in ecosystems. Water stress in plants occurs whenever transpirational demand for water

vapor in the atmosphere exceeds water supply to the root system. Water supply is not only a function of the available water in the soil, but is also a function of the ability of the roots of a plant to take up water from the soil, better known as root hydraulic conductivity (Fiscus, 1975).

Roots are generally the most important rate-limiting barrier to water movement (Lopushinsky, 1964; Emmert, 1972; Shaner and Boyer, 1976a, b). Therefore, it is very important to examine the root hydraulic conductivity (L_p) to understand the water relations of plants.

Hansen (1974) examined the root hydraulic conductance of the seminal root of wheat seedlings. Six to seven-day-old seedlings which had only a seminal root were transferred to perspex tubes, and hydraulic conductivity of individual 1 cm regions of single seminal roots were measured. Root hydraulic conductance was highest between 2 and 6 cm behind the tip. The highest conductivity was due to the presence of mature xylem vessels and non-suberized tissue surface in this region. Outside of this 4 cm region, the conductance decreased dramatically.

Sands et al. (1982) also found the average hydraulic conductivity was 2.5-fold higher in the unsuberized roots than in the suberized roots of eight-month-old loblolly pine.

Syvertsen et al. (1983) studied the effects of temperature and flooding on L_p of citrus. Two-month-old rough

lemon (Citrus jambhiri Lush.) and sour orange (C. aurantium L.) seedlings were grown at constant soil temperatures of 16, 24, or 33° C for three months. Root hydraulic conductivity was highest in seedlings grown at a soil temperature of 16° C for both species. Since there was more evaporation from the soil at 33° C than at the cooler temperature, the plants grown at 33° C might have experienced more water stress, and water stress might decrease L_p .

Markhart et al. (1979) analyzed the effects of temperature on water transport in soybean by using an Arrhenius plot. For the first experiment, soybean was grown at a 28/33° C thermoperiod for 30 days and L_p was measured at various temperatures. The break point in the slope occurred at 14.7° C, which means roots of soybeans showed increased activation energy at temperatures below 14° C. In a second experiment, they compared soybeans grown at a 28/23° C thermoperiod with those grown at a 17/11° C thermoperiod. The break point was shifted from 14° C to 8.7° C, which indicated that the chill sensitivity of L_p would be relieved somewhat by low temperature acclimation. The authors concluded the membrane might be altered by the chilling acclimation.

Later, the increase in activation was interpreted in terms of cell membrane properties which affected the conducting path at the lower temperature. The cause of the conductance behavior of the roots was examined by Markhart

et al. (1980), who measured the total fatty acids in the root system. Chilling-sensitive soybean and chilling-tolerant broccoli were grown at a thermoperiod of 29/23° C or 17/11° C for two to four weeks. Double bond index and the linolenic: linoleic acid ratio of fatty acids was higher in both plants grown at a 17/11° C than 29/23° C thermoperiod. There was no significant difference between chilling-sensitive soybean and chilling-tolerant broccoli grown at a 17/11°C thermoperiod, however, the chilling-tolerant broccoli showed a greater increase in double bond index and the linolenic: linoleic acid ratio of fatty acids than chilling-sensitive soybean.

In general, chilling temperatures increase the unsaturated fatty acids (Markhart et al., 1980) and high quantities of unsaturated fatty acids in membrane phospholipids may prevent phase transitions at chilling temperatures and maintain the fluidity and hydraulic conductance of membranes.

It is apparent that abscisic acid (ABA) plays a key role in the regulation of physiological processes which maintain water balance in plants (Karmoker and Van Steveninck, 1978). Increases in ABA by water stress have been well documented (Beardsell and Cohen, 1975; Bengston et al., 1977; Harrison and Walton, 1975). Therefore, any possible effects of ABA on root hydraulic conductance might be important for water balance in plants. At the present

time, however, ABA effects on L_p are inconclusive.

Most of the early experiments regarding the effects of ABA on conductance utilized decapitated roots. Collins and Kerrigan (1974) examined the effects of kinetin and ABA on water transport in isolated maize roots. Water flow rate was increased by 53% with addition of 1 mM ABA, and 0.1 mM ABA showed maximum stimulation effects. In contrast, application of 1 mM kinetin dramatically decreased water flow rate.

Glinka (1973) also found a similar stimulation effect of ABA on root exudation in decapitated sunflower plants, and the increase was related to stimulation in permeability of plant cell membranes.

Tal and Imber (1971) reported ABA caused a stimulation in exudation rate from decapitated roots of tomato. Application of ABA (10 mg l^{-1}), five times a day for three days, significantly increased exudation more than two-fold in both mutant and normal plants.

Stimulatory effects of ABA were criticized by Glinka (1977) on the grounds that decapitated roots were used, an effect which might have no relevance in an intact root of a transpiring plant. In a study on effects of ABA and hydrostatic pressure gradient on water movement (Glinka, 1977), five-day-old sunflowers were decapitated and the cut stumps were connected to a graduated pipette. The other end of the pipette was connected to a suction system and the pressure

was monitored with a mercury manometer. Suction applied at 50 cmHg increased by three-fold the exudation rate compared with no suction applied. Application of 27 mM ABA increased water flow rate by 180-190% at all of the suction applied from 0 to 60 cmHg.

Water flow rate can be altered by ion transport as well as L_p . Therefore, ABA-induced changes in water flow rate need to be separated into two portions. Glinka (1980) examined the effects of ABA on water flow rate and release of ions to xylem of sunflower root systems bathed in either a nutrient or 0.2 mM CaSO_4 solution. ABA application increased K^+ and NO_3^- fluxes by 250% and 230%, respectively, for roots bathed in CaSO_4 solution, whereas, in nutrient solution, both fluxes were increased by 60%. Flow rate also increased 358% in roots bathed in CaSO_4 solution relative to the nutrient solution which indicates that ABA increased the flow of ions from the vacuoles to the cytoplasm. In nutrient solution, root hydraulic conductivity was increased three-fold by ABA treatment, whereas in CaSO_4 solution, it was enhanced five-fold. The author concluded that ABA application not only increased the flow of ions from the vacuole to the cytoplasm, but also directly enhanced root hydraulic conductivity.

Karmoker and Van Steveninck (1978) reported similar stimulation effects of ABA on flow rate with bean plants. ABA increased volume flow rate by six to eight fold in

excised bean root systems bathed in 0.5 mM KCl + 0.1 mM CaSO₄ solution: One mM ABA treatment showed the maximum stimulation effect. The authors also examined the interaction of ABA and carbonyl cyanide-m-chlorophenyl hydrazone (CCCP) on K⁺ transport in excised bean root systems. The application of CCCP inhibited K⁺ transport and nullified the stimulatory effects of ABA on volume flow rate. Therefore, the authors concluded that the observed stimulatory effect of ABA was due to changes in ion transport rate rather than a direct change of root hydraulic conductivity.

Erlandsson et al. (1978) reported contradictory effects of ABA on ion and water uptake in sunflower roots. K-uptake was decreased by 30-70% with treatment of 20mM ABA, however, water uptake was not significantly changed.

Cram and Pitman(1972) found a similar inhibitory effect of ABA on ion uptake in excised barley and maize roots. Application of 27 mM ABA reduced uptake of K, Rb, and Cl ions to 20% of the control value. However, water flow rate was also significantly reduced by ABA treatment, and this reduction was due to the inhibition of potassium secretion rather than the change of root hydraulic conductivity.

In the later studies, Pitman and Wellfare (1978) examined the effect of ABA on ion transport and hydraulic conductivity, separately, in barley seedling. Application of ABA did not significantly change root hydraulic conductivity. However, ABA significantly reduced K⁺, Na⁺, Mg²⁺,

Ca²⁺, and phosphate in the exudate. The authors concluded that the reduction of water flow rate by ABA treatment was due to the inhibition of ion transport into the xylem rather than to direct reduction of permeability of the roots to water.

Fiscus (1981) criticized much of the past research about effects of ABA on water flow rate. Effects of ABA on L_p have been difficult to determine in excised roots with little or no hydrostatic pressure difference applied since the volume flux in these systems has been dominated by ion transport. In studies of the effects of ABA on hydraulic conductance and ion transport through bean root systems, Fiscus (1981) applied ABA at a rate of 0.1 mg cm⁻² leaf area with 4.1 bars of hydrostatic pressure. The high pressure applied would rapidly dilute and wash away the solutes from the osmotically active sites and would not affect the volume flow rate. The peak response of volume flow rate was correlated with the peak release of solutes. After the initial peak release of solutes was complete, solute flux steadily increased but volume flow rate steadily decreased. From the results, Fiscus concluded that ABA might increase ion transport, but decrease root hydraulic conductivity of bean roots.

Effects of ABA on water and ion transport are inconclusive because of lack of agreement at the present time.

Root hydraulic conductivity can also be affected by

root age (Fiscus and Markhart, 1979), mycorrhizae (Nelsen and Safir, 1982; Nelsen et al., 1981), nutrients (Radin and Eidenbock, 1984; Morgan, 1986), and disease (Dawson and Weste, 1982; Olsen et al., 1983; Tzeng et al., 1985).

In bean plants, Fiscus and Markhart (1979) observed a rapid increase in root hydraulic conductivity until 15-day-old and then a decrease.

Although Sands et al. (1982) did not find any effects of mycorrhizal infection on Lp in loblolly pine seedlings, Nelsen and Safir (1982) found root hydraulic conductance was significantly increased by mycorrhizal infection in onion plants grown in low soil P conditions. However, there were no significant effects of mycorrhizal infection on Lp in plants grown under high soil P conditions. The authors concluded that P nutrition might be a very important factor involved in mycorrhizal effects on water relations in onion plants. A similar interaction between nutrient status and mycorrhizal infection had been reported by Safir et al. (1972). Mycorrhizal infection significantly increased Lp in soybean plants, but the application of nutrients to the soil nullified the stimulatory effects of mycorrhizae.

Some soil-borne fungi which cause root rot of plants are known to induce abnormal water relations (Dawson and Weste, 1982; Olsen et al., 1983; Tzeng et al., 1985). Infection by Phytophthora cinnamomi caused a significant reduction in transpiration, relative water content and leaf

water potential of Isopogon creatophyllus plants which are highly susceptible to P. cinnamomi. Eucalyptus goniocalyx which is resistant to P. cinnamomi showed less infection-associated variation (Dawson and Weste, 1982).

Tzeng et al. (1985) also reported infection by Verticillium dahliae caused a lower water potential than that measured in healthy cotton plants. Olsen et al. (1985) found that resistance to water flow in roots of cotton plants infected by Phymatotrichum omnivorum was increased more than ten-fold compared with healthy plants, which was the main cause of wilting in diseased plants.

Nutrients are also known to affect root hydraulic conductivity in plants. Radin and Eidenbock (1984) reported low levels of phosphorus in nutrient solution decreased root hydraulic conductivity of cotton plants, resulting in limitation of leaf expansion.

Morgan (1986) examined the effects of N nutrition on the water relations and gas exchange of wheat (Triticum aestivum L.) plants. Three-week-old plants were treated with full-strength Hewitt's nutrient solution containing 12 or 1 mM NO₃⁻ N. High-N plants showed greater sensitivity of leaf water potential and leaf conductance to CO₂ as well as greater photosynthetic capacity compared with low-N plants.

LITERATURE CITED

- Abouguendia, Z. M. and L. A. Baschak. 1987. Response of two western Canadian conifers to simulated acidic precipitation. *Water, Air, & Soil Pollut.* 33:15-12.
- Adams, H. S., S. L. Stephenson, T. J. Blasing, and D. N. Duvick. 1985. Growth-trend declines of spruce and fir in Appalachian subalpine forests. *Environ. Exp. Bot.* 25:315-325.
- Baes III, C. F. and S. B. Mclaughlin. 1984. Trace elements in tree rings: Evidence of recent and historical air pollution. *Science* 224:494-497.
- Baldwin, H.I. 1934. Germination of the red spruce. *Plant Physiol.* 9:491-532.
- Barnes, R. L. 1972a. Effects of chronic exposure to ozone on soluble sugar and ascorbic acid contents of pine seedlings. *Can. J. Bot.* 50:215-219.
- Barnes, R. L. 1972b. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. *Environ. Pollut.* 3:133-8.
- Beck, D. E. and L. Della-Bianca. 1981. Yellow-poplar: Characteristics and management. *USDA For. Serv. Agric. Handb.* 583, 91pp.
- Bengston C., C. O. Falk and S. Larson. 1977. The aftereffect of water stress on transpiration rate and changes in abscisic acid content of young wheat plants. *Physiol. Plant.* 41:149-154.
- Benoit, L. F., J. M. Skelly, L. D. Moore and L. S. Dochinger. 1982. Radial growth reductions of Pinus strobus L. correlated with foliar ozone sensitivity as an indicator of ozone-induced losses in eastern forests. *Can. J. For. Res.* 12:673-678.
- Bennett, J. P. and R. J. Oshima. 1976. Carrot injury and yield response to ozone. *J. Amer. Soc. Hort. Sci.* 101:638-639.
- Bennett, J. P., R. J. Oshima and L. F. Lippert. 1979. Effects of ozone on injury and dry matter partitioning in pepper plants. *Environ. Expt. Bot.* 19:33-39.
- Berry, C. R. 1974. Age of pine seedlings with primary needles affects sensitivity to ozone and sulfur diox-

ide. *Phytopathology* 64:207-209.

- Blanpied, G. D. 1979. Effect of artificial rain water pH and calcium concentration on the calcium and potassium in apple leaves. *HortScience* 14:706-708.
- Bosch, Chr., E. Pfannkuch, K. E. Rehfuess, K. H. Runkel, P. Schramel and M. Senser. 1986. Einfluß einer Düngeung mit Magnesium und Calcium, von Ozon und saurem Nebel auf Frostharte, Ernährungszustand und Biomasseproduktion junger Fichten (*Picea abies* [L.] Karst.). *Forstw. Cbl.* 105:218-229.
- Bruck, R. I. 1984. Decline of montane boreal ecosystems in central Europe and the southern Appalachian Mountains. In Research and Development Conference. Technical Association of the Pulp and Paper Industry. Atlanta, GA. pp. 159-163.
- Butler L. K. and T. W. Tibbitts. 1979. Stomatal mechanisms determining genetic resistance to ozone in *Phaseolus vulgaris* L. *J. Amer. Soc. Hort. Sci.* 104:213-216.
- Carey, A. C., E. A. Miller and G. T. Geballe. 1984. *Armillaria mellea* and decline of red spruce. *Plant Disease* 68:794-795.
- Chappelka III, A. H., B. I. Chevone and T. E. Burk. 1985. Growth response of yellow-poplar (*Liriodendron tulipifera* L.) seedlings to ozone, sulfur dioxide, and simulated acidic precipitation, alone and in combination. *Environ. Exp. Bot.* 25:233-244.
- Chappelka III, A. H. and B. I. Chevone. 1986. White ash seedling growth response to ozone and simulated acid rain. *Can. J. For. Res.* 16:786-790.
- Cogbill, C. V. and C. E. Likens. 1974. Acid precipitation in the northeastern United States. *Water Resour. Res.* 10:1133-1137.
- Collins, J. C. and A. P. Kerrigan. 1974. The effect of kinetin and abscisic acid on water and ion transport in isolated maize roots. *New Phytol.* 73:309-314.
- Constantinidou, H. A. and T. T. Kozlowski. 1979. Effects of sulfur dioxide and ozone on *Ulmus americana* seedlings. II. Carbohydrates, proteins, and lipids. *Can. J. Bot.* 57:176-184.
- Cooley, D. R. and W. J. Manning. 1987. The impact of ozone

- on assimilate partitioning in plants: A review. *Environ. Pollut.* 47:95-113.
- Cornic, G. 1987. Interaction between sublethal pollution by sulphur dioxide and drought stress. The effect on photosynthetic capacity. *Physiol. Plantarum* 71:115-119.
- Cram, W. J. and M. G. Pitman. 1972. The action of abscisic acid on ion uptake and water flow in plant roots. *Aust. J. Biol. Sci.* 25:1125-1132.
- Dawson, P. and G. Weste. 1982. Changes in water relations associated with infection by Phytophthora cinnamomi. *Aust. J. Bot.* 30:393-340.
- Dean, C. E., and D. R. Davis. 1967. Ozone and soil moisture in relation to the occurrence of weather fleck on florida cigar-wrapper tobacco in 1966. *Plant Dis. Reprtr.* 51:72-75.
- Delmas, R. J., A. Aristarain and M. Legrand. 1980. Acidity of Antarctic snow: A natural reference level for acid rains. pp. 104-105. In Ecological impact of acid precipitation. D. Drablos and A. Tollan (eds.). SNSF Project, Oslow, Norway.
- Dochinger, L. S. 1976. Effects of soil applications of acidified solutions on growth and survival of forest tree species. *Proc. Amer. Phytopathol. Soc.* 3:304.
- Duchelle, S. F., J. M. Skelly and B. I. Chevone. 1982. Oxidant effects on forest tree seedling growth in the Appalachian Mountain. *Water, Air & Soil Pollut.* 12:363-373.
- Duchelle, S. F., J. M. Skelly, T. L. Sharik, B. I. Chevone, Y. S. Yang and J. E. Nellessen. 1983. Effects of ozone on the productivity of natural vegetation in a high meadow of the Shenandoah Park of Virginia. *J. Environ. Mgt.* 17:299-308.
- Elliott, C. L., J. C. Eberhardt and E. G. Brennan. 1987. The effect of ambient ozone pollution and acidic rain on the growth and chlorophyll content of green and white ash. *Environ. Pollut.* 44:61-70.
- Emmert, F. H. 1972. Effect of time, water flow, and pH on centripetal passage of radiophosphorus across roots of intact plants. *Plant Physiol.* 50:332-335.
- Erlandsson G. S., S. Pettersson and S. Svensson. 1978. Rapid effects on ion uptake in sunflower roots. *Phy-*

- siol. Plant. 43:380-384.
- Evans, L. S., N. F. Gmur and F. Dacosta. 1978. Foliar response of six clones of hybrid poplar to simulated acid rain. *Phytopathology* 68:847-856.
- Evans, L., N. F. Gmur and D. Mancini. 1982. Effects of simulated acidic rain on yields of Raphanus sativus, Lactuca sativa, triticum aestivum and Medicago sativa. *Environ. Exp. Bot.* 22:445-453.
- Evans, L. S. 1984a. Botanical aspects of acidic precipitation. *The Botanical Review* 50:449-490.
- Evans, L. S. 1984b. Acidic precipitation effects on terrestrial vegetation. *Ann. Rev. Phytopathol.* 22:397-420.
- Fairfax, J. A. W. and N. W. Lepp. 1975. Effect of simulated 'acid rain' on cation loss from leaves. *Nature, Lond.* 255:324-325.
- Ferenbaugh, R. W. 1976. Effects of simulated acid rain on phaseolus vulgaris L. (Fabaceae). *Amer. J. Bot.* 63:283-288.
- Fiscus, E. L. 1975. The interaction between osmotic- and pressure-induced water flow in plant roots. *Plant Physiol.* 55:917-922.
- Fiscus, E. L. 1981. Effects of abscisic acid on the hydraulic conductance of and total ion transport through Phaseolus root systems. *Plant Physiol.* 68:169-174.
- Fiscus, E. L. and A. H. Markhart. 1979. Relationships between root system water transport properties and plant size in Phaseolus. *Plant Physiol.* 64:770-773.
- Forberg, E., H. Aarnes and S. Nilsen. 1987. Effects of ozone on net photosynthesis oat (Avena sativa) and duckweed (Lemna gibba). *Environ. Pollut.* 47:285-291.
- Foster, K. W., H. Timm, C. K. Labanaukas and R. J. Oshima. 1983. Effects of ozone and sulfur dioxide on tuber yield and quality of potatoes. *J. Environ. Qual.* 12:75-80.
- Fowells, H. A. 1965. Silvics of forest trees of the United States. USDA For. Serv. Agric. Handb. 271, pp. 256-265.
- Friedland, A. J., A. H. Johnson and T. G. Siccama. 1984a. Trace metal content of the forest floor in the Green Mountains of Vermont: Spatial and temporal patterns.

- Water Air & Soil Pollut. 21:161-170.
- Friedland, A. J., A. H. Johnson, T. G. Siccama and D. L. Lader. 1984b. Trace metal profiles in the forest floor of New England. *Soil Sci. Soc. Amer. J.* 48:422-425.
- Glinka Z. 1973. Abscisic acid effect on root exudation related to increased permeability to water. *Plant Physiol.* 51:217-219.
- Glinka Z. 1980. Abscisic acid promotes both volume flow and ion release to the xylem in sunflower roots. *Plant Physiol.* 65:537-540.
- Hansen, G. K. 1974. Resistance to water transport in soil and young wheat plants. *Acta Agric. Scand.* 24:37-48.
- Hammer, C. U. 1977. Past volcanism revealed by Greenland ice sheet impurities. *Nature* 270:482-486.
- Harkov, R. and E. Brennan. 1980. The influence of soil fertility and water stress on the ozone response of hybrid poplar trees. *Phytopathology* 70:991-994.
- Harrison, M. A., and D. C. Walton. 1975. Abscisic acid metabolism in water stressed bean leaves. *Plant Physiol.* 56:250-254.
- Heagle, A. S., D. E. Body, and C. E. Neely. 1974. Injury and yield responses of soybean to chronic doses of ozone and sulfur dioxide in the field. *Phytopathology* 64:132-136.
- Heagle, A. S., M. B. Letchworth and C. A. Mitchell. 1983. Injury and yield responses of peanuts to chronic doses of ozone in open-top field chambers. *Phytopathology* 73:551-555.
- Heggestad, H. E. and J. T. Middleton. 1959. Ozone in high concentrations as cause of tobacco leaf injury. *Science* 129:208-210.
- Heggestad, H. E., T. J. Gish, F. H. Lee, J. H. Bennett and L. W. Douglass. 1985. Interaction of soil moisture stress and ambient ozone on growth and yield of soybeans. *Phytopathology* 75:472-477.
- Howell, R. K., E. J. Koch and L. P. Rose., Jr. 1979. Field assessment of air pollution-induced soybean yield losses. *Agron. J.* 71:285-288.
- Jacobson, J. S. 1982. Ozone and the growth and productivity

- of agricultural crops. In: Effects of gaseous air pollution in agriculture and horticulture. M. H. Unsworth and D. P. Ormrod (eds.). pp. 293-304. Butterworths Scientific, London.
- Jensen, K. F. 1973. Response of nine forest tree species to chronic ozone fumigation. *Plant Dis. Repr.* 57:914-917.
- Jensen, K. F. 1981a. Ozone fumigation decreased the root carbohydrate content and dry weight of green ash seedlings. *Environ. Pollut. (ser. A)*. 26:147-152.
- Jensen, K. F. 1981b. Growth analysis of hybrid poplar cuttings fumigated with ozone and sulphur dioxide. *Environ. Pollut.* 26:243-50.
- Jensen K. F. 1983. Growth relationships in silver maple seedlings fumigated with O₃ and SO₂. *Can. J. For. Res.* 3:298-302.
- Jensen, K. F. 1985. Response of yellow poplar seedlings to intermittent fumigation. *Environ. Pollut. (ser. A)*. 38:183-191.
- Jensen, K. F. and B. R. Roberts. 1986. Changes in yellow poplar stomatal resistance with SO₂ and O₃ fumigation. *Environ. Pollut. (ser. A)*. 41:235-245.
- Johnson A. H., T. G. Siccama, D. Wang, R. S. Turner and T. H. Barringer. 1981. Recent changes in patterns of tree growth rate in the New Jersey pinelands: a possible effect of acid rain. *J. environ. Qual.* 10:427-430.
- Johnson, A. H., T. G. Siccama and A. J. Friedland. 1982. Spatial and temporal patterns of lead accumulation in the forest floor in the northeastern United States. *J. Environ. Qual.* 11:577-580.
- Johnson, A. H. 1983. Red spruce decline in the northeastern U. S.: Hypotheses regarding the role of acid rain. *JAPCA* 33:1049-1054.
- Johnson, A. H. and T. G. Siccama. 1983. Acid deposition and forest decline. *Environ. Sci. Tech.* 17:294-305.
- Johnson, A. H. and T. G. Siccama. 1984. Decline of red spruce in the northern Appalachians: Assessing the possible role of acid deposition. *TAPPI* 67:68-72.
- Junge, C. E. and R. T. Werby. 1958. The concentration of chloride, sodium, potassium, calcium and sulfate in rain water over the U. S. *J. Meteorol.* 15:417-425.

- Karmoker J. L. and R. F. M. Van Steveninck. 1978. Stimulation of volume flow and ion flux by abscisic acid in excised root systems of Phaseolus vulgaris L. cv. Redland Pioneer. *Planta* 141:37-43.
- Khatamian, H., N. O. Adedipe, and D. P. Ormrod. 1973. Soil-plant-water aspects of ozone phytotoxicity in tomato plants. *Plant and Soil* 38:531-541.
- Kramer, P. J. 1983. *Water Relations of plants*. Academic Press, New York. 489 pp.
- Kress, L. W. 1978. Growth impact of O₃, SO₂ and NO₂ singly and in combination on loblolly pine (Pinus taeda L.) and American sycamore (Plantanus occidentalis L.). Ph.D. Dess. VPI & SU, Blacksburg, VA. 201 pp.
- Kress, L. W. and J. M. Skelly. 1982. Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. *Plant Disease* 66:1149-1152.
- Langway, C. C., H. Oeschger, B. Alder and B. Renaud. 1965. Sampling polar ice for radiocarbon dating. *Nature* 206:500-501.
- Lee, J. J. and D. E. Weber. 1979. The effects of simulated acid rain on seedling emergence and growth of eleven woody species. *Forest Sci.* 25:393-398.
- Likens, G. E. and T. J. Butler. 1981. Recent acidification of precipitation in North America. *Atmos. Environ.* 15:1103-1109.
- Lioy, P. J. and P. J. Samson. 1979. Ozone concentration patterns observed during the 1976-1977 long range transport study. *Environ. Int'l.* 2:77-83.
- Lopushinsky, W. 1964. Effects of water movement on ion movement into the xylem of tomato roots. *Plant Physiol.* 39:494-501.
- MacDowell, F. D. H. 1965. Stages of ozone damage to respiration of tobacco leaves. *Can. J. Bot.* 43:419-27.
- Mahoney, M. J., J. M. Skelly, B. I. Chevone and L. D. Moore. 1984. Response of yellow poplar (Liriodendron tulipifera L.) seedling shoot growth to low concentrations of O₃, SO₂ and NO₂. *Can. J. For. Res.* 14:150-153.
- Markhart, A. H. III, E. L. Fiscus, A. W. Naylor, and P. J. Kramer. 1979. Effect of temperature on water and ion

- transport in soybean and broccoli systems. *Plant Physiol.* 64:83-87.
- Markhart, A. H. III, M. M. Peet, N. Sionit and P. J. Kramer. 1980. Low temperature acclimation of root fatty acid composition, leaf water potential, gas exchange and growth of soybean seedlings. *Plant Cell Environ.* 3:435-441.
- Mateev, A. A. 1970. Chemical hydrology of regions of East Antarctica. *J. Geophys. Res.* 75:3686-3690.
- Matziris, D, I. and G. Nakos. 1977. Effects of simulated acid rain on juvenile characteristics of Aleppo pine (*Pinus halepensis* Mill). *Forest ecology and management* 1:267-272.
- McColl, J. G. and R. Johnson. 1983. Effects of simulated acid rain on germination and early growth of Douglas-fir and ponderosa pine. *Plant and Soil* 74:125-129.
- McFee, W. W. 1983. Sensitivity ratings of soils to acid deposition: A review. *Environ. Exp. Bot.* 23:203-210.
- McLaughlin, S. B., R. K. McConathy, D. Duvick and L. K. Mann. 1982. Effects of chronic air pollution stress on photosynthesis, carbon allocation, and growth of white pine trees. *For. Sci.* 28:60-70.
- McLaughlin, S. B. and R. K. McConathy. 1983. Effects of SO₂ and O₃ on allocation of ¹⁴C-labeled photosynthate in *Phaseolus vulgaris*. *Plant Physiol.* 73:630-635.
- McLaughlin, S. B. 1985. Effects of air pollution on forests: A critical review. *JAPCA* 35:512-534.
- Meyer, F. H. 1987. Der Verzweigungsindex, ein Indikator für Schaden am Feinwurzelsystem. *Forstw. Cbl.* 106:84-92.
- Miller, P. R., J. R. Parmeter, Jr., B. H. Flick and C. W. Martinez. 1969. Ozone dosage response of ponderosa pine seedlings. *JAPCA* 19:435-438.
- Miller, P. L., M. H. McCulchan and H. P. Milligan. 1972. Oxidant air pollution in the Central Valley, Sierra Nevada Foothills, and Mineral King Valley of California. *Atmos. Environ.* 6:623-633.
- Mooi, J. 1980. Influence of ozone on growth of two poplar cultivars. *Plant Disease* 64:772-773.
- Morgan, J. A. 1986. The effects of N nutrition on the water

- relations and gas exchange characteristics of wheat (Triticum aestivum L.). *Plant Physiol.* 80:52-58.
- Nelsen, C. E., N. C. Bolgiano, S. C. Furutani, G. R. Safir and B. H. Zandstra. 1981. Interaction of vesicular-mycorrhizal infection and soil phosphorus levels in field grown onion plants. *J. Amer. Soc. Hort. Sci.* 106:786-788.
- Nelsen, C. E. and G. R. Safir. 1982. The water relations of well-watered, mycorrhizal, and non-mycorrhizal onion plants. *J. Amer. Soc. Hort. Sci.* 107:271-274.
- Neufeld, B. H., J. A. Jernstedt and B. L. Haines. 1985. Direct foliar effects of simulated acid rain: I. Damage, growth and gas exchange. *New Phytol.* 99:389-405.
- Ogata, G. and E. V. Maas. 1973. Interactive effects of salinity and ozone on growth and yield of garden beet. *J. Environ. Qual.* 2:518-520.
- Olson, M. W., I. J. Misaghi, D. Goldstein, and R. B. Hine. 1983. Water relations in cotton plants infected with Phymatotrichum. *Phytopathology* 73:213-216.
- Olszyk, D. M. and T. W. Tibbitts. 1981. Stomatal response and leaf injury of Pisum sativum L. with SO₂ and O₃ exposures. II. Influence of moisture stress and time of exposure. *Plant Physiol.* 67:545-549.
- Oshima, R. J., J. P. Bennett and P. K. Braegelmann. 1978. Effect of ozone on growth and assimilate partitioning in parsley. *J. Amer. Soc. Hort. Sci.* 103:348-350.
- Pell, E. J. and E. Brennan. 1973. Changes in respiration, photosynthesis, adenosine 5'-triphosphate and total adenylate content of ozonated Pinto Bean foliage as they relate to symptom expression. *Plant Physiol.* 51:378-381.
- Pitman, M. G. and D. Wellfare. 1978. Inhibition of ion transport in excised barley roots by abscisic acid; relation to water permeability of the roots. *J. Exp. Bot.* 29:1125-1138.
- Pratt, G. C., R. C. Hendrickson, B. I. Chevone, D. A. Christopherson, M. V. O'Brien and S. V. Krupa. 1983a. Ozone and oxides of nitrogen in the rural upper-midwestern U. S. A. *Atmos. Environ.* 17:2013-2033.
- Pratt, G. C., K. W. Kromroy and S. V. Krupa. 1983b. Effects of ozone and sulphur dioxide on injury and foliar con-

- centrations of sulphur and chlorophyll in soybean Glycine max. Environ. Pollut. (ser. A). 32:91-99.
- Proctor, J. T. A. 1983. Effects of simulated sulfuric acid rain on apple tree foliage nutrient, yield and fruit quality. Environ. Exp. Bot. 23:167-174.
- Puckett, L. J. 1982. Acid rain, air pollution, and tree growth in southeastern New York. J. Environ. Qual. 11:376-381.
- Radin, J. W. and M. P. Eidenbock. 1984. Hydraulic conductance as a factor limiting leaf expansion of phosphorus-deficient cotton plants. Plant Physiol. 75:372-377.
- Raynal, D. J., J. R. Roman and W. Eichenlaub. 1982a. Response of Tree seedlings to acid precipitation - I. Effect of substrate acidity on seed germination. Environ. Exp. Bot. 22:377-383.
- Raynal, D. J., J. R. Roman, and W. M. Eichenlaub. 1982b. Response of tree seedlings to acid precipitation: II. Effect of simulated acidified canopy throughfall on sugar maple seedling growth. Environ. Exp. Bot. 22:385-392.
- Reich, P. B., R. G. Amundson and J. P. Lassoie. 1982. Reductions in soybean yield after exposure to ozone and sulfur dioxide using a linear gradient exposure technique. Water, Air & Soil Pollut. 17:29-36.
- Reich, P. B. 1983. Effects of low concentrations of O₃ on net photosynthesis, dark respiration, and chlorophyll contents in aging hybrid poplar leaves. Plant Physiol. 73:291-296.
- Reich, P. B., and R. G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566-570.
- Reich, P. B. and J. P. Lassoie. 1985. Influence of low concentrations of ozone on growth, biomass partitioning and leaf senescence in young hybrid poplar plants. Environ. Pollut. (ser. A). 39:39-51.
- Reich, P. B., A. W. Schoettle, and R. G. Amundson. 1986a. Effects of O₃ and acidic rain on photosynthesis and growth in sugar maple and northern red oak seedlings. Environ. Pollut. (ser. A). 40:1-15.
- Reich, P. B., A. W. Schoettle, R. M. Raba, and R. G. Amundson. 1986b. Response of soybean to low concentration of

- Reich, P. B., A. W. Schoettle, R. M. Raba, and R. G. Amundson. 1986b. Response of soybean to low concentration of ozone: I. Reductions in leaf and whole plant net photosynthesis and leaf chlorophyll content. *J. Environ. Qual.* 15:31-36.
- Reich, P. B., A. W. Schoettle, H. F. Stroo, J. Troiano, and R. G. Amundson. 1987. Effects of ozone and acid rain on white pine (*Pinus strobus*) seedlings grown in five soils. I. Net photosynthesis and growth. *Can. J. Bot.* 65:977-987.
- Rich, S. and N. C. Turner. 1972. Importance of moisture on stomatal behavior of plants exposed to O₃. *J. Air Pollut. Control Assoc.* 22:718-721.
- Richards, B. L., J. T. Middleton and W. B. Hewitt. 1958. Air pollution with relation to agronomic crops: V. Oxidant stipple to grape. *Agron. J.* 50:559-561.
- Roberts, B. R. 1987. Photosynthetic response of yellow-poplar seedlings to the antioxidant chemical ethylene-diurea. *J. Arboriculture* 13:154-157.
- Rosen, P. M., R. C. Musselman and W. J. Kender. 1978. Relationship of stomatal resistance sulfur dioxide and ozone injury in grapevines. *Sci. Hort.* 8:137-142.
- Safir, G. R., J. S. Boyer and J. W. Gerdemann. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. *Plant Physiol.* 49:700-703.
- Sands, R., E. L. Fiscus and C. P. P. Reid. 1982. Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentiation and mycorrhizal infection. *Aust. J. Plant Physiol.* 9:559-569.
- Scherbatskoy, T. and R. M. Klein. 1983. Response of spruce and birch foliage to leaching by acidic mists. *J. Environ. Qual.* 12:189-195.
- Scott, J. T., T. G. Siccama, A. H. Johnson and A. R. Breisch. 1984. Decline of red spruce in the Adirondacks, New York. *Bull. Torrey Bot. Club* 111:438-444.
- Seiler, J. R. and D. J. Paganelli. 1987. Photosynthesis and growth response of red spruce and loblolly pine to soil-applied lead and simulated acid rain. *For. Sci.* 33:668-675.
- Shafer, S. R., A. S. Heagle and D. M. Camberato. 1987. Effects of chronic doses of ozone on field-grown

- loblolly pine: Seedling responses in the first year. JAPCA 37:1179-1184.
- Shaner, D. L. and J. S. Boyer. 1976a. Nitrate reductase activity in maize (Zea mays L.) leaves. I. Regulation by nitrate flux. Plant physiol. 58:499-504.
- Shaner, D. L. and J. S. Boyer. 1976b. Nitrate reductase activity in maize (Zea mays L.) leaves. II. Regulation by nitrate flux at low leaf water potential. Plant Physiol. 58:505-509.
- Siccama, T. G., M. Bliss and H. W. Vogelmann. 1982. Decline of red spruce in the Green Mountains of Vermont. Bull. Torrey Bot. Club 109:162-168.
- Skeffington, R. A. and T. M. Roberts. 1985. The effects of ozone and acid mist on Scots pine saplings. Oecologia (Berlin) 65:201-206.
- Skelly, J. M., B. I. Chevone and Y. S. Yang. 1982. Effects of ambient concentrations of air pollutants on vegetation indigenous to the Blue Ridge Mountains of Virginia. In Acid Rain: A water Resources Issue for the 80's. R. Herrmann and A. I. Johnson (eds.). American Water Resources Association, Bethesda, MD. pp. 69-74.
- Sprugel, D. G., J. E. Miller, R. N. Muller, H. J. Smith and P. B. Xerikos. 1980. Sulfur dioxide effects on yield and seed quality in field-grown soybeans. Phytopathology 70:1129-1133.
- Stone, L. L. and J. M. Skelly. 1974. The growth of two forest tree species adjacent to a periodic source of air pollution. Phytopathology 64:773-778.
- Syvertsen, J. P., R. M. Zablutowicz and M. L. Smitil, Jr. 1983. Soil temperature and flooding effects on two species of citrus: I. Plant growth and hydraulic conductivity. Plant and Soil 72:3-12.
- Tal, M. and D. Imber. 1971. Abnormal stomatal behavior and hormonal imbalance in Flaca, a wilted mutant of tomato. III. Hormonal effects on the water status of the plant. Plant Physiol. 47:849-850.
- Tamm, C. O. and E. B. Cowling. 1977. Acidic precipitation and forest vegetation. Water, Air & Soil Pollut. 7:503-511.
- Taylor, G. E., Jr., R. J. Norby, S. B. McLaughlin, A. H. Johnson and R. S. Turner. 1986. Carbon dioxide assimilation

- lation and growth of red spruce (Picea rubens Sarg.) seedlings in response to ozone - precipitation chemistry and soil type. *Oecologia* 70:163-171.
- Tingey, D. T., W. W. Heck and R. A. Reinert. 1971. Effect of low concentrations of ozone and sulfur dioxide on foliage growth and yield of radish. *J. Amer. Soc. Hort. Sci.* 96:369-371.
- Tingey, D. T., R. A. Reinert, C. Wickliff and W. W. Heck. 1973. Chronic ozone or sulfur dioxide exposures, or both, affect the early vegetative growth of soybean. *Can. J. Plant. Sci.* 53:875-879.
- Tingey, D. T., R. G. Wilhour and C. Standley. 1976. The effects of chronic ozone exposure on the metabolite content of ponderosa pine seedlings. *For. Sci.* 22:234-241.
- Tingey, D. T., G. L. Thutt, M. L. Gumpertz and W. E. Hogsett. 1982. Plant water status influences ozone sensitivity of bean plants. *Agric. Environ.* 7:243-254.
- Tingey, D. T. and W. E. Hogsett. 1985. Water stress reduces ozone injury via a stomatal mechanism. *Plant physiol.* 77:944-947.
- Tseng E. C., J. R. Seiler and B. I. Chevone. 1988. Effects of ozone and water stress on greenhouse-grown Fraser fir seedling growth and physiology. *Environ. Exp. Bot.* (In Press).
- Tveite, B. 1980. Effects of acid precipitation on soil and forest. 8. Foliar nutrient concentrations in field experiments. In D. Drablos and A. Tollan, (eds.). *Proceedings International conference on the Ecological Impact of Acid Precipitation*, Sandefjord, Norway. pp. 204-205.
- Tzeng, D. D., R. J. Wakeman and J. E. DeVay. 1985. Relationships among verticillium wilt development, leaf water potential, phenology, and lint yield in cotton. *Physiol. Plant Pathology* 26:73-81.
- Vogelmann, H. W., G. J. Badger, M. Bliss and R. M. Klein. 1985. Forest decline on Camels Hump, Vermont. *Bull. Torrey Bot. Club.* 112:274-287.
- Ward, M. M. 1980. Variation in the response of loblolly pine to ozone. M.S. Thesis, VPI & SU, Blacksburg, VA. 201 pp.

- Weiss M. and R. Agerer. 1986. Reaktionen des Warzelsystems von Picea abies (L.) Karst. auf Mineralstoffernahrung und auf Belastung des Sprosses mit ozon und saurem Nebel. Forstw. Cbl. 105:230-233.
- Winner, W. E., I. S. Cotter, H. R. Powers, Jr. and J. M. Skelly. 1987. Screening loblolly pine seedling responses to SO₂ and O₃: Analysis of families differing in resistance to Fusiform rust disease. Environ. Pollut. 47:205-220.
- Wood, T. and F. H. Bormann. 1974. Effects of an artificial acid mist upon the growth of Betula alleghaniensis Britt. Environ. Pollut. (ser. A). 7:259-268.
- Wood, T. and F. H. Bormann. 1975. Increases in foliar leaching caused by acidification of an artificial mist. Ambio. 4:169-171.
- Wood, T. and F. H. Bormann. 1977. Short-term effects of a simulated acid rain upon the growth and nutrient relations of Pinus strobus L. Water, Air, & Soil Pollut. 7:479-488.
- Woodman, J. N. and E. B. Cowling. 1987. Airborne chemicals and forest death. Environ. Sci. Technol. 21:120-126.
- Yang, Y. S., J. M. Skelly and B. I. Chevone. 1982. Clonal response of eastern white pine to O₃, SO₂ and NO₂ exposure singly and in combination. Can. J. For. Res. 12:803-808.
- Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983a. Effects of long-term ozone exposure on photosynthesis and dark respiration of eastern white pine. Environ. Sci. Tech. 17:371-373.
- Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983b. Effects of short-term ozone exposure on net photosynthesis, dark respiration and transpiration of three eastern white pine clones. Environ. Int'l. 9:265-269.

CHAPTER I

GROWTH RESPONSE AND DROUGHT SUSCEPTIBILITY OF RED SPRUCE SEEDLINGS EXPOSED TO SIMULATED ACIDIC RAIN AND OZONE

INTRODUCTION

Red spruce (Picea rubens Sarg.) is a long-lived, shade-tolerant tree that is commonly present in the cool, moist climates at high elevations of the Appalachian Mountains. Recently, an accelerated decline of red spruce has been reported in the northern Appalachians in the Green Mountains (Siccama et al., 1982) on Camels Hump (Siccama et al., 1982; Vogelmann et al., 1985), Vermont and on Whiteface Mountain in New York (Scott et al., 1984) as well as in the mid (Adams et al., 1985) and southern Appalachians (Bruck, 1984). Even though many possible causes of this decline have been suggested (Friedland et al., 1984a, b; Johnson and Siccama, 1983, 1984; Johnson and McLaughlin, 1986; Carey et al., 1984), none have been established conclusively at present. High acid inputs and elevated concentrations of heavy metals, in addition to ozone stress, have been strongly suspected as contributing factors for the decline (Johnson and Siccama, 1984). Growth reduction of mature trees in the northeast, beginning in the early 1960's, appeared to have been initiated by a

severe drought (Johnson and Siccama, 1983, 1984). A similar coincidence between the initiation of growth decline and drought was reported in the mid-Appalachian forests (Adams et al., 1985). For the recent extensive decline of red spruce in the northeastern United States, the hypothesis has been suggested that acidic deposition, heavy metal inputs, and, possibly, ozone stress can impair root growth and root physiology to such an extent that affected trees become highly drought susceptible (Johnson and Siccama, 1984).

The objectives of this present research were to investigate the effects of simulated acidic rain and ozone on growth and drought susceptibility of red spruce seedlings by measuring biomass, foliar nutrient status, root hydraulic conductivity, and gas exchange rates.

MATERIALS AND METHODS

Plant Materials

One-year-old red spruce seedlings were obtained from a Vermont Nursery and transplanted into 10 cm diameter plastic pots containing Altavista soil (fine-loamy, mixed, thermic, Aquic Hapludult) which was collected from the A horizon at the Reynolds Homestead Agricultural Experiment Station (Patrick County, VA). Soil was screened through a 0.5 cm mesh and mixed with sand in ratio of 5:1 (v/v).

Prior to transplanting, the fresh weight of each seedling was measured.

All seedlings were grown in a greenhouse supplied with charcoal-filtered air (mean hourly ozone concentration < 0.025 ppm). The photoperiod was supplemented with 1000 W sodium lamps to provide a 16-hour daylength with 580 ± 30 $\mu\text{Mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD). Ozone fumigations and simulated acidic rain applications were begun when the majority of seedlings started to break bud.

Ozone and Acid Rain Exposures

Seedlings were exposed to ozone in a continuous stirred tank reactor system (CSTR) (Heck et al., 1978). Ozone fumigations occurred for four consecutive hours, three days a week for ten continuous weeks at 0.00 or 0.10 $\mu\text{l l}^{-1}$ (ppm). Exposure conditions within the CSTRs were maintained at $27 \pm 2^\circ \text{C}$, $55 \pm 10\% \text{RH}$, and 600 ± 30 $\mu\text{Mol m}^{-2} \text{s}^{-1}$ PPFD at plant canopy height. Ozone was generated by UV discharge using a Welsbach ozone generator (Model T-408) and monitored in each CSTR on a time-shared system with a Bendix chemiluminescent ozone monitor (Model 8002). The ozone monitor was checked for zero and span drift weekly and a five-point calibration was conducted monthly using a CSI Photocal 3000 ozone calibrator. The ozone calibrator was verified against a Dasibi UV photometer at EPA/RTP.

All seedlings were exposed to simulated acidic precipitation in the greenhouse area using a rainfall simulator developed on the principle of droplet formation from needle tips (Chevone et al., 1984). Treatments consisted of simulated rain events at pH 3.0 (1000 meq H^+l^{-1}), and 5.6 (2.5 meq H^+l^{-1}). Analytical grade chemicals and deionized water were used in the preparation of simulated rain solutions. Major anionic and cationic concentrations approximated the mean concentrations found in rain in southwestern Virginia (Skelly et al., 1982). See Table A1 (Appendix) for ion concentrations. The pH of the solutions were adjusted by adding a mixture of 1.0 M H_2SO_4 and 0.5 M HNO_3 (2:1, v/v) or 1.0 M NaOH.

Applications of simulated acidic rain were conducted for one hour, twice each week at a rainfall intensity of 0.75 cm hr^{-1} . Ozone exposures and rain exposures occurred on different days during each week.

Growth Measurements

Rate of growth was determined by measuring seedling heights from a marking line to the top of the terminal branch, every two weeks, starting with the first day of fumigation. Ten seedlings per treatment were harvested for fresh and dry shoot and root weight after five and ten weeks of treatment. Dry weight were measured after drying in oven at $60^\circ C$ for 48 hours, and these data were used for

determining relative growth rate (RGR) and for use in allometric growth analyses.

Drought Stress and Associated Measurements

After the ten-week fumigation and acid rain treatment period, the seedlings were exposed to one last acidic rain event to bring the soil water content to field capacity. Water was then withheld from all seedlings to simulate a drought. Beginning the day after watering and continuing every day, net photosynthesis (Pn) and transpiration (Ts) were measured on twelve seedlings per treatment with a LI-6000 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE). Each branch was marked to insure repetitive measurements and placed into a quarter-liter cuvette for determining gas exchange rates. Average environmental variables within the cuvette were $32 \pm 3^\circ\text{C}$, $39 \pm 8\%$ RH, 845 ± 70 $\mu\text{Mol m}^{-2} \text{s}^{-1}$ PPFD and 360 ± 30 ppm CO_2 concentration. After each gas exchange measurement, seedling water potential (Wp) was measured on a small branch using a pressure chamber (Scholander et al., 1965). The gas exchange measurements continued until the mean net photosynthetic rate dropped below five percent of the initial rate for that treatment. Water-use efficiency (WUE) was calculated as Pn divided by Ts.

After the first drought cycle, six seedlings per treatment were harvested to measure root hydraulic conductivity

(Lp). The remaining six seedlings per treatment were rewatered with the appropriate simulated acidic rain solution, and submitted to a second drought cycle. Net photosynthesis, T_s , W_p , and L_p were measured as in the first drought cycle.

Root hydraulic conductivity, as described by Fiscus (1975, 1977, 1981a) and Ramos and Kaufmann (1979), was also determined on six seedlings per treatment after five and ten weeks of air pollutant treatment. The seedlings used for the gas exchange measurements were rewatered with the appropriate acidic rain treatment and maintained in the laboratory at 25°C for two to three hours. The stem was then cut at 4 cm above the soil surface and the soil was carefully washed away from the root system. The root system was submerged in a 0.4-liter pressure chamber containing half strength Hoagland solution at 23±1°C with the stem protruding through a silicon seal. A calibrated pipet was connected to the stump using a small piece of rubber tubing, and the air pressure within the chamber was increased slowly to a final constant pressure of 5 bar. Exudation rate was measured every 15 minutes for at least one hour after a constant flow rate occurred (usually 30-60 min.). L_p was calculated as the exudation rate ($nl\ s^{-1}$) per unit root dry weight (g) and pressure (bar).

Foliar Nutrition Analysis

Needles were collected from seedlings used for dry weight measurement, and ground with a Cyclone Sample Mill (UD Co., Boulder, CO) through a 0.1 cm screen. One gram of ground needle was ashed at 550° C for 5.5 hours and dissolved in 25 ml of concentrated HCl. Concentrations of P, K, Ca, Mg, Al, and S were measured by using inductively coupled plasma (ICP) spectrometry, and total nitrogen was measured by the micro-Kjeldahl method in the Soil Testing and Plant Analysis Laboratory, Virginia Polytechnic Institute and State University.

Experimental Design

The experiments were designed as a randomized factorial combination of two ozone (0.00 and 0.10 ppm) and two pH (3.0 and 5.6) treatments. Thirty-two seedlings per treatment were used, thus providing ten replicates for biomass after five and ten weeks of treatment and six replicates for gas exchange measurements after the first and second drought cycle. Standard analysis of variance (ANOVA) was used to analyze the single treatment effects and interactions between ozone and simulated acidic rain. Analysis of covariance (ANCOVA) was performed to adjust the data by a significant covariate such as initial height or initial fresh weight. Duncan's new multiple range test was used to compare the data among combined treatments. Stepwise

regression analysis was performed to determine a best-fit model for the relationship between gas exchange measurements and branch water potential. The slopes and intercepts of the regression models between treatments were tested by a t-test using dummy-variable model (Kleinbaum and Kupper, 1978).

RESULTS

Effects of Treatments on Growth of Red Spruce Seedlings

Initial seedling height was found to be a significant covariate ($P=0.001$) for shoot height growth, and initial total fresh weight was a significant covariate ($P=0.001$) for fresh weight increment, total dry weight, and RGR. These growth variables were adjusted by the appropriate covariate.

Fresh weight increment and dry weight were significantly reduced by 14% and 6%, respectively, after the 10-wk treatment with ozone. Ozone exposure did not result in any changes in root and shoot dry weight, root to shoot ratio and RGR. The application of simulated rain at pH 3.0 increased fresh weight increment compared to pH 5.6 (Table 1). There were no significant interactions between ozone and simulated acid rain for any measured variable. However, biomass was always lower in seedlings exposed to 0.1 ppm ozone in combination with the solution at pH 5.6 (Table

Table 1. Changes in mean fresh weight increment (FWT), total dry weight (DWT), root to shoot ratio (RSR), and relative growth rate (RGR) of one-year-old red spruce after 10 wks of treatment with ozone and simulated acidic rain.

	OZONE ⁴			RAIN pH ⁵			INTERACTION			
	0.0	0.1	5.6	3.0	5.6	11.16	0.0+5.6	0.0+3.0	0.1+5.6	0.1+3.0
FWT ¹	13.05	11.28†	11.16	13.17†	12.75	13.36	9.58	12.98		
DWT ²	6.87	6.42†	6.54	6.75	6.89	6.84	6.18	6.65		
RSR	0.41	0.38	0.40	0.39	0.41	0.40	0.37	0.38		
RGR ³	0.10	0.09	0.09	0.10	0.10	0.10	0.08	0.10		

¹FWT = Final fresh weight - Initial fresh weight (gm)

²gm

³gm gm⁻¹ wk⁻¹

⁴Ozone (ppm)/treatment across all rain pHs

⁵Rain pH across all ozone treatments

Daggers indicate the significant differences at the 0.05 (t) level

No significant interactions between ozone and rain pH occurred

FWT, DWT, and RGR are adjusted for initial fresh weight

1). Treatment with artificial rain at pH 3.0 significantly increased shoot height growth (SHG) compared with pH 5.6 (Figure 1). This stimulation effect of solution acidity became significant after ten weeks of treatment. The SHG was always higher (6 to 11%) in seedlings exposed to 0.1 ppm ozone + pH 3.0 solution compared with any other treatment throughout the 10 weeks of the experiment. However, this increase in SHG was not statistically significant.

Effects of Treatments on Foliar Nutrient Content

Only total foliar nitrogen content was significantly increased as a result of ozone fumigation (Table 2). However, application of simulated acid rain at pH 3.0 significantly increased potassium and sulphur compared with pH 5.6. There was no significant interaction between ozone and acid rain on any nutrient analyzed except Ca. Foliar Ca concentration was lower in seedlings exposed to 0.1 ppm ozone + pH 5.6 compared with 0.1 ppm ozone + pH 3.0.

Effects of Pollutant Treatments on Drought Response of Seedlings

Root hydraulic conductivity (L_p) of red spruce seedlings was significantly reduced by aging and drought stress regardless of air pollutant treatment. The L_p in all treatments decreased about 40% between week five and ten of the exposure period. After the second drought cycle, L_p was

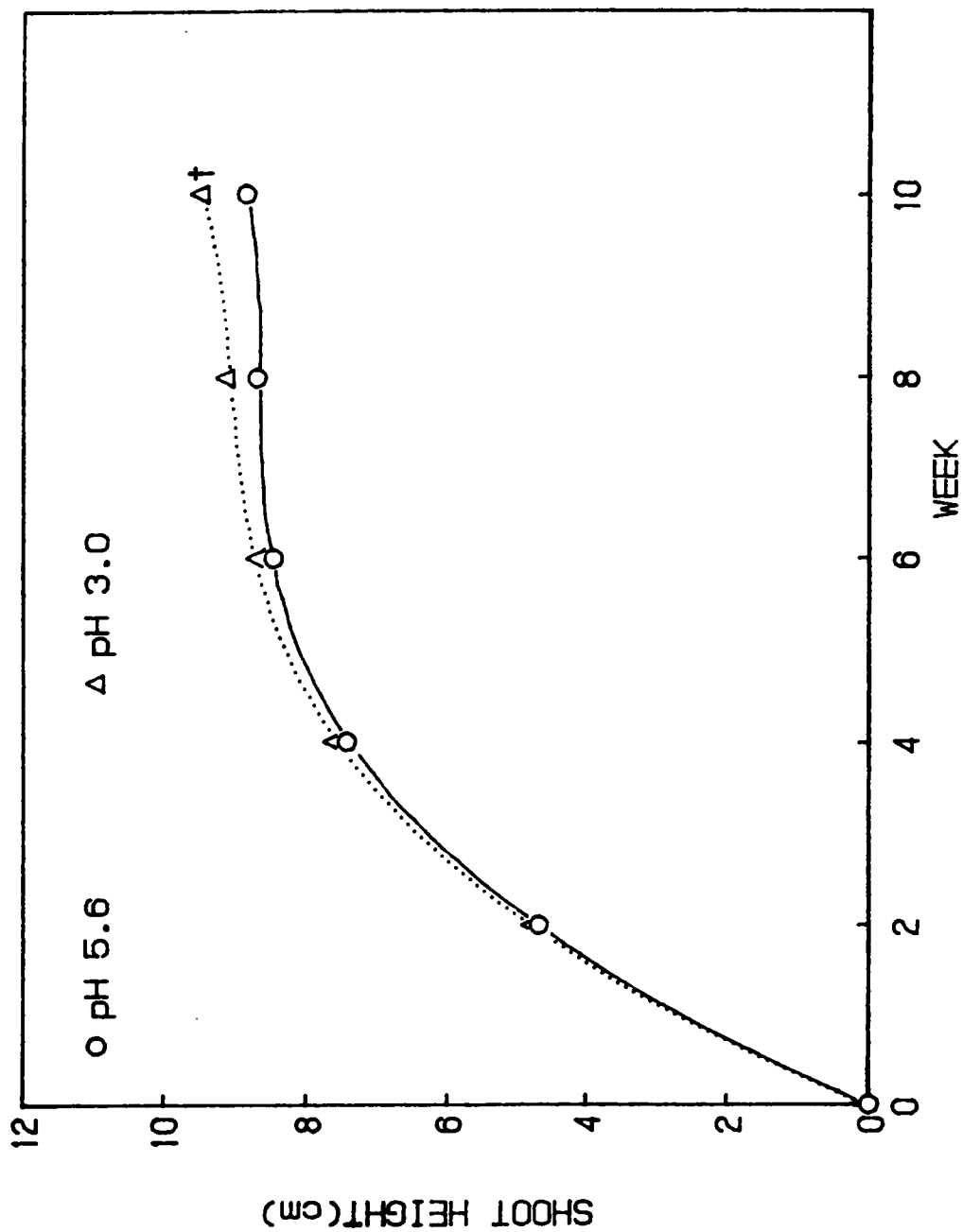


Figure 1. Changes in cumulative shoot height growth (cm) of one-year-old red spruce seedlings during 10 wks of treatment with simulated rain. Dagger (†) indicates statistically significant differences at $P = 0.05$. Data are adjusted for initial height.

Table 2. Summary of results of needle analysis of one-year-old red spruce seedlings after ten weeks of treatment with ozone and simulated acidic rain. Values (% per gram needle dry weight) are means of five samples.

	N			P			K		
	Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
	0.0	0.1		0.0	0.1		0.0	0.1	
pH 3.0	0.89	0.96	0.93	0.085	0.083	0.084	0.29	0.31	0.30**
pH 5.6	0.90	1.01	0.95	0.078	0.089	0.084	0.22	0.24	0.23
X ²	0.90	0.99*		0.082	0.086		0.26	0.27	

	Ca			Mg			S		
	Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
	0.0	0.1		0.0	0.1		0.0	0.1	
pH 3.0	0.31 ^{ab}	0.34 ^a	0.32	0.11	0.11	0.11	0.062	0.060	0.060**
pH 5.6	0.31 ^{ab}	0.27 ^b	0.29	0.10	0.10	0.10	0.045	0.042	0.044
X ²	0.31	0.30		0.1	0.10		0.053	0.051	

¹Averaged across all ozone levels

²Averaged across all pH levels

*,**Means indicate significant differences at 5% (*) or 1% (**) level within ozone or simulated acidic rain treatment across all other treatments

Means within interaction lacking (or with common) letters are not significantly different at 5% level by Duncan's new multiple range test

significantly less by 33% in seedlings exposed to 0.1 ppm ozone compared with control seedlings (Table 3).

Simulated acid rain at pH 3.0 resulted in a Lp 24% higher than treatment with pH 5.6 after five weeks of exposure, but this stimulation effect was less evident after ten weeks of treatment. After the first drought cycle seedlings exposed to pH 3.0 had a significant greater Lp as compared with seedlings exposed to solution at pH 5.6, but there was no significant difference between pH treatments after the second drought cycle (Table 3).

Significant interactions between ozone and simulated acidic rain on Lp were observed after five weeks of air pollutant treatment and after the first drought cycle. Lp was significantly higher in seedlings exposed to 0.1 ppm ozone + pH 3.0 rain compared with any other treatments for both periods (Table 3).

When Pn, Ts, and WUE were measured immediately after the ten-week pollutant exposure period, there were no significant effects of ozone or simulated rain on any measurement (Table 4).

The relationship between net photosynthetic rate and branch water potential was examined by regression analysis using several model parameters. The best-fit model for all treatment combinations was of the form $Pn = a + b \ln(wp)$. Coefficients of determination for the relationship were significantly changed between the first and second drought

Table 3. Changes in mean root hydraulic conductivity¹ of one-year-old red spruce seedlings during and after 10 wks of pollutant treatment with ozone and simulated acid rain. All seedlings were submitted to two drought cycles after 10 wks of treatment.

Week	OZONE ²		RAIN pH ³			INTERACTION		
	0.0	0.1	5.6	3.0	0.0+5.6	0.0+3.0	0.1+5.6	0.1+3.0
5	73.9a	84.5a	70.7a	87.8a†	77.2a	70.6a	64.1a	105a†
10	44.2b	49.9b	44.3b	49.8b	40.8b	47.7b	47.8b	51.9b
11 ⁴	16.4c	20.8c	15.5c	21.7c†	15.4c	17.4c	15.5c	26.0c†
12 ⁵	17.3c	11.7d†	15.4c	13.6d	18.2c	16.4c	12.6c	10.7d

¹nl g⁻¹ s⁻¹ bar⁻¹

²Ozone (ppm) treatment across all rain pHs

³Rain pH across all ozone treatments

⁴First drought stress cycle

⁵Second drought stress cycle

Daggers indicate the significant differences at the .05 (†) or .01 (‡) level

Means within each column with common letters are not significantly different at 0.05 level by Duncan's new multiple range test.

Table 4. Changes in net photosynthesis (Pn), transpiration (Ts), and water-use efficiency (WUE) of one-year-old red spruce seedlings after 10 wks of treatment with ozone and simulated acidic rain.

	OZONE ⁴		RAIN pH ⁵			INTERACTION		
	0.0	0.1	3.0	5.6	0.0+3.0	0.0+5.6	0.1+3.0	0.1+5.6
Pn ¹	4.04	3.88	4.22	3.70	4.06	4.01	4.38	3.39
Ts ²	1.78	2.03	2.01	1.80	1.85	1.70	2.16	1.91
WUE ³	2.13	1.86	1.89	2.10	1.90	2.36	1.89	1.83

¹Pn = mgCO₂g⁻¹hr⁻¹

²Ts = gH₂Og⁻¹hr⁻¹

³WUE = mgCO₂/gH₂O

⁴Ozone (ppm) treatment across all rain pHs

⁵Rain pH across all ozone treatments

No means within single treatments or interactions were significantly different (p = 0.05).

cycles. The slope parameter for the first cycle was -3.33 compared with only -1.66 for the second cycle, indicating a more rapid decline in photosynthesis with decreasing branch water potential during the first drought cycle (Table 5). No significant differences were detected between seedlings exposed to ozone and simulated acidic rain during the first drought stress period, however, a significant shift in the response of photosynthesis to decreasing branch water potential occurred during the second drought cycle. Net photosynthetic rate of seedlings pre-exposed to 0.1 ppm ozone + pH 3.0 solution decreased more rapidly than any other treatment as branch water potential decreased. No significant effects of ozone and simulated acidic rain, alone, on the relationship between photosynthesis and branch water potential were observed during either of the drought cycles (Table 5).

The relationship between transpiration and branch water potential also was examined by regression analysis. Only the intercept parameter was significantly changed between the first and second drought cycles. The intercept parameter for the first cycle was 1.28 compared with 1.02 for the second cycle. There were no significant differences between seedlings exposed to ozone or simulated acidic rain during both drought cycles (Table 6).

Table 5. Regression models and coefficients of determination for net photosynthesis (Pn) of one-year-old red spruce seedlings during two drought cycles following exposure to ozone and simulated acidic rain¹

Cycle	O ₃ (ppm)	Rain pH	Model		R ²	n
First Cycle	0.0	5.6	Pn = 2.16	-3.41 ln(Wp)	.50	34
	0.0	3.0	Pn = 2.02	-3.68 ln(Wp)	.50	34
	0.1	5.6	Pn = 2.02	-2.67 ln(Wp)	.50	27
	0.1	3.0	Pn = 2.30	-3.61 ln(Wp)	.50	31
Second Cycle	0.0	5.6	Pn = 1.47	-1.24 ln(Wp)	.46	27
	0.0	3.0	Pn = 1.00	-0.83 ln(Wp)	.46	26
	0.1	5.6	Pn = 1.37	-1.84 ln(Wp)	.46	19
	0.1	3.0	Pn = 2.14†	-3.11‡ ln(Wp)	.46	27
First cycle ²			Pn = 2.11	-3.33 ln(Wp)	.46	126
Second cycle ²			Pn = 1.47†	-1.66‡ ln(Wp)	.46	99

¹Pn = mgCO₂g⁻¹hr⁻¹; Wp = branch water potential, MPa

²Across all treatments

Daggers indicate the significant differences from 0.0 ppm ozone + pH 5.6 within drought cycles or from the first cycle at 0.05 (†) or 0.01 (‡) levels

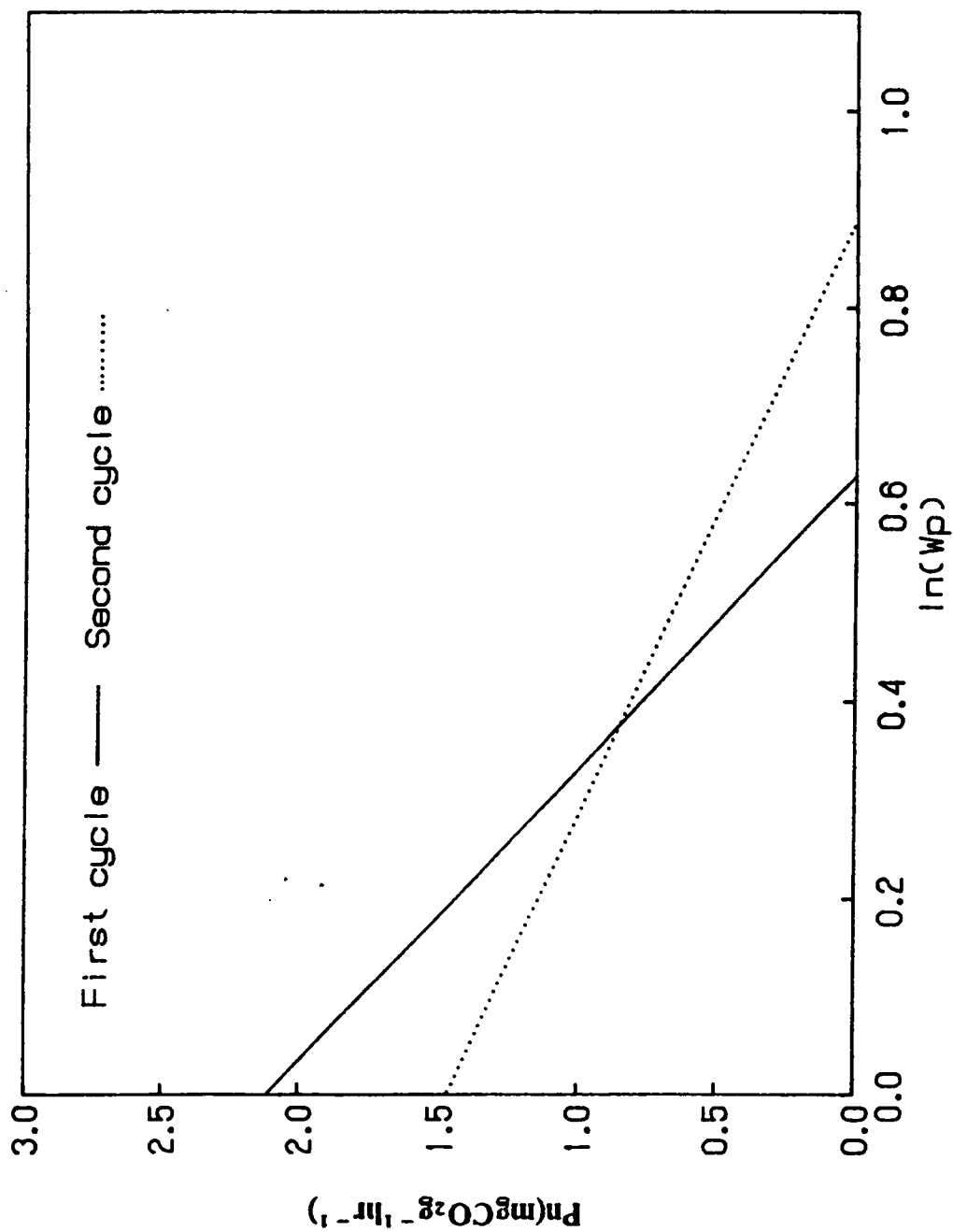


Figure 2. Predicted regression response of net photosynthesis (P_n) in one-year-old red spruce seedlings across all air pollutant treatments during the first and second drought cycle. See Table 5 for the regression models.

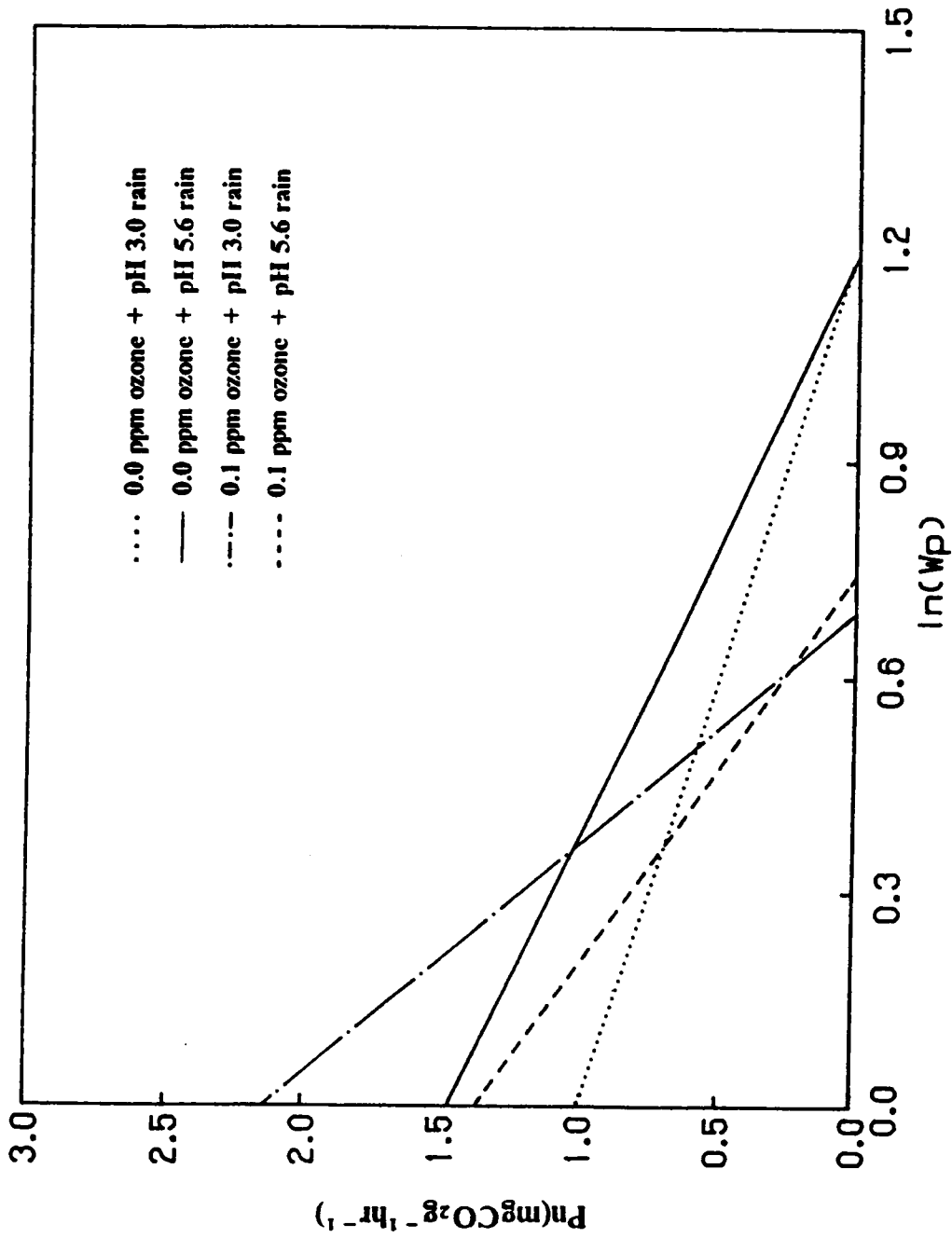


Figure 3. Predicted regression response of net photosynthesis (P_n) in one-year-old red spruce seedlings as affected by the 10-wk exposure to ozone and simulated rain during the second drought cycle. See Table 5 for the regression models.

Table 6. Regression models and coefficients of determination for transpiration (Ts) of one-year-old red spruce seedlings during two drought cycles following exposure to ozone and simulated acidic rain¹

Cycle	O ₃ (ppm)	Rain pH	Model		R ²	n
First Cycle	0.0	5.6	Ts =	1.16 -1.02 ln(Wp)	.58	34
	0.0	3.0	Ts =	1.23 -1.30 ln(Wp)	.58	34
	0.1	5.6	Ts =	1.26 -1.22 ln(Wp)	.58	25
	0.1	3.0	Ts =	1.49 -1.32 ln(Wp)	.58	31
Second Cycle	0.0	5.6	Ts =	0.96 -0.88 ln(Wp)	.50	25
	0.0	3.0	Ts =	1.05 -0.94 ln(Wp)	.50	24
	0.1	5.6	Ts =	0.96 -0.69 ln(Wp)	.50	19
	0.1	3.0	Ts =	1.13 -1.26 ln(Wp)	.50	25
First cycle ²			Ts =	1.28 -1.19 ln(Wp)	.54	124
Second cycle ²			Ts =	1.02‡ -0.93 ln(Wp)	.54	93

¹Ts = gH₂Og⁻¹hr⁻¹; Wp = branch pressure potential, MPa

²Across all treatments

Daggers indicate the significant differences at 0.01 (‡) levels

DISCUSSION

The results of this study demonstrated that ozone and simulated acid rain altered certain growth patterns of red spruce seedlings. Ozone treatment significantly inhibited fresh weight increment and total dry weight after ten weeks exposure. Both the root and shoot dry weight were slightly reduced by the ozone treatment, although changes were not statistically significant, resulting in no change in the root to shoot ratio. Shoot elongation was not altered by ozone fumigation throughout ten weeks of treatment. In contrast, Taylor et al. (1986) found a significant increase in root biomass after fumigation with 0.12 ppm ozone. However, inhibitory effects of ozone on plant growth have been reported in conifers. Reduction in height growth was observed in loblolly pine seedlings after exposure to 0.05-0.10 ppm ozone (Kress, 1978; Ward, 1980; Kress and Skelly, 1982; Winner et al., 1987). Ward (1980) also observed a significant reduction in total dry weight in loblolly pine seedlings after fumigation with 0.1 ppm ozone.

Taylor et al. (1986) also observed a slight increase in root and shoot biomass of red spruce seedlings after application of simulated rain (pH=4.1) and mist (pH=3.6), although these changes were not significant. Our results support those of Taylor et al. (1986), however, the

significant stimulation effect of simulated acid rain at pH 3.0 was detected only by using mean fresh weight increment (final fresh weight - initial fresh weight) during ten weeks of exposure. The increase observed only in fresh weight increment might be due to seedlings being more succulent or that the net increase in dry weight during ten weeks of pollutant treatments might be statistically insignificant because of the high variation in initial biomass.

The stimulation effects of acid rain on growth of tree species also were observed in eastern white pine (*Pinus strobus*) (Wood and Bormann, 1977; Reich et al., 1987), yellow-poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), shagbark hickory (*Carya ovata*), and Douglas-fir (*Pseudotsuga menziesii*) (Lee and Weber, 1979). All authors suggested that the stimulation of seedling growth was due to N fertilization, rather than to an acidity effect, because of the positive correlation between foliar N content and nitrate levels in simulated acid rain (Wood and Bormann, 1977). Reich et al. (1987) found that the highest stimulation effect on seedling growth by simulated rain occurred in white pine seedlings planted in soil with the lowest N content. However, foliar concentration of N was not changed by a pH 3.0 solution in our study. Alternatively, accumulation of K due to the pH 3.0 solution in our study may have stimulated fresh weight increment since K concentration was lower than the critical defi-

ciency level of 0.4% (Zech et al., 1985). The stimulation effect of simulated rain at pH 3.0 on fresh weight increment also may be explained partially by increased Lp, which can stimulate leaf expansion resulting in increased fresh weight (Radin and Eidenbock, 1984), or which might make seedlings more succulent.

Results from the foliar nutrient analysis showed that only potassium and sulphur were significantly increased in seedlings exposed to rain at pH 3.0 compared with pH 5.6. The accumulated sulphur concentration was lower than the threshold value of 0.12% for sulphur toxicity (Zech et al., 1985). A similar sulphur accumulation was observed in needles of white spruce exposed to simulated rain at pH 2.6 without any change of growth (Abouguendia and Baschak, 1987). Tveite (1980) also found an increase of sulphur and sulphate in current and previous year needles of Norway spruce and Scotch pine saplings after three to six years of treatment with ground water acidified with sulphuric acid.

A significant reduction in Lp as seedlings aged was observed in red spruce in this study. A similar result was reported in soybean plants (Fiscus, 1981b), and was suggested to result from an increase in the proportion of suberized roots. Sands et al. (1982) found that the average Lp was 2.5 fold higher in unsuberized roots than in the suberized roots of eight-month-old loblolly pine seedlings. Another factor which can alter Lp is drought stress. After

the first drought cycle in this study, L_p decreased 35-44% from pre-drought levels. This result is in agreement with similar findings on the reduction of L_p by drought stress reported in citrus (Ramos and Kaufman, 1979; Levy *et al.*, 1983). The reduced L_p might be due to changes in permeability of the root cell membranes or to increased suberin deposition in the cell walls of the cortical cells (Ramos and Kaufmann, 1979).

The photosynthetic acclimation of red spruce to low water potential during the second drought cycle is in agreement with similar findings in sunflower (Matthews and Boyer, 1984) and loblolly pine (Seiler and Johnson, 1985, 1988). Matthews and Boyer (1984) observed a 0.3 to 0.4 MPa shift in the response of photosynthesis to decreasing leaf water potential when plants are submitted to a continuous low water potential of -0.7 to -1.1 MPa. Seiler and Johnson (1985) also reported approximately a 0.5 MPa shift when seedlings were watered only when pre-dawn needle water potential fell below -1.4 MPa. In our study, a 0.5 MPa shift occurred after drought stress, and this change might be due to the osmotic adjustment or chloroplastic acclimation within the needle tissue (Seiler and Johnson, 1985, 1988).

The photosynthetic response with decreasing branch water potential, observed in seedlings exposed to 0.1 ppm ozone + pH 3.0 solution could have resulted, in part, from

changes in root hydraulic conductivity. During the second drought cycle, in seedlings exposed to 0.1 ppm ozone + pH 3.0 solution, the predicted net photosynthetic rate at -1.0 MPa water potential (y intercept) was significantly higher, and the slope was significantly more negative than any other treatment. Therefore the photosynthetic rate decreased more rapidly with decreasing water potential. The regression equations also show that P_n at $\ln(W_p)=0$ is the same for both drought cycles for 0.1 ppm ozone + 3.0 pH treatment and that the slopes are also similar. The significant changes that were observed in the second drought cycle with the other treatments did not occur in the 0.1 ppm ozone + 3.0 pH treatment. Therefore, P_n was more susceptible to drought stress under these pollutant conditions. The higher L_p observed after the first drought cycle in seedlings exposed to 0.1 ppm ozone + pH 3.0 rain might affect the ability of stomata to stabilize plant water budget and maintain optimum water use efficiency (Kuppers, 1984). Syvertsen and Graham (1985) observed a similar correlation between root hydraulic conductivity and leaf gas exchange in citrus rootstocks. Carrizo citrange, which had a higher L_p than sour orange, exhibited a more rapid decrease in transpiration rate during a drought cycle and recovered more slowly after rewatering than sour orange. The relationship between transpiration and branch water potential, although not statistically significant, showed

trends similar to the response of photosynthesis with decreasing water potential. The slope of the regression equation was more negative ($P=0.17$) in seedlings exposed to 0.1 ppm ozone + pH 3.0 rain and transpiration rates declined more quickly with decreasing water potential in this treatment after the second drought cycle. During prolonged or cyclic drought stress, seedlings experiencing rain at pH 3.0 and 0.1 ppm ozone in combination may maintain lower photosynthetic rates for longer periods of time than seedlings not exposed to these conditions.

Ozone inhibition of photosynthesis has been reported in many plant species (Miller et al., 1969; Barnes, 1972; Pell and Brennan, 1973; Yang et al., 1983a, b; Reich, 1983; Reich et al., 1986a, b, 1987; Reich and Amundson, 1985). In our studies, however, there were no effects of ozone and simulated rain on the rates of photosynthesis, transpiration, or water use efficiency in red spruce seedlings at the end of ten weeks of pollutant treatment. Taylor et al. (1986) reported similar results with one-year-old red spruce seedlings treated with 0.12 ppm ozone in combination with simulated rain (pH. 4.1 or 5.1) and mist (pH 3.6 or 5.1) for four months. Rates of photosynthesis and transpiration were not affected by any of the treatments.

In our study, during the second drought stress cycle, the photosynthetic rate of seedlings exposed to simulated rain at pH 3.0 in combination with 0.1 ppm ozone decreased

more rapidly in response to increasing branch water potential than other treatments. Therefore, the acidity of rain in combination with elevated ozone concentrations may affect red spruce seedlings by changing the susceptibility of photosynthetic capacity to drought stress. The causal factor(s) responsible for the air pollutant-induced differential response of photosynthetic decline with decreasing water potential are not known at present. We suggest that the combination of acidity with high ozone concentrations interferes with the normal response of Lp to drought stress resulting in alterations of gas exchange rates. Additional research is necessary to assess the potential detrimental effects of ozone, simulated acidic rain, and moisture stress.

LITERATURE CITED

- Abouguendia, Z. M. and L. A. Baschak. 1987. Response of two western Canadian conifers to simulated acidic precipitation. *Water, Air, & Soil Pollut.* 33:15-12.
- Adams, H. S., S. L. Stephenson, T. J. Blasing, and D. N. Duvick. 1985. Growth-trend declines of spruce and fir in Appalachian subalpine forests. *Environ. Exp. Bot.* 25:315-325.
- Barnes, R. L. 1972. Effects of chronic exposure to ozone on photo-synthesis and respiration of pines. *Environ. Pollut.* 3:133-8.
- Bruck, R. I. 1984. Decline of montane boreal ecosystems in central Europe and the southern Appalachian Mountains. In Research and Development Conference. Technical Association of the Pulp and Paper Industry. Atlanta, GA. pp.159-163.
- Carey, A. C., E. A. Miller and G. T. Geballe. 1984. Armillaria mellea and decline of red spruce. *Plant Disease* 68:794-795.
- Chevone, B. I., Y. S. Yang, W. E. Winner, I. Storcks-Cotter and S. J. Long. 1984. A rainfall simulator for laboratory use in acidic precipitation studies. *JAPCA* 31:355-359.
- Fiscus, E. L. 1975. The interaction between osmotic- and pressure-induced water flow in plant roots. *Plant Physiol.* 55:917-922.
- Fiscus, E. L. 1977. Determination of hydraulic and osmotic properties of soybean root system. *Plant Physiol.* 59:1013-1020.
- Fiscus, E. L. 1981a. Analysis of the components of area growth of bean root systems. *Crop Sci.* 21:909-913.
- Fiscus, E. L. 1981b. Effects of abscisic acid on the hydraulic conductance of and total ion transport through Phaseolus root systems. *Plant Physiol.* 68:169-174.
- Friedland, A. J., A. H. Johnson and T. G. Siccama. 1984a. Trace metal content of the forest floor in the Green Mountains of Vermont: Spatial and temporal patterns. *Water Air & Soil Pollut.* 21:161-170.

- Friedland, A. J., A. H. Johnson, T. G. Siccama and D. L. Lader. 1984b. Trace metal profiles in the forest floor of New England. *Soil Sci. Soc. Amer. J.* 48:422-425.
- Heck, W. W., R. B. Philbeck and J. A. Dunning. 1978. A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants. USDA-ARS Paper ARS-S-181.
- Johnson, A. H. and S. B. McLaughlin. 1986. The nature and timing of the deterioration of red spruce in the northern Appalachian Mountains. In National Research Council Acid Deposition: Long-term trends, National Academy Press. Washington, D. C.
- Johnson, A. H. and T. G. Siccama. 1983. Acid deposition and forest decline. *Environ. Sci. Tech.* 17:294-305.
- Johnson, A. H. and T. G. Siccama. 1984. Decline of red spruce in the northern Appalachians: Assessing the possible role of acid deposition. *TAPPI* 67:68-72.
- Kleinbaum, D. G. and L. L. Kupper. 1978. Applied regression analysis and other multivariable methods. *Dexbury Press. Boston, MA.* pp. 556.
- Kuppers, M. 1984. Carbon relations and competition between woody species in a Central European hedgerow: II. Stomatal responses, water use, and hydraulic conductivity in the root/leaf pathway. *Oecologia* 64:344-354.
- Kress, L. W. 1978. Growth impact of O₃, SO₂, and NO₂ singly and in combination on loblolly pine (*Pinus taeda* L.). Ph.D. Dissertation, VPI&SU, Blacksburg, VA. pp.201.
- Kress, L. W., J. M. Skelly. 1982. Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. *Plant Disease* 66:1149-1152.
- Lee, J. J. and D. E. Weber. 1979. The effects of simulated acid rain on seedling emergence and growth of eleven woody species. *Forest Sci.* 25:393-398.
- Levy, Y., J. P. Syvertsen and S. Nemec. 1983. Effect of drought stress ad VAM on citrus transpiration and hydraulic conductivity of roots. *New Phytol.* 93:61-66.
- Matthew, M. A. and J. S. Boyer. 1984. Acclimation of photosynthesis to low leaf water potentials. *Plant Physiol.* 74:161-166.
- Miller, P. R., J. R. Parmeter, Jr., B. H. Flick and C. W.

- Martinez. 1969. Ozone dosage response of ponderosa pine seedlings. JAPCA 19:435-438.
- Pell, E. J. and E. Brennan. 1973. Changes in respiration, photosynthesis, adenosine 5'-triphosphate and total adenylate content of ozonated Pinto Bean foliage as they relate to symptom expression. Plant Physiol. 51:378-381.
- Radin, J. W. and M. P. Eidenbock. 1984. Hydraulic conductance as a factor limiting leaf expansion of phosphorus-deficient cotton plants. Plant Physiol. 75:372-377.
- Ramos, C. and M. R. Kaufmann. 1979. Hydraulic resistance of rough lemon roots. Physiol. Plant 45:311-314.
- Reich, P. B. 1983. Effects of low concentrations of O₃ on net photosynthesis, dark respiration, and chlorophyll contents in aging hybrid poplar leaves. Plant Physiol. 73:291-296.
- Reich, P. B., and R. G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566-570.
- Reich, P. B., A. W. Schoettle, and R. G. Amundson. 1986a. Effects of O₃ and acidic rain on photosynthesis and growth in sugar maple and northern red oak seedlings. Environ. Pollut. (ser. A). 40:1-15.
- Reich, P. B., A. W. Schoettle, R. M. Raba, and R. G. Amundson. 1986b. Response of soybean to low concentration of ozone: I. Reductions in leaf and whole plant net photosynthesis and leaf chlorophyll content. J. Environ. Qual. 15:31-36.
- Reich, P. B., A. W. Schoettle, H. F. Stroo, J. Troiano, and R. G. Amundson. 1987. Effects of ozone and acid rain on white pine (Pinus strobus) seedlings grown in five soils. I. Net photosynthesis and growth. Can. J. Bot. 65:977-987.
- Sands, R., E. L. Fiscus and C. P. P. Reid. 1982. Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentiation and mycorrhizal infection. Aust. J. Plant Physiol. 9:559-569.
- Scholander, P. E., H. T. Hammel, E. D. Bradstreet and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 48:339-346.
- Scott, J. T., T. G. Siccama, A. H. Johnson and A. R.

- Breisch. 1984. Decline of red spruce in the Adirondacks, New York. Bull. Torrey Bot. Club 111:438-444.
- Seiler, J. R. and J. D. Johnson. 1985. Photosynthesis and transpiration of loblolly pine seedlings as influenced by moisture-stress conditioning. For. Sci. 31:742-749.
- Seiler, J. R. and J. D. Johnson. 1988. Physiological and morphological responses of three half-sib families of loblolly pine to water-stress conditioning. For. Sci. (In press)
- Siccama, T. G., M. Bliss and H. W. Vogelmann. 1982. Decline of red spruce in the Green Mountains of Vermont. Bull. Torrey Bot. Club 109:162-168.
- Skelly, J. M., B. I. Chevone and Y. S. Yang. 1982. Effects of ambient concentrations of air pollutants on vegetation indigenous to the Blue Ridge Mountains of Virginia. In Acid Rain: A Water Resources Issue for the 80's. R. Herrmann and A. I. Johnson (eds.). American Water Resources Association, Bethesda, MD. pp. 69-74.
- Syvertsen, J. P. and J. H. Graham. 1985. Hydraulic conductivity of roots, mineral nutrition and leaf gas exchange of citrus rootstocks. J. Amer. Soc. Hort. Sci. 110:865-869.
- Taylor, G. E., Jr., R. J. Norby, S. B. McLaughlin, A. H. Johnson and R. S. Turner. 1986. Carbon dioxide assimilation and growth of red spruce (Picea rubens Sarg.) seedlings in response to ozone - precipitation chemistry and soil type. Oecologia 70:163-171.
- Tveite, B. 1980. Effects of acid precipitation on soil and forest. 8. Foliar nutrient concentrations in field experiments. In D. Drablos and A. Tollan, (eds.). Proceedings International conference on the Ecological Impact of Acid Precipitation, Sandefjord, Norway. pp. 204-205.
- Vogelmann, H. W., G. J. Badger, M. Bliss and R. M. Klein. 1985. Forest decline on Camels Hump, Vermont. Bull. Torrey Bot. Club. 112:274-287.
- Ward, M. M. 1980. Variation in the response of loblolly pine to ozone. M.S. Thesis. VPI&SU, Blacksburg, VA. pp.201.
- Winner, W. E., I. S. Cotter, H. R. Powers, Jr, J. M. Skelly. 1987. Screening loblolly pine seedlings responses to SO₂ and O₃: Analysis of families differing

- in resistance to Fusiform rust disease. Environ. Pollut. 47:205-220.
- Wood, T. and F. H. Bormann. 1977. Short-term effects of a simulated acid rain upon the growth and nutrient relations of Pinus strobus L. Water, Air, & Soil Pollut. 7:479-488.
- Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983a. Effects of long-term ozone exposure on photosynthesis and dark respiration of eastern white pine. Environ. Sci. Tech. 17:371-373.
- Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983b. Effects of short-term ozone exposure on net photosynthesis, dark respiration and transpiration of three eastern white pine clones. Environ. Int'l. 9:265-269.
- Zech, W., TH. Suttner, and E. Popp. 1985. Elemental analyses and physiological responses of forest trees in SO₂-polluted areas of NE-Bavaria. Water, Air, and Soil Pollut. 25:175-183.

CHAPTER II

GROWTH RESPONSES OF THREE SOUTHEASTERN COMMERCIAL TREE SPECIES EXPOSED TO SIMULATED ACIDIC RAIN AND OZONE

INTRODUCTION

Ozone and acidic precipitation are the two most widespread airborne pollutants impacting forest ecosystems in the eastern United States (Cogbill and Likens, 1974; Liroy and Samson, 1979; Skelley et al., 1982). During the spring and early summer, when forest vegetation is in a rapid growth phase, mean hourly ambient ozone concentrations generally range from 0.050 to 0.065 ppm (Duchelle et al., 1982; Yang and Chevone, 1982) and can exceed 0.09 ppm for several hours daily during ozone episodes (Duchelle et al., 1983). Ambient ozone concentrations are known to reduce growth of sensitive forest species under both laboratory and field experimental conditions (Kress and Skelly, 1982; Miller et al., 1972; Skelly et al., 1982), probably through inhibition of photosynthesis and retention of photosynthate in foliage (McLaughlin et al., 1982; Yang et al., 1983a, b).

Acidic precipitation is considered a major environmental concern, primarily in the eastern United States. Although acidic impacts to sensitive lakes have been

reported, effects on terrestrial vegetation remain inconclusive. The recent decline of red spruce throughout the northeast (Johnson and Siccama, 1983, 1984) may, in part, result from acidic deposition. The decline of mature trees in the northeast, beginning in the early 1960's, appeared to have been initiated by a severe drought. However, high acid inputs and elevated concentrations of heavy metals, in addition to ozone stress, may be contributing factors (Johnson and Siccama, 1984).

The effect of acidic rain on the leaching of calcium and the mobilization of aluminum in soils has been implicated in the decline of German forest (Ulrich, 1981). Feeder root length in Norway spruce was shown to decrease as the molar ratio of calcium to aluminum decreased in soil solution. A similar relationship between soil calcium and aluminum has not been found in declining spruce stands in the United States. (Johnson, 1983).

Ozone and simulated acid rain have been reported to induce magnesium and calcium deficiency in Norway spruce (Picea abies [L.] Karst.) (Bosh et al., 1986, Weiss and Agerer, 1986) and magnesium and zinc deficiency in spruce and fir (Abies alba) (Zech and Popp, 1983). These micronutrient deficiencies resulted in the expression of symptoms similar to those in declining spruce stands and the application of fertilizer containing magnesium-calcium or magnesium brought about a revitalization of affected trees

(Bosch et al., 1986; Weiss and Agerer, 1986; Zech and Popp, 1983; Kaupenjohann et al., 1987) in Germany. Friedland et al. (1984a, b, 1985) observed winter injury in a declining red spruce site, and proposed that this injury might be related to excessive nitrogen deposition which could retard the development of frost hardiness and predispose the foliage to freezing during winter time.

The potential exists for ozone and acid rain to interact with the nutrient status and growth of forest tree species. However, scientific evidence demonstrating effects of acid rain and ozone, alone and in combination, on terrestrial vegetation is inconclusive. The combined effects of ozone and acidic precipitation on tree growth and nutrient concentrations have not yet been investigated adequately.

The purposes of this research were to examine the effects of ozone and simulated acid rain, alone and in combination, on foliar nutrient concentrations and the growth of loblolly pine, yellow-poplar, and sweetgum seedlings.

MATERIALS AND METHODS

Plant Materials

One-year-old, mixed seed lot, loblolly pine (Pinus taeda L.) seedlings were obtained from the Virginia Department of Forestry, New Kent Forestry Center (Providence

Forge, VA). One-year-old yellow-poplar (Liriodendron tulipifera L.) and sweetgum (Liquidamba styraciflua L.) seedlings were obtained from a commercial source (Hillis Nursery Co., Inc., McMinnville, TN). All seedlings were transplanted into 10 cm (loblolly pine) or 15 cm (yellow-poplar and sweetgum) diameter plastic pots containing Altavista soil (fine-loamy, mixed, thermic, Aquic Hapludult) which was collected from the A horizon at the Reynolds Homestead Agricultural Experiment Station (Patrick County, VA). Soil was screened with a 0.5 cm mesh and mixed with sand in ratio of 5:1 (v/v). Prior to transplanting, the fresh weight of each seedling was measured.

All seedlings were grown in greenhouse supplied with charcoal-filtered air (mean hourly ozone concentration < 0.025 ppm). The photoperiod was supplemented with 1000 W sodium lamps to provide a 16-hour daylength with $580 \pm 30 \mu\text{Mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD). Ozone fumigations and simulated acidic rain applications were begun when the majority of the seedlings started to break bud.

Ozone and Acid Rain Exposures

Seedlings were exposed to ozone in a continuous stirred tank reactor system (CSTR) (Heck et al., 1978). Ozone fumigations occurred for four consecutive hours, three days a week for ten continuous weeks at 0.00 or 0.10 ppm. Expo-

sure conditions within the CSTRs were maintained at $27 \pm 2^\circ$ C, $55 \pm 5\%$ RH, and $600 \pm 30 \text{ uMol m}^{-2} \text{ s}^{-1}$ PPFD at plant canopy height. Ozone was generated by UV discharge using a Welsbach ozone generator (Model T-408) and monitored in each CSTR on a time-shared system with a Bendix chemiluminescent ozone monitor (Model 8002). The ozone monitor was checked for zero and span drift weekly and a five-point calibration was conducted monthly using a CSI Photocal 3000 ozone calibrator. The ozone calibrator was verified against a Dasibi UV photometer at EPA/RTP.

All seedlings were exposed to simulated precipitation at pH 3.0 or 5.6 in the greenhouse area using a rainfall simulator developed on the principle of droplet formation from needle tips (Chevone et al., 1984). Major anionic and cationic concentrations in solutions approximated the mean concentrations found in rain in southwestern Virginia (Skelly et al., 1982), and were prepared as described by Chevone et al. (1984). See Table A1 (Appendix) for ion concentrations. The pHs of the various solutions were adjusted by adding a mixture of 1.0 M H_2SO_4 and 0.5 M HNO_3 (2:1, v/v) or 1.0 M NaOH.

Applications of simulated acidic rain were conducted for one hour, twice each week at a rainfall intensity of 0.75 cm hr^{-1} . Ozone exposures and rain exposures occurred on different days during each week.

Growth Measurements

Rate of growth was determined by measuring seedling height from a marking line to the terminal branch (loblolly pine) or the terminal bud (yellow-poplar and sweetgum) every two weeks, starting with the first day of fumigation. Ten seedlings per treatment were harvested for fresh and dry shoot and root weight after five and ten weeks of treatment. Dry weights were measured after drying in oven at 60°C for 48 hours, and these data were used for determining relative growth rate (RGR) and for use in allometric growth analyses. After ten weeks of treatment, presence or absence of foliar injury was recorded.

The growth rate was also determined by measuring a apparent plastochron duration (Besnard-Wibaut, 1981). Apparent plastochron duration (APD) is the time interval (d) between breaking two successive capsules of leaf primordia (yellow-poplar) or the time (d) which separates the formation of two 5 mm long successive leaves (sweetgum). The average number of APD among treatments was calculated for the entire ten-week exposure.

Foliar Nutrition Analysis

Needles or leaves, depending upon the tree species, were collected from seedlings used for dry weight measurement, and ground with a Cyclone Sample Mill (UD Co., Boulder, CO) through a 0.1 cm screen. One gram of ground needle

was ashed at 550° C for 5.5 hours and dissolved in 25 ml of concentrated HCl. Concentrations of P, K, Ca, Mg, Al, and S were measured using inductively coupled plasma (ICP) spectrometry, and total nitrogen was measured by the micro-Keldahl method in the Soil Testing and Plant Analysis Laboratory, Virginia Polytechnic Institute and State University.

Experimental Design

The experiments were designed as a randomized factorial combination of two ozone (0.00 ppm and 0.10 ppm) and two pH (3.0 and 5.6) treatments. Thirty-two seedlings per treatment were used, thus providing ten replicates for biomass after five and ten weeks of treatment, and six replicates for gas exchange measurements after the first and second drought cycle. Standard analysis of variance (ANOVA) techniques were used to analyze the single treatment effects and interaction between ozone and simulated acidic rain. Analysis of covariance (ANCOVA) was performed to adjust the data by a significant covariate such as initial height or initial fresh weight. Duncan's new multiple range test was used to compare the data among combined treatments.

RESULTS

Growth response of loblolly pine seedlings

Fresh weight increment (FWT) and RGR were significantly greater in seedlings exposed to simulated rain at pH 3.0 compared to pH 5.6 across all ozone treatments at the end of the ten-week exposure. However, there was no significant effect of ozone or any interaction between ozone and solution pH on any biomass variable (Table 7). Shoot height growth (SHG) of loblolly pine seedlings was not altered by any single treatment, however, a significant interaction between ozone and solution pH was evident beginning the fourth week of treatments (Figure 4). Throughout the treatment period, SHG was significantly lower in seedlings exposed to 0.1 ppm ozone + pH 5.6 than in seedlings exposed to either 0.1 ppm ozone + pH 3.0 or 0.0 ppm ozone + pH 5.6.

Growth response of yellow-poplar seedlings

No growth measurements, except the apparent plastochron duration (APD) were significantly affected by any treatment in yellow-poplar seedlings (Table 8 and Figure 5). APD was approximately 30% higher in seedlings exposed to 0.1 ppm ozone + pH 5.6 solution than any other treatment (Table 9). Visible foliar injury developed on approximately 75% of the plants submitted to simulated rain at pH 3.0 across all ozone treatments. Symptoms began to appear primarily on

Table 7. Changes in fresh weight increase (FWT), total dry weight (DWT), root to shoot ratio (RSR), and relative growth rate (RGR) of one-year-old loblolly pine after 10 wks of exposure to ozone and simulated rain.

	OZONE ³		RAIN pH ⁴			INTERACTION			
	0.0	0.1	3.0	5.6	12.08	0.0+3.0	0.0+5.6	0.1+3.0	0.1+5.6
FWT ¹	12.71	12.46	13.08†	12.08	12.08	13.27	12.15	12.90	12.01
DWT ¹	5.40	5.35	5.38	5.36	5.36	5.41	5.38	5.35	5.34
RSR	.340	.317	.339	.317	.317	.345	.335	.333	.300
RGR ²	.129	.127	.131†	.125	.125	.133	.126	.129	.125

¹gm

²gm gm⁻¹ wk⁻¹

³Ozone (ppm) treatment across all rain pHs

⁴Rain pH across all ozone treatments

Daggers indicate the significant differences at the 0.05 (†) levels

No significant interactions between ozone and rain pH occurred

FWT, DWT, and RGR are adjusted for initial fresh weight

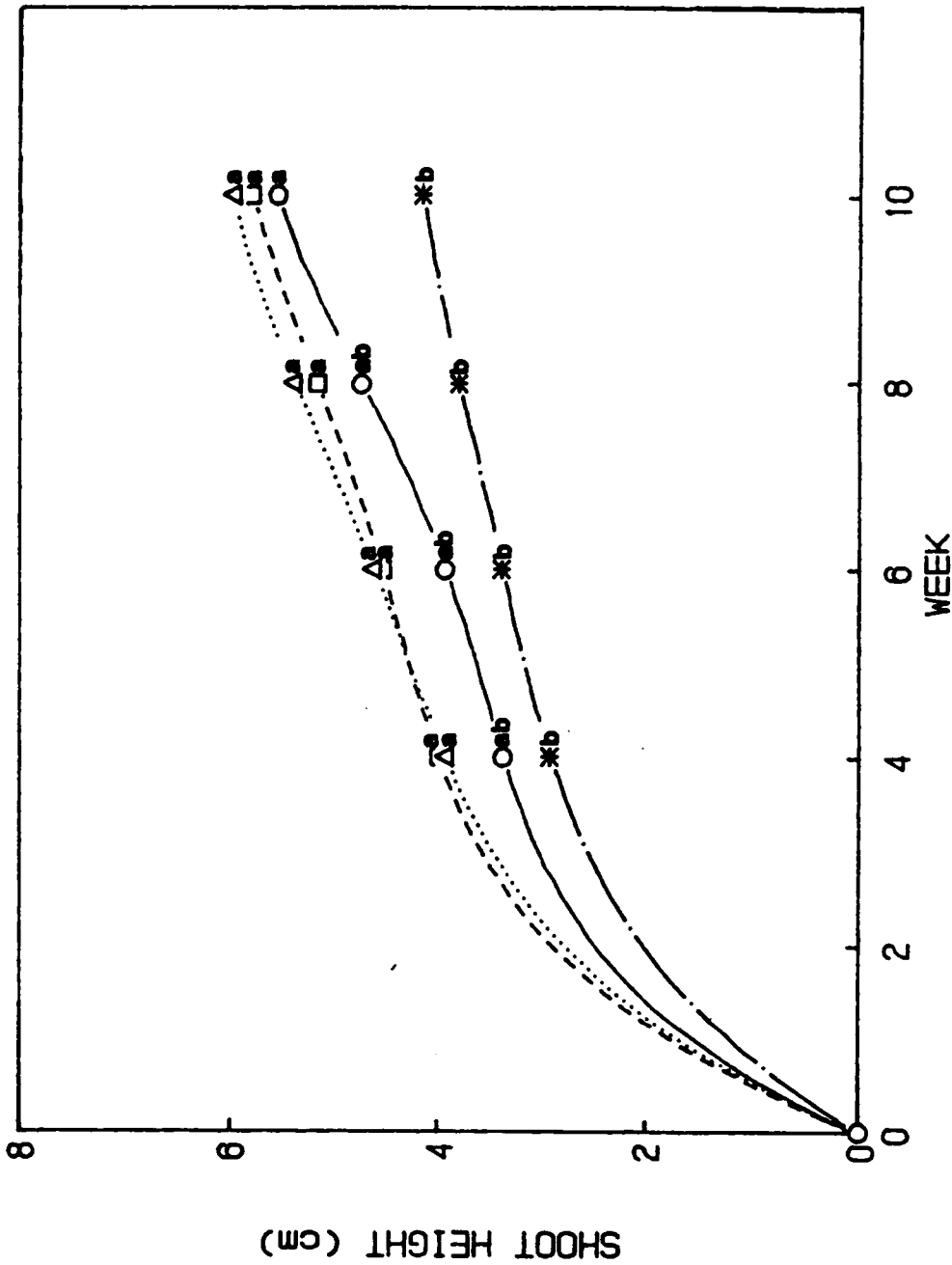


Figure 4. Changes in cumulative shoot height growth (cm) of one-year-old loblolly pine seedlings during 10 wks of treatment with ozone and simulated rain: 0.0 ppm ozone + pH 3.0 (○), 0.0 ppm ozone + pH 5.6 (△), 0.1 ppm ozone + pH 3.0 (□), 0.1 ppm ozone + pH 5.6 (*). Means with common letters are not significantly different at $P = 0.05$ by Duncan's new multiple range test. Data are adjusted for initial seedling height.

Table 8. Changes in mean fresh weight increment (FWT), total dry weight (DWT), root to shoot ratio (RSR), and relative growth rate (RGR) of one-year-old yellow-poplar after 10 wks of treatment with ozone and simulated rain.

	OZONE ³			RAIN pH ⁴			INTERACTION			
	0.0	0.1	5.96	3.0	5.6	6.00	0.0+3.0	0.0+5.6	0.1+3.0	0.1+5.6
FWT ¹	6.58	2.31	5.96	6.54	6.00	6.70	6.70	6.47	6.38	5.54
DWT ¹	2.51	2.31	2.31	2.42	2.41	2.50	2.50	2.51	2.33	2.29
RSR	.428	.449	.449	.432	.446	.405	.405	.452	.459	.439
RGR ²	.103	.098	.098	.102	.100	.103	.103	.103	.100	.096

¹ gm

² gm gm⁻¹ wk⁻¹

³ Ozone (ppm) treatment across all rain pHs

⁴ Rain pH across all ozone treatments

No significant effects of ozone and rain pH, alone and in combination, occurred

FWT, DWT, and RGR are adjusted for initial fresh weight

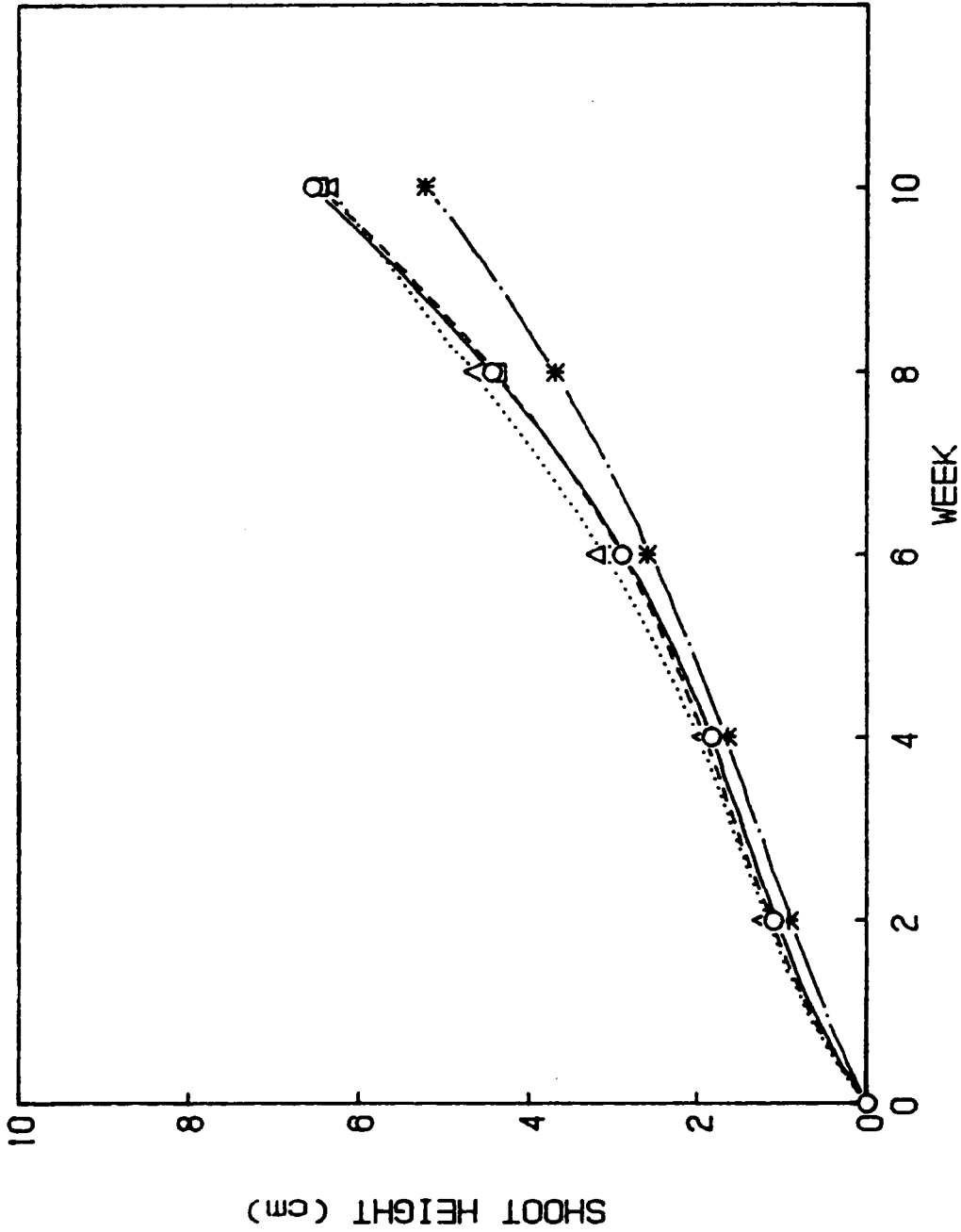


Figure 5. Changes in cumulative shoot height growth (cm) of one-year-old yellow-poplar seedlings during 10 wks of treatment with ozone and simulated rain: 0.0 ppm ozone + pH 3.0 (O), 0.0 ppm ozone + pH 5.6 (Δ), 0.1 ppm ozone + pH 3.0 (□), 0.1 ppm ozone + pH 5.6 (*). No significant effect of ozone and simulated rain occurred. Data are adjusted for initial height.

Table 9. Apparent plastochron duration (days) of one-year-old sweetgum (SG) and yellow-poplar (YP) seedlings during 10 wks of treatment with ozone and simulated acid rain.

species	OZONE ¹		RAIN pH ²		INTERACTION			
	0.0	0.1	3.0	5.6	0.0+3.0	0.0+5.6	0.1+3.0	0.1+5.6
SG	4.91	5.88‡	4.78	6.01‡	4.77a	5.06a	4.79a	6.97b
YP	6.68	7.97	6.97	7.68	7.30a	6.07a	6.63a	9.30b

¹Ozone (ppm) treatment across all rain pHs

²Rain pH across all ozone treatments

Daggers indicate the significant differences at 0.01 (‡) levels within single treatments. Means within rows of interaction with common letters are not significantly different at .05 level by Duncan's new multiple range test.

new, fully expanded leaves five weeks after treatment initiation, and were characterized by necrotic circular lesions (0.4-1.4 mm diameter) on the adaxial leaf surface. However, no ozone symptoms were observed through the ten weeks of treatment.

Growth response of sweetgum seedlings

Ozone significantly reduced SHG of sweetgum seedlings (Figure 6) and this reduction became apparent after six weeks of fumigation, and resulted in approximately 24% less than control seedlings at the end of the ten-week fumigation. There were no significant changes on FWT, RGR, total dry weight (DWT), and root to shoot ratio (RSR) as a result of ozone or simulated rain treatment (Table 10). There were significant effects of single and combined effects of ozone and simulated acid rain on APD (Table 9). APD was significantly increased by 19.8% and 25.7% in seedlings exposed to 0.1 ppm ozone and pH 5.6 solution, respectively, and resulted in 46.1% higher APD in seedlings exposed to 0.1 ppm ozone + pH 5.6 solution compared with seedlings exposed to 0.0 ppm ozone + pH 3.0 solution, which means additive combination effect of 0.1 ppm ozone and pH 5.6 solution on APD.

Visible foliar symptoms were observed on approximately 95% of the plants exposed to 0.1 ppm ozone regardless of simulated rain pH. Visible foliar injury first appeared on

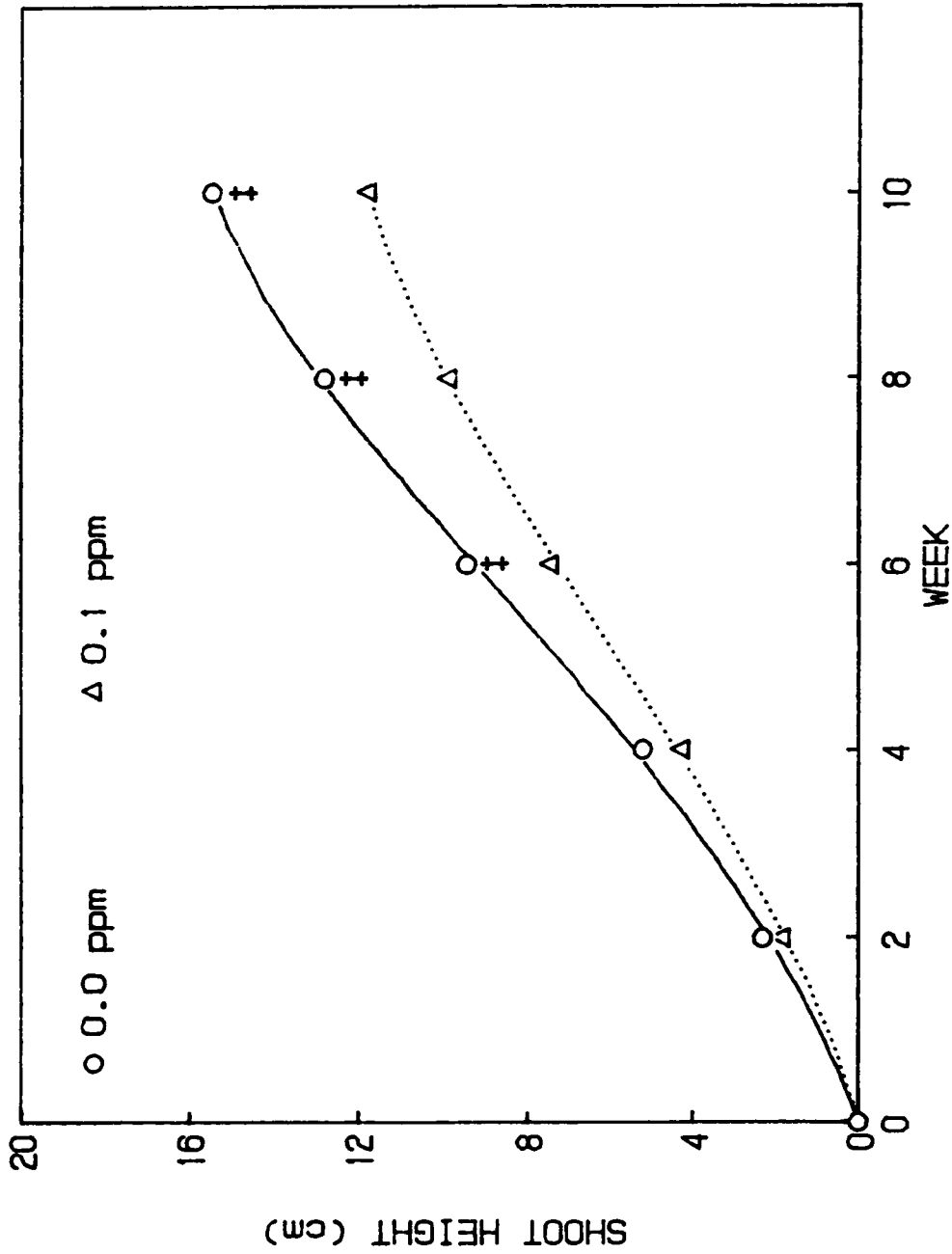


Figure 6. Changes in cumulative shoot height growth (cm) of one-year-old sweetgum seedlings during 10 wks of treatment with ozone. Dagger (†) indicates statistically significant differences at $P = 0.01$. Data are adjusted for initial height.

Table 10. Changes in mean fresh weight increment (FWT), total dry weight (DWT), root to shoot ratio (RSR), and relative growth rate (RGR) of one-year-old sweetgum seedlings after 10 wks of treatment with ozone and simulated acidic rain.

	OZONE ³		RAIN pH ⁴			INTERACTION		
	0.0	0.1	3.0	5.6	0.0 + 3.0	0.0 + 5.6	0.1 + 3.0	0.1 + 5.6
FWT ¹	27.32	24.94	26.57	25.69	28.10	26.54	25.05	24.84
DWT ¹	10.13	9.50	9.82	9.81	10.23	10.03	9.40	9.59
RSR	.361	.349	.357	.353	.353	.368	.360	.339
RGR ²	.164	.156	.163	.158	.169	.160	.157	.155

¹gm

²gm gm⁻¹ wk⁻¹

³Ozone (ppm) treatment across all rain pHs

⁴Rain pH across all ozone treatments

No significant effects of ozone and rain pH, alone and in combination, occurred
 FWT, DWT, and RGR are adjusted for initial fresh weight

the older leaves after four weeks of fumigation initiation, and was characterized by premature red pigmentation with small brown necrotic lesions on the adaxial leaf surface. There was no visible foliar injury due to the simulated acid rain.

Effects of ozone and simulated acid rain on foliar nutrient concentrations

The effects of ozone and simulated acid rain treatment on foliar nutrient concentrations are presented in Tables 11, 12, and 13. Phosphorus and sulphur were significantly greater in seedlings exposed to simulated rain at pH 3.0 compared with pH 5.6 for all three species, and significant interactions between ozone and solution pH occurred in S concentration in loblolly pine and sweetgum seedlings. Foliar S content was higher in loblolly pine seedlings exposed to 0.1 ppm ozone + pH 3.0 than in seedlings exposed to any other treatment. Whereas, in sweetgum, foliar S concentration was higher in seedlings exposed to 0.0 ppm ozone + pH 3.0 than in seedlings exposed to any other treatment. Ozone significantly increased S in loblolly pine and Ca in sweetgum seedlings, in contrast, ozone fumigation significantly reduced Ca in yellow-poplar and reduced concentrations of S and Mg in sweetgum seedlings.

Table 11. Summary of results of needle analysis of one-year old loblolly pine after ten weeks of treatment with ozone and simulated acid rain. Values (% per gram needle dry weight) are means of five samples.

	N			P			K		
	Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
	0.0	0.1		0.0	0.1		0.0	0.1	
pH 3.0	0.63	0.69	0.66	0.081	0.078	0.079*	0.32	0.33	0.33
pH 5.6	0.58	0.64	0.61	0.075	0.073	0.074	0.32	0.31	0.32
X ²	0.61	0.67		0.078	0.075		0.32	0.32	

	Ca			Mg			S		
	Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
	0.0	0.1		0.0	0.1		0.0	0.1	
pH 3.0	0.22	0.21	0.21	0.097	0.099	0.098	0.035b	0.040a	0.038**
pH 5.6	0.21	0.21	0.21	0.095	0.096	0.095	0.031c	0.031c	0.031
X ²	0.21	0.21		0.096	0.097		0.033	0.036*	

¹Averaged across all ozone levels

²Averaged across all pH levels

*.***Means indicate significant differences at 5% (*) or 1% (**) level within ozone or simulated acidic rain treatment across all other treatments

Means within interaction lacking (or with common) letters are not significantly different at 5% level by Duncan's new multiple range test

Table 12. Summary of results of foliar analysis of one-year-old yellow-poplar after ten weeks of treatment with ozone and simulated acid rain. Values (% per gram leaf dry weight) are means of five samples.

	N			P			K		
	Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
	0.0	0.1		0.0	0.1		0.0	0.1	
pH 3.0	2.60	2.35	2.48	0.12	0.12	0.14*	1.19	1.06	1.12
pH 5.6	2.52	2.44	2.48	0.10	0.10	0.11	0.99	1.03	1.01
X ²	2.56	2.40		0.10	0.09		1.09	1.05	

	Ca			Mg			S		
	Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
	0.0	0.1		0.0	0.1		0.0	0.1	
pH 3.0	1.02	0.90	0.96	0.34	0.31	0.32	0.088	0.100	0.094**
pH 5.6	0.96	0.84	0.90	0.33	0.29	0.31	0.065	0.072	0.069
X ²	0.99	0.87*		0.33	0.30		0.077	0.086	

¹Averaged across all ozone levels

²Averaged across all pH levels

*,**Means indicate significant differences at 5% (*) or 1% (**) level within ozone or simulated acidic rain treatments across all other treatments

Table 13. Summary of results of foliar analysis of one-year-old sweetgum after ten weeks of treatment with ozone and simulated acid rain. Values (% per gram leaf dry weight) are means of five samples.

	N			P			K		
	Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
	0.0	0.1		0.0	0.1		0.0	0.1	
pH 3.0	1.72	1.74	1.73*	0.16	0.16	0.16*	1.20	1.19	1.20
pH 5.6	1.60	1.66	1.63	0.14	0.14	0.14	1.16	1.17	1.17
X ²	1.66	1.70		0.15	0.15		1.18	1.18	

	Ca			Mg			S		
	Ozone		X ¹	Ozone		X ¹	Ozone		X ¹
	0.0	0.1		0.0	0.1		0.0	0.1	
pH 3.0	0.82a	0.82a	0.82	0.27	0.22	0.25	0.084a	0.071b	0.078**
pH 5.6	0.77b	0.86a	0.81	0.26	0.21	0.23	0.062c	0.060c	0.061
X ²	0.79	0.84*		0.26	0.22*		0.073	0.066**	

¹Averaged across all ozone levels

²Averaged across all pH levels

* ** Means indicate significant differences at 5% (*) or 1% (**) level within ozone or simulated acidic rain treatment across all other treatments

Means within interaction lacking (or with common) letters are not significantly different at 5% level by Duncan's new multiple range test

DISCUSSION

Detrimental effects of ozone on the growth of loblolly pine seedlings have been reported in both field (Shafer et al., 1987) and laboratory studies (Kress, 1978; Kress and Skelly, 1982; Ward, 1980; Winner et al., 1987; Shafer et al., 1987), which is in contrast to results obtained in the present study. However, total dose of ozone that seedlings received was higher in most of the previous reports than in the present experiment. For example, Winner et al. (1987) and Ward (1980) reported that total doses of 14.0 and 33.6 ppm hr ozone, respectively, resulted in a significant reduction of height growth, respectively, whereas only 12 ppm hr ozone was used in the current study. In a field study, Shafer et al. (1987) found that reductions of shoot height, stem diameter, and dry weight occurred after five months of treatment with a total dose of at least 77.3 ppm hr ozone. In the present study, although ozone did not result in any statistically significant effect on SHG of loblolly pine seedlings, the growth suppression increased gradually to result in a reduction of 14% ($P=0.053$) at the end of the ten-week fumigation, which is similar to the amount of growth suppression reported by Shafer et al. (1987).

Ozone alone has been reported to have little effect on the growth of yellow-poplar seedlings in most laboratory studies (Jensen, 1973; Kress and Skelly, 1982; Mahoney et

al., 1984; Chappelka et al., 1985), which is consistent with the current results. In contrast, Duchelle et al. (1982) found that ambient concentration of ozone (monthly average was 0.05 ppm) significantly reduced the height growth of yellow-poplar by 44% in a field study. Jensen (1985) also observed suppression of growth in yellow-poplar with 0.1 ppm ozone in a laboratory study.

In this present study, although FWT, DWT, and RGR of yellow-poplar were not affected significantly by ozone or simulated acid rain, in general, treatments with ozone and pH 5.6 solution resulted in lower biomass accumulation compared with 0.0 ppm ozone and pH 3.0 solution. The repressive effects of ozone and pH 5.6 solution were additive for all biomass variables. Therefore, the potential exists for a detrimental effect of ozone or for the interactive effects of ozone and simulated acid rain on growth of yellow-poplar.

In this present study, sweetgum is the most sensitive species to ozone in terms of SHG, APD, and foliar injury. Sweetgum was the only species exhibited significant foliar symptom when treated with 0.05 ppm ozone among ten eastern forest species examined by Kress and Skelly (1982). In this study, only sweetgum seedlings showed foliar ozone injury. APD was the most sensitive indicator for ozone and simulated acid rain in sweetgum and yellow-poplar.

Most of the previous studies on the effect of air pol-

lutants on plant growth (Reich and Amundson, 1985; Reich et al., 1986, 1987; Skeffington and Roberts, 1985; Elliot et al., 1987) have reported no significant interactions between ozone and simulated rain pH, which is supported by our studies with yellow-poplar and sweetgum seedlings. However, loblolly pine seedlings showed a significant interaction between ozone and solution pH for SHG.

A fertilization effect of simulated acid rain found in loblolly pine is in agreement with the report of Wells et al. (1973). These authors found, in the Southeastern Coastal Plain, that, in loblolly pine needles, a concentration of 0.1 percent P was the critical level, above this concentration, tree height was not increased by P fertilization whereas below this concentration growth was usually increased. According to the present results, application of simulated rain at pH 3.0 significantly increased P concentration for all three species, however, only loblolly pine seedlings (needle P content was less than 0.08 % per g dry weight) exhibited a significant increase of growth in terms of fresh weight increment and relative growth rate.

Although simulated acid rain contained very high concentrations of S and N, only foliar S concentration was increased in all species except sweetgum. The stimulation effect of simulated rain at pH 3.0 on S concentration supports the results reported by Abouguendia and Baschak (1987) for white spruce (Picea glauca). The increase in

foliar S was not above the threshold value of 0.12 % (Zech et al., 1985), and did not result in any growth alteration.

German researchers found Mg and Ca deficiencies were induced by high SO₂ concentration (Zech et al., 1983) and by the combination of ozone and simulated acid rain (Bosch et al., 1986, Weiss and Agerer, 1986). Magnesium and Ca fertilization resulted in a revitalization of trees which had exhibited Mg-deficiency symptoms by air pollutants (Kampenjohann et al., 1987; Bosch et al., 1986; Weiss and Agerer, 1986). Magnesium is known to play many essential roles in the processes of plant metabolism. Magnesium is a constituent of the chlorophyll molecule, and is necessary for full activity of many enzymes involved in carbohydrate metabolism and in the synthesis of nucleic acids. In this present study, the Mg content was significantly reduced in seedlings exposed to 0.1 ppm ozone compared with control plants. A reduced Mg concentration may affect Pn and cause a reduction in SHG and APD. The effects of simulated acid rain on foliar nutrient concentration were variable among tree species. Similar results have been reported in the literature. Wood and Bormann (1977) found declines of K, Mg, and Ca concentrations at pH's of 3.0 and below, and a positive correlation of nitrogen level in plant tissue with nitrate level in simulated rain. However, Tveite (1980) reported increases of concentrations of sulphate, P, Mn, and Fe by treatment with ground water acidified with only

sulphuric acid. When peat moss (Abouguensia and Baschak, 1987) or fertilizer (Takemoto et al., 1987) were added to potting media, no significant alterations were observed for any foliar nutrients. Therefore, interpretation of the results should be performed with care based on soil characteristics, fertilizer, simulated rain constituents, and experimental environments (field, greenhouse, temperature, humidity, etc.). The results from this study indicate that ozone and/or simulated acidic rain can alter the growth and foliar nutrient status of forest trees under laboratory conditions. However, additional research is necessary using different tree ages, soil type, and field test to determine if such effects found in the laboratory conditions are occurring in natural forest ecosystems.

LITERATURE CITED

- Abouguendia, Z. M. and L. A. Baschak. 1987. Response of two western Canadian conifers to simulated acidic precipitation. *Water, Air, & Soil Pollut.* 33:15-12.
- Besnard-Wilbaut, C. 1981. Effectiveness of gibberellins and 6-benzyladenine on flowering of Arabidopsis thaliana. *Physiol. Plant* 53:205-212.
- Bosch, Chr., E. Pfannkuch, K. E. Rehfuss, K. H. Runkel, P. Schramel and M. Senser. 1986. Einfluß einer Dünung mit Magnesium und Calcium, von Ozon und saurem Nebel auf Frostharte, Ernährungszustand und Biomasseproduktion junger Fichten (Picea abies [L.] Karst.). *Forstw. Cbl.* 105:218-229.
- Chappelka III, A. H., B. I. Chevone and T. E. Burk. 1985. Growth response of yellow-poplar (Liriodendron tulipifera L.) seedlings to ozone, sulfur dioxide, and simulated acidic precipitation, alone and in combination. *Environ. Exp. Bot.* 25:233-244.
- Chevone, B. I., Y. S. Yang, W. E. Winner, I. Storcks-Cotter and S. J. Long. 1984. A rainfall simulator for laboratory use in acidic precipitation studies. *JAPCA* 31:355-359.
- Cogbill, C. V. and C. E. Likens. 1974. Acid precipitation in the northeastern United States. *Water Resour. Res.* 10:1133-1137.
- Duchelle, S. F., J. M. Skelly and B. I. Chevone. 1982. Oxidant effects on forest tree seedling growth in the Appalachian Mountain. *Water, Air & Soil Pollut.* 12:363-373.
- Duchelle, S. F., J. M. Skelly, T. L. Sharik, B. I. Chevone, Y. S. Yang and J. E. Nellessen. 1983. Effects of ozone on the productivity of natural vegetation in a high meadow of the Shenandoah Park of Virginia. *J. Environ. Mgt.* 17:299-308.
- Elliott, C. L., J. C. Eberhardt and E. G. Brennan. 1987. The effect of ambient ozone pollution and acidic rain on the growth and chlorophyll content of green and white ash. *Environ. Pollut.* 44:61-70.
- Friedland, A. J., A. H. Johnson and T. G. Siccama. 1984a. Trace metal content of the forest floor in the Green Mountains of Vermont: Spatial and temporal patterns.

- Water Air & Soil Pollut. 21:161-170.
- Friedland, A. J., A. H. Johnson, T. G. Siccama and D. L. Lader. 1984b. Trace metal profiles in the forest floor of New England. *Soil Sci. Soc. Amer. J.* 48:422-425.
- Friedland, A. J., G. A. Lawley and R. A. Gregory. 1985. Investigations of nitrogen as a possible contributor to red spruce (*Picea rubens* Sarg.) decline. In *Proc. Air Pollutants Effects on Forest Ecosystems*. The Acid Rain Foundation, St. Paul, MN. pp. 95-106.
- Heck, W. W., R. B. Philbeck and J. A. Dunning. 1978. A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants. USDA-ARS Paper ARS-S-181.
- Jensen, K. F. 1973. Response of nine forest tree species to chronic ozone fumigation. *Plant Dis. Reptr.* 57:914-917.
- Jensen, K. F. 1985. Response of yellow poplar seedlings to intermittent fumigation. *Environ. Pollut. (ser. A)*. 38:183-191.
- Johnson, A. H. 1983. Red spruce decline in the northeastern U. S.: Hypotheses regarding the role of acid rain. *JAPCA* 33:1049-1054.
- Johnson, A. H. and T. G. Siccama. 1983. Acid deposition and forest decline. *Environ. Sci. Tech.* 17:294-305.
- Johnson, A. H. and T. G. Siccama. 1984. Decline of red spruce in the northern Appalachians: Assessing the possible role of acid deposition. *TAPPI* 67:68-72.
- Kaupenjohann, M., W. Zech, R. Hantschel and R. Horn. 1987. Ergebnisse von Dungungsversuchen mit Magnesium an vermutlich immissionsgeschädigten Fichten (*Picea abies* [L.] Karst.) im Fichtelgebirge. *Forstw. Cbl.* 106:78-84.
- Kress, L. W. 1978. Growth impact of O₃, SO₂ and NO₂ singly and in combination on loblolly pine (*Pinus taeda* L.) and American sycamore (*Plantanus occidentalis* L.). Ph.D. Dess. VPI & SU, Blacksburg, VA. 201 pp.
- Kress, L. W. and J. M. Skelly. 1982. Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. *Plant Disease* 66:1149-1152.
- Lioy, P. J. and P. J. Samson. 1979. Ozone concentration patterns observed during the 1976-1977 long range transport study. *Environ. Int'l.* 2:77-83.

- Mahoney, M. J., J. M. Skelly, B. I. Chevone and L. D. Moore. 1984. Response of yellow poplar (Liriodendron tulipifera L.) seedling shoot growth to low concentrations of O₃, SO₂ and NO₂. Can. J. For. Res. 14:150-153.
- McLaughlin, S. B., R. K. McConathy, D. Duvick and L. K. Mann. 1982. Effects of chronic air pollution stress on photosynthesis, carbon allocation, and growth of white pine trees. For. Sci. 28:60-70.
- Miller, P. L., M. H. McCulchan and H. P. Milligan. 1972. Oxidant air pollution in the Central Valley, Sierra Nevada Foothills, and Mineral King Valley of California. Atmos. Environ. 6:623-633.
- Reich, P. B., and R. G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566-570.
- Reich, P. B., A. W. Schoettle, and R. G. Amundson. 1986. Effects of O₃ and acidic rain on photosynthesis and growth in sugar maple and northern red oak seedlings. Environ. Pollut. (ser. A). 40:1-15.
- Reich, P. B., A. W. Schoettle, R. M. Raba, and R. G. Amundson. 1987. Response of soybean to low concentration of ozone: I. Reductions in leaf and whole plant net photosynthesis and leaf chlorophyll content. J. Environ. Qual. 15:31-36.
- Shafer, S. R., A. S. Heagle and D. M. Camberato. 1987. Effects of chronic doses of ozone on field-grown loblolly pine: Seedling responses in the first year. JAPCA 37:1179-1184.
- Skeffington, R. A. and T. M. Roberts. 1985. The effects of ozone and acid mist on Scots pine saplings. Oecologia (Berlin) 65:201-206.
- Skelly, J. M., B. I. Chevone and Y. S. Yang. 1982. Effects of ambient concentrations of air pollutants on vegetation indigenous to the Blue Ridge Mountains of Virginia. In Acid Rain: A water Resources Issue for the 80's. R. Herrmann and A. I. Johnson (eds.). American Water Resources Association, Bethesda, MD. pp. 69-74.
- Takemoto, B. K., D. S. Shriner and J. W. Johnston, Jr. 1987. Physiological responses of soybean (Glycine max Merr) to simulated acid rain and ambient ozone in the field. Water, Air & Soil Pollut. 33:373-384.

- Tveite, B. 1980. Effects of acid precipitation on soil and forest. 8. Foliar nutrient concentrations in field experiments. In D. Drablos and A. Tollan, (eds.). Proceedings International conference on the Ecological Impact of Acid Precipitation, Sandefjord, Norway. pp. 204-205.
- Ulrich, B. 1981. Eine okosystemare Hypothese uber die Ursachen des Tannensterbens (Abies alba Mill.). Forstw. Cbl. 100:228-236.
- Ward, M. M. 1980. Variation in the response of loblolly pine to ozone. M.S. Thesis, VPI & SU, Blacksburg, VA. 201 pp.
- Weiss M. and R. Agerer. 1986. Reaktionen des Warzelsystems von Picea abies (L.) Karst. auf Mineralstoffernahrung und auf Belastung des Sprosses mit ozon und saurem Nebel. Forstw. Cbl. 105:230-233.
- Wells, C. G., D. M. Crutchfield, N. M. Bereny and C. B. Davey. 1973. Soil and foliar guidelines for phosphorus fertilization of loblolly pine. U.S.D.A. Forest Service Research Paper SE-110, October, 1973.
- Winner, W. E., I. S. Cotter, H. R. Powers, Jr. and J. M. Skelly. 1987. Screening loblolly pine seedling responses to SO₂ and O₃: Analysis of families differing in resistance to Fusiform rust disease. Environ. Pollut. 47:205-220.
- Wood, T. and F. H. Bormann. 1977. Short-term effects of a simulated acid rain upon the growth and nutrient relations of Pinus strobus L. Water, Air, & Soil Pollut. 7:479-488.
- Yang, Y. S. and B. I. Chevone. 1982. Characterization of ambient ozone in the Blue Ridge Mountains of Virginia. Phytopathology 72:712.
- Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983a. Effects of long-term ozone exposure on photosynthesis and dark respiration of eastern white pine. Environ. Sci. Tech. 17:371-373.
- Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983b. Effects of short-term ozone exposure on net photosynthesis, dark respiration and transpiration of three eastern white pine clones. Environ. Int'l. 9:265-269.
- Zech, W. and E. Popp. 1983. Magnesiummangel, einer der

grunde fur das fichten-und tannensterben in NO-Bayern.
Forstw. Cbl. 102:50-55.

Zech, W., T. H. Suttner and E. Popp. 1985. Elemental analyses and physiological responses of forest trees in SO₂-polluted areas of NE-Bavaria. Water, Air, & Soil Pollut. 25:175-183.

CHAPTER III

WATER RELATIONS AND PHYSIOLOGY OF TREE SPECIES AS INFLUENCED BY DROUGHT AND AIR POLLUTANTS

INTRODUCTION

Ozone, among gaseous pollutants, is considered to have the greatest harmful effect on plants throughout most of the eastern United States. Although early air pollution research focused on foliar symptoms (Yang et al., 1983a), it now is well known that ozone can alter plant growth and yield without causing any visible symptoms (Heck et al., 1982; Duchelle et al., 1982, 1983; Reich and Amundson, 1984; Shafer et al., 1987). Ozone-induced growth reductions in plants have been explained through inhibition of photosynthesis (Miller et al., 1969; Barnes, 1972; Yang et al., 1982, 1983a, b; Reich et al., 1983, 1986, 1987) and through changes in photosynthate allocation (McLaughlin and McConathy, 1983; McLaughlin et al., 1982). However, the responses of plants to ozone is quite variable due to differences in environmental conditions and genetic variation (Tingey and Taylor, 1982).

Acidic precipitation is also a significant air pollution problem in the eastern United States and Europe

(Evans, 1984a, b). Many reports on acid rain have appeared within the last ten years that show inhibitory (Matziris and Nakos, 1977; Dochinger, 1976; Raynal et al., 1982) or stimulatory (Wood and Bormann, 1974, 1977; Lee and Weber, 1979; Reich et al., 1987) as well as no effect (McColl and Johnson, 1983; Abouguendia and Baschak, 1987) on plant growth. Although researchers have tried to explain growth impact of acid rain through changes in photosynthesis (Reich et al., 1986, 1987; Neufeld et al., 1985), this mechanism of action has not been conclusively demonstrated.

Root hydraulic conductivity (L_p) is known to have a role in controlling water relations in plants, and can be affected by many factors including root age (Fiscus and Markhart, 1979), root temperature (Smit-Spinks et al., 1983; Markhart et al., 1979, 1980), degree of suberization (Sands et al., 1982), hormone concentration (Glinka, 1973, 1977, 1980; Tal and Imber, 1971; Fiscus 1981b), disease (Dawson and Weste, 1982, 1984; Tzeng et al., 1985), vesicular-arbuscular mycorrhizae (Nelsen and Safir, 1982; Safir et al., 1972), and nutrients (Radin and Eidenbock, 1984; Radin and Boyer, 1982). However, the effects of ozone and acid rain on the L_p of plants have not been investigated.

The decline of mature coniferous trees in the northeastern United States, beginning in the early 1960's, appears to have been initiated by a severe drought (Johnson and

Siccama, 1983, 1984). The similar synchronous occurrence between the initiation of growth decline and drought was also reported in mid-Appalachian forests (Adams et al., 1985). A hypothesis has been suggested that acid deposition, heavy metal inputs, and possibly, ozone stress can impair root growth and root physiology to such an extent that affected trees become highly drought susceptible (Johnson and Siccama, 1984). Although effects of ozone and water stress in combination have been studied (Khatamian et al., 1973; Olszyk and Tibbitts, 1981; Tingey et al., 1982; Tseng et al., 1988; Tingey and Hogsett, 1985), the effect of drought stress on plants already subjected to ozone and acid rain has not been investigated. Cornic (1987) reported a significant decline in photosynthesis after rehydration of plants exposed to SO₂, which suggests that the potential for a change of drought susceptibility exists in plants pre-exposed to other pollutants such as ozone and acid rain.

The objective of this study was to examine the hypothesis that ozone and acidic inputs can alter the drought susceptibility of three southeastern commercial forest trees, loblolly pine (Pinus taeda L.), yellow-poplar (Liriodendron tulipifera L.), and sweetgum (Liquidamba styraciflua L.) subjected to severe moisture stress. Drought response was characterized by measuring Lp, water potential, and foliar gas exchange processes.

MATERIALS AND METHODS

Plant Materials

One-year-old, mixed seed lot, loblolly pine seedlings were obtained from the Virginia Department of Forestry, New Kent Forestry Center (Providence Forge, VA). One-year-old yellow-poplar and sweetgum seedlings were obtained from a commercial source (Hillis Nursery Co., Inc., McMinnville, TN). All seedlings were transplanted into 10 cm (loblolly pine) or 15 cm (yellow-poplar and sweetgum) diameter plastic pots containing Altavista soil (fine-loamy, mixed, thermic, Aquic Hapludult) which was collected from the A horizon at Reynolds Homestead Agricultural Experiment Station (Patrick County, VA). Soil was screened through a 0.5 cm mesh and mixed with sand in ratio of 5:1 (v/v). Prior to transplanting, the fresh weight of each seedlings was measured. All seedlings were grown in a greenhouse supplied with charcoal-filtered air (mean hourly ozone concentration < 0.025 ppm). The photoperiod was supplemented with 1000 W sodium lamps to provide a 16-hour daylength with 580 ± 30 $\mu\text{Mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD). Ozone fumigations and simulated acidic rain applications were begun when the majority of seedlings started to break bud.

Ozone and Acid Rain Exposures

Seedlings were exposed to ozone in a continuous stirred tank reactor system (CSTR) (Heck et al., 1978). Ozone fumigations occurred for four consecutive hours, three days a week for ten continuous weeks at 0.00 or 0.10 $\mu\text{l l}^{-1}$ (ppm). Exposure conditions within the CSTRs were maintained at $27 \pm 2^\circ \text{C}$, $55 \pm 5\% \text{RH}$, and $600 \pm 30 \mu\text{Mol m}^{-2} \text{s}^{-1}$ PPFD at plant canopy height. Ozone was generated by UV discharge using a Welsbach ozone generator (Model T-408) and monitored in each CSTR on a time-shared system with a Bendix chemiluminescent ozone monitor (Model 8002). The ozone monitor was checked for zero and span drift weekly and a five-point calibration was conducted monthly using a CSI Photocal 3000 ozone calibrator. The ozone calibrator was verified against a Dasibi UV photometer at EPA/RTP.

All seedlings were exposed to simulated acidic precipitation in the greenhouse area using a rainfall simulator developed on the principle of droplet formation from needle tips (Chevone et al., 1984). Major anionic and cationic concentrations in solutions were approximated the mean concentrations found in rain in southwestern Virginia (Skelly et al., 1982) and prepared as described by Chevone et al. (1984). See Table A1 (Appendix) for ion concentrations. The pH of the various solutions were adjusted to the desired pH by adding the mixture of 1.0 M H_2SO_4 and 0.5 M HNO_3 (2:1, v/v) or 1.0 M NaOH.

Applications of simulated acidic rain were conducted for one hour, twice each week at a rainfall intensity of 0.75 cm hr^{-1} . Ozone exposures and rain exposures occurred on different days during each week.

Drought Stress and Associated Measurements

After the ten-week fumigation and acid rain treatment period, the seedlings were exposed to one last acidic rain event to bring the soil water content to field capacity. Water was then withheld from all seedlings to simulate a drought. Beginning the day after watering and continuing every day, net photosynthesis (Pn), stomatal conductance (Cs), and transpiration (Ts) were measured on twelve seedlings per treatment with a LI-6000 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE). Each branch of loblolly pine seedlings or each leaf of yellow-poplar and sweetgum seedlings was marked to insure repetitive measurements and placed into a quarter-liter (loblolly pine) or a liter cuvette (yellow-poplar and sweetgum) for determining gas exchange rates. Average environmental variables within the cuvette were $32 \pm 3^\circ\text{C}$, $39 \pm 8\%$ RH, $845 \pm 70 \text{ uMol m}^{-2} \text{ s}^{-1}$ PPFD and $360 \pm 30 \text{ ppm CO}_2$ concentration. After each gas exchange measurement, seedling water potential (Wp) was measured on a fascicle or leaf using a pressure chamber (Scholander et al., 1965). The gas exchange measurements continued until the mean net photosynthetic rate dropped below five percent

of the initial rate for that treatment. Water-use efficiency (WUE) was calculated as P_n divided by T_s .

After the first drought cycle, six seedlings per treatment were harvested to measure root hydraulic conductivity (L_p). The remaining six seedlings per treatment were rewatered with the appropriate simulated acidic rain solution, and submitted to a second drought cycle. Net photosynthesis, T_s , W_p , and L_p were measured as in the first drought cycle.

Root hydraulic conductivity, as described by Fiscus (1975, 1977, 1981a) and Ramos and Kaufmann (1979), was also determined on six seedlings per treatment after five and ten weeks of air pollutant treatment. The seedlings used for gas exchange measurements were rewatered with an appropriate acidic rain treatment and maintained in the laboratory at 25°C for two to three hours. The stem was then cut at 4 cm above the soil surface and the soil was carefully washed away from the root system. The root system was submerged in a 0.4-liter pressure chamber containing half strength Hoagland solution at 23±1°C with the stem protruding through a silicon seal. A calibrated pipet was connected to the stump using a small piece of rubber tubing, and the air pressure within the chamber was increased slowly to a final constant pressure of 5 bar. Exudation rate was measured every 15 minutes for at least one hour after a constant flow rate occurred (usually 30-60 min.).

L_p was calculated as the exudation rate (nl s^{-1}) per unit root dry weight (g) and pressure (bar).

Experimental Design

The experiments were designed as a randomized factorial combination of two ozone treatments (0.00 and 0.10 ppm) and two pH values (3.0 and 5.6). Thirty-two seedlings per treatment were used, thus providing ten replicates for biomass after five and ten weeks of treatment, and six replicates for gas exchange measurements after the first and second drought cycle. Standard analysis of variance (ANOVA) was used to analyze the single treatment effects and interactions between ozone and simulated acidic rain. Duncan's new multiple range test was used to compare the data among combined treatments. Stepwise regression analysis was performed to determine the best-fit model for relationship between photosynthesis rate and water potential. The slopes and intercepts of the regression models between treatments were tested by a t-test using the dummy-variable model (Kleinbaum and Kupper, 1978).

RESULTS**Ozone and simulated rain effects on the gas exchange rates**

Ten weeks of exposure to ozone had a minimal effect on the gas exchange rates of the tree seedlings (Table 14). However, moisture stress significantly altered gas exchange rates and these changes were influenced by ozone treatment in loblolly pine and sweetgum. Loblolly pine seedlings exposed to 0.1 ppm ozone exhibited a significant increase in Pn and Ts after the first drought cycle compared with control seedlings. In sweetgum seedlings after moisture stress, all measurements except WUE were significantly reduced in seedlings exposed to 0.1 ppm ozone compared to control seedlings. However, yellow-poplar exhibited no significant effect of ozone on any gas exchange rate (Table 14).

Ten weeks of treatment with simulated rain at pH 3.0 resulted in greater Pn and Ts rates in loblolly pine compared to seedlings treated with pH 5.6 solution (Table 15). Photosynthesis and Ts were not affected in yellow-poplar and sweetgum by the ten-week treatment with simulated rain. However, Cs was reduced after ten weeks of treatment with simulated rain at pH 3.0 compared with pH 5.6. Following moisture stress, significant differences in gas exchange rates were developed when pre-treated with simulated rain.

Table 14. Changes in net photosynthesis (Pn), stomatal conductance (Cs), transpiration (Ts), and water-use efficiency (WUE) of one-year-old loblolly pine, yellow-poplar, and sweetgum seedlings after 10 wks of fumigation (FG) and the first drought cycle (DC).

	Ozone ppm	Loblolly pine		Yellow-poplar(Yp)		Sweetgum(Sg)	
		FG	DC	FG	DC	FG	DC
Pn ¹	0.0	4.87	2.75	0.27	0.19	0.23	0.15
	0.1	4.50	3.68‡	0.22	0.20	0.21	0.11†
Cs ²	0.0	--	--	0.52	0.45	0.72	0.33
	0.1	--	--	0.48	0.41	0.72	0.26†
Ts ³	0.0	1.07	0.94	46.9	46.6	68.2	39.1
	0.1	1.10	1.47‡	46.5	47.7	72.2	33.3†
WUE ⁴	0.0	4.84	2.91	5.78	4.43	3.51	4.07
	0.1	4.20	2.56	5.09	4.32	3.31	3.29

¹Pn(Loblolly) = mgCO₂g⁻¹hr⁻¹

¹Pn(Yp & Sg) = mgCO₂m⁻²s⁻¹

²Cs = cm/s

³Ts(Loblolly) = gH₂Og⁻¹hr⁻¹

³Ts(Yp & Sg) = mgH₂Om⁻²s⁻¹

⁴WUE = mgCO₂/gH₂O

Daggers indicate the significant differences at 0.05 (†) or 0.01 (‡) levels within treatment periods

--Cs data for loblolly pine were not available

Table 15. Changes in net photosynthesis (Pn), stomatal conductance (Cs), transpiration (Ts), and water-use efficiency (WUE) of one-year-old loblolly pine, yellow-poplar, and sweetgum seedlings after 10 wks of acid rain treatment (AT) and the first drought cycle (DC).

	RAIN	Loblolly pine		Yellow-poplar(Yp)		Sweetgum(Sg)	
	pH	AT	DC	AT	DC	AT	DC
Pn ¹	3.0	5.24‡	3.20	0.23	0.17‡	0.22	0.14
	5.6	4.14	3.22	0.27	0.22	0.22	0.11
Cs ²	3.0	--	--	0.43‡	0.43	0.71	0.32
	0.1	--	--	0.58	0.43	0.73	0.26
Ts ³	3.0	1.18†	1.28	43.7	47.6	71.7	40.6‡
	5.6	0.99	1.14	49.7	46.7	69.0	31.6
WUE ⁴	3.0	4.54	3.31	5.27	3.69	3.26	3.54
	5.6	4.50	3.52	5.61	5.05†	3.56	3.82

¹Pn(Loblolly) = mgCO₂g⁻¹hr⁻¹

¹Pn(Yp & Sg) = mgCO₂m⁻²s⁻¹

²Cs = cm/s

³Ts(Loblolly) = gH₂Og⁻¹hr⁻¹

³Ts(Yp & Sg) = mgH₂Om⁻²s⁻¹

⁴WUE = mgCO₂/gH₂O

Daggers indicate the significant differences at 0.05 (†) or 0.01 (‡) levels within treatment periods.

--Cs data for loblolly pine were not available.

Yellow-poplar seedlings exposed to pH 3.0 solution showed a significant reduction of Pn and WUE after the first drought cycle, Transpiration rate of sweetgum was significantly increased in seedlings exposed to pH 3.0 solution compared to pH 5.6 after the first drought cycle (Table 15).

There was no significant interaction between ozone and simulated rain for any measurement in all species.

Ozone and simulated rain effects on gas exchange and water relations

The relationship between gas exchange rate and foliar water potential (Wp) was examined by regression analysis using several model parameters. The best-fit model for all treatments was of the form $P_n = a + b \ln(W_p)$ for loblolly pine. In yellow-poplar and sweetgum, VPD (vapor pressure deficit) was also a significant variable the model due likely to the responsiveness of their stomata. The best-fit models for yellow-poplar and sweetgum were of the form $P_n = a + b \ln(W_p) + c (VPD)$ and $P_n = a + b (W_p) + c (VPD)$, respectively. The VPD (KPa) was calculated by the following equation (Jones, 1983):

$$VPD = 0.61078 * (1-R.H.) * \exp [17.269 * T / (237.3 + T)]$$

where R.H. and T are relative humidity in decimal and chamber temperature in °C, respectively.

The relationship between Pn or Ts and Wp during moisture stress was affected by pre-exposure to ozone and/or

simulated rain. In loblolly pine, pre-treatment with ozone and/or pH 3.0 solution resulted in an increase in Pn and Ts at -1.0 MPa needle Wp (y-intercept) and also altered the physiological response of Pn and Ts to needle Wp compared to 0.0 ppm ozone + pH 5.6 solution (Table 16 and Figures 7, 8, 9, and 10). During the first drought cycle, loblolly pine seedlings exposed to pH 3.0 solution had a higher y-intercept and more negative slope for Pn than seedlings exposed to pH 5.6 solution (Table 16 and Figure 7). During the second drought cycle, seedlings exposed to 0.1 ppm ozone had a higher y-intercept and more negative slope for Pn and Ts in loblolly pine (Figures 8 and 10). These changes in slope due to the treatments with simulated rain or ozone indicate that seedlings exposed to air pollutants decreased photosynthesis rate more rapidly than control seedlings as needle Wp declined. However, a lower Pn rate due to treatments with ozone and simulated rain occurred only when water potential dropped below -2.0 MPa (Figures 7 and 8).

In yellow-poplar, seedlings pre-exposed to pH 3.0 solution had a more negative slope for Pn than seedlings exposed to pH 5.6 solution during the first drought cycle. During the second drought cycle, the slope for Pn of seedlings exposed to 0.0 ppm ozone + pH 3.0 solution was more negative than seedlings exposed to any other treatment (Table 17). Cs and Ts showed similar trends with Pn,

Table 16. Regression models and coefficients of determination for net photosynthesis (Pn) and transpiration (Ts) of one-year-old loblolly pine seedlings during two drought cycles following exposure to ozone and simulated acidic rain¹.

Cycle	O ₃ (ppm)	Rain pH	Model		R ²	n
First Cycle	0.0	5.6	Pn = 3.20	-3.09 ln(Wp)	.69	59
	0.0	3.0	Pn = 4.49‡	-4.63‡ ln(Wp)	.69	54
	0.1	5.6	Pn = 3.50	-3.10 ln(Wp)	.69	58
	0.1	3.0	Pn = 4.07‡	-4.03 ln(Wp)	.69	51
Second Cycle	0.0	5.6	Pn = 3.17	-2.39 ln(Wp)	.69	21
	0.0	3.0	Pn = 4.02	-3.27 ln(Wp)	.69	23
	0.1	5.6	Pn = 4.67‡	-4.22‡ ln(Wp)	.69	23
	0.1	3.0	Pn = 5.52‡	-5.69‡ ln(Wp)	.69	22
First Cycle	0.0	5.6	Ts = 0.80	-0.67 ln(Wp)	.65	62
	0.0	3.0	Ts = 0.93	-0.78 ln(Wp)	.65	60
	0.1	5.6	Ts = 0.84	-0.64 ln(Wp)	.65	58
	0.1	3.0	Ts = 0.97†	-0.88 ln(Wp)	.65	54
Second Cycle	0.0	5.6	Ts = 0.91	-0.75 ln(Wp)	.54	21
	0.0	3.0	Ts = 1.15	-0.99 ln(Wp)	.54	23
	0.1	5.6	Ts = 1.45†	-1.40 ln(Wp)	.54	23
	0.1	3.0	Ts = 2.00‡	-2.20‡ ln(Wp)	.54	22

¹Pn = mgCO₂g⁻¹hr⁻¹; Ts = gH₂Og⁻¹hr⁻¹; Wp = needle water potential

Daggers indicate the significant differences from 0.0 ppm ozone + pH 5.6 within drought cycles at 0.05 (†) or 0.01 (‡) levels

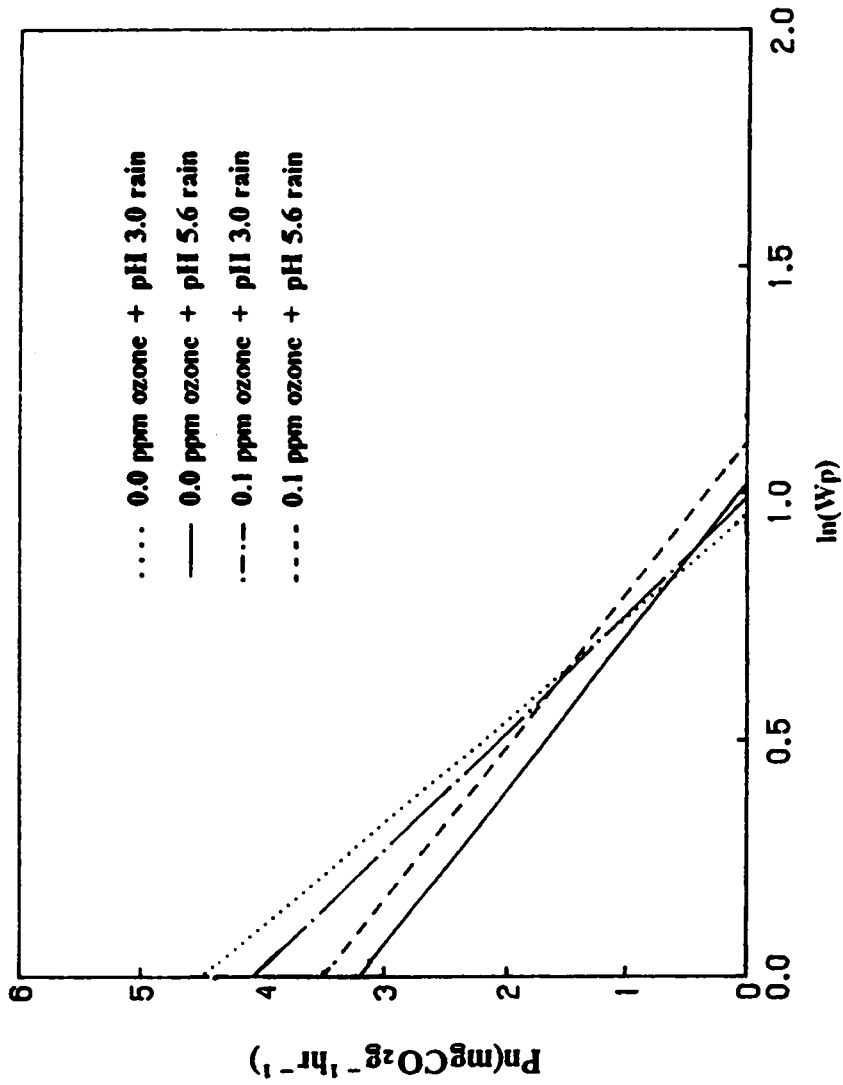


Figure 7. Predicted regression response of net photosynthesis (P_n) in one-year-old loblolly pine seedlings as affected by the 10-wk exposure to ozone and simulated rain during the first drought cycle. See Table 19 for the regression models.

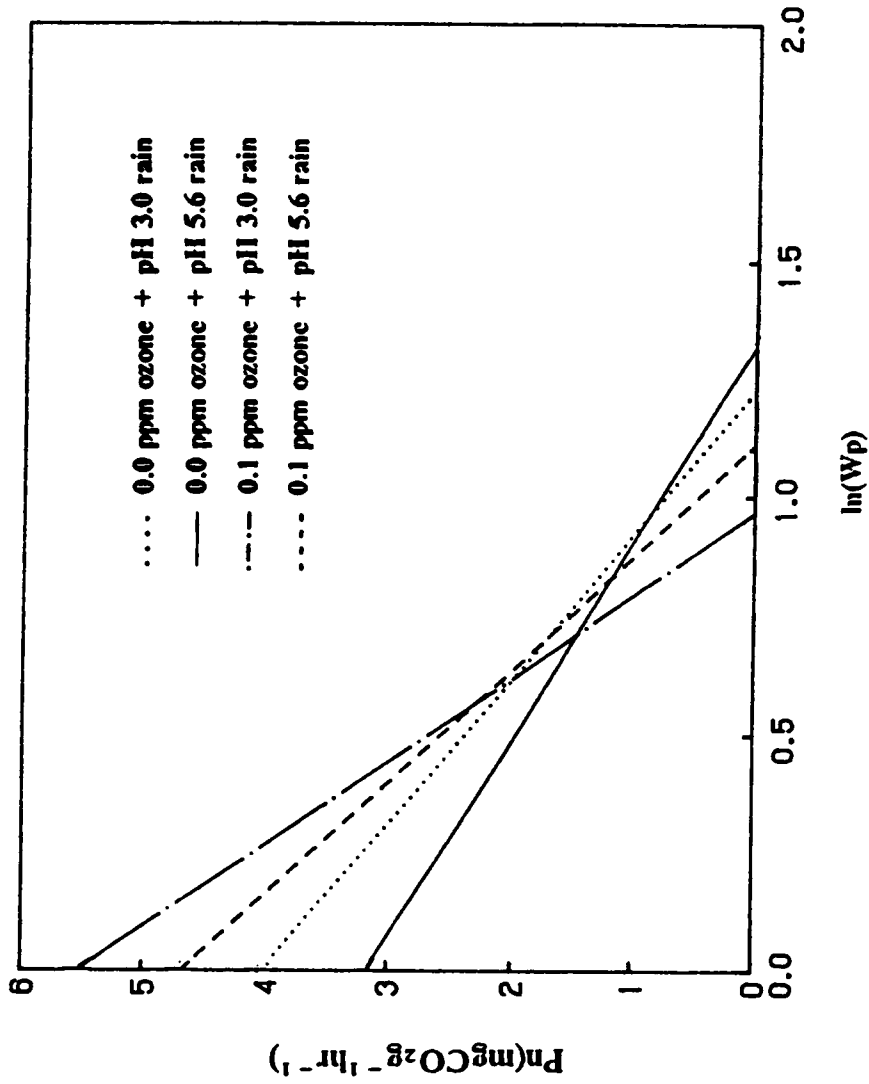


Figure 8. Predicted regression response of net photosynthesis (P_n) in one-year-old loblolly pine seedlings as affected by the 10-wk exposure to ozone and simulated rain during the second drought cycle. See Table 19 for the regression models.

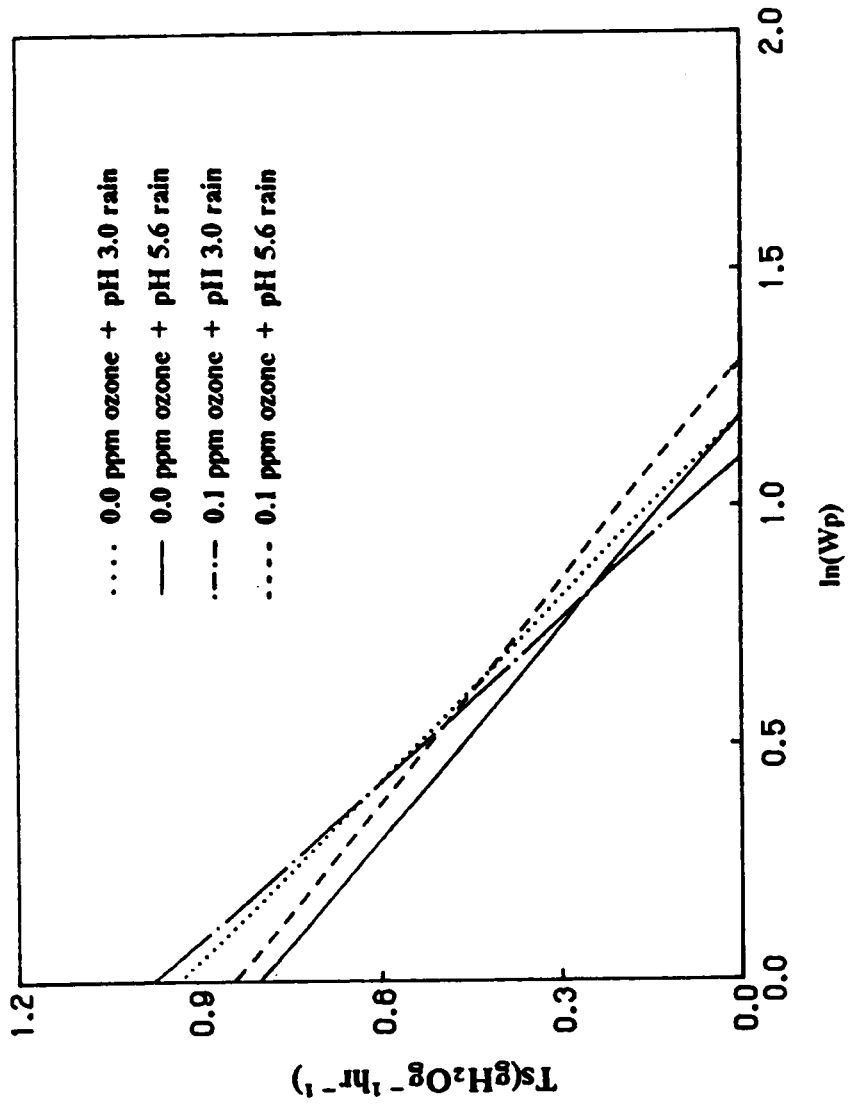


Figure 9. Predicted regression response of transpiration (T_s) in one-year-old loblolly pine seedlings as affected by the 10-wk exposure to ozone and simulated rain during the first drought cycle. See Table 19 for the regression models.

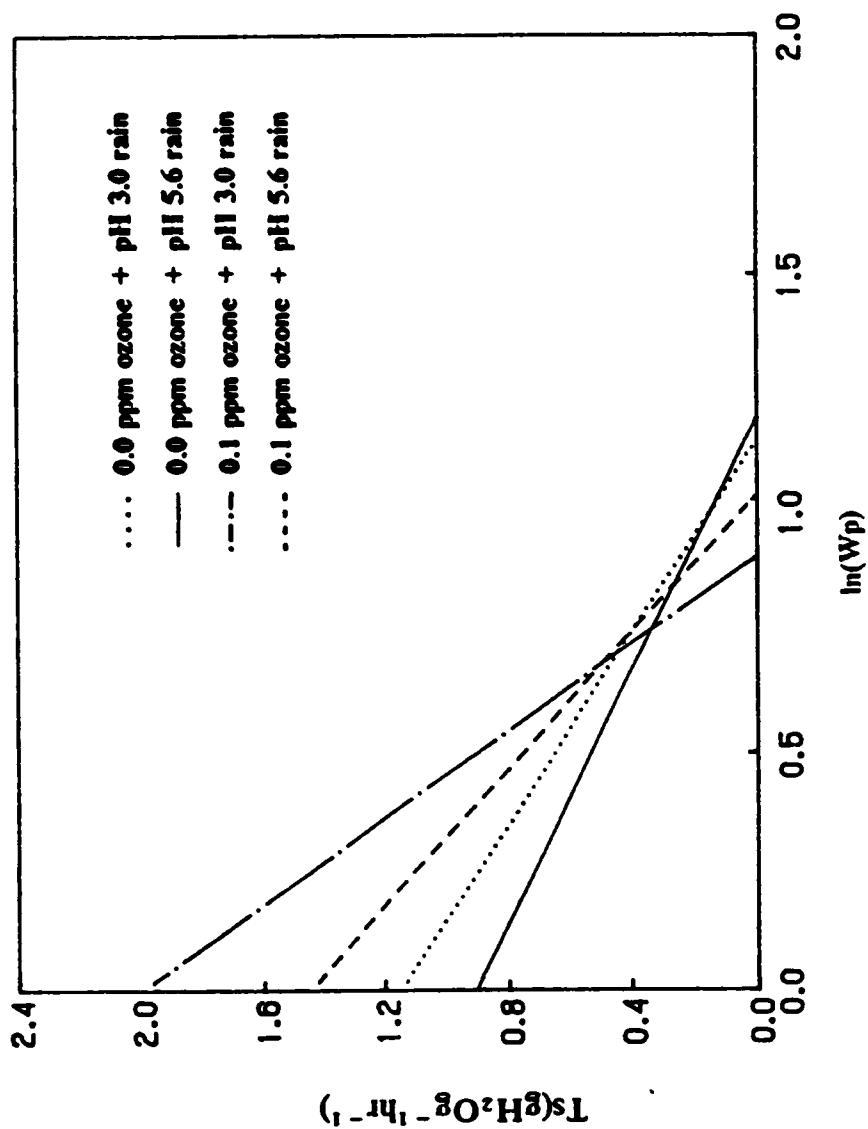


Figure 10. Predicted regression response of transpiration (T_s) in one-year-old loblolly pine seedlings as affected by the 10-wk exposure to ozone and simulated rain during the second drought cycle. See Table 19 for the regression models.

Table 17. Regression models and coefficients of determination for net photosynthesis (Pn) of one-year-old yellow-poplar seedlings during two drought cycles following exposure to ozone and simulated acidic rain¹.

Cycle	O ₃ (ppm)	pH	Model			R ²	n
First cycle	0.0	5.6	Pn = 0.087	-0.293 ln(Wp)	-0.02 VPD	.49	34
	0.0	3.0	Pn = 0.249	-0.132† ln(Wp)	-0.07 VPD	.49	33
	0.1	5.6	Pn = 0.136	-0.188 ln(Wp)	-0.02 VPD	.49	35
	0.1	3.0	Pn = 0.296	-0.108† ln(Wp)	-0.07 VPD	.49	29
Second cycle	0.0	5.6	Pn = 0.177	-0.178 ln(Wp)	-0.03 VPD	.60	27
	0.0	3.0	Pn = 0.153	-0.272† ln(Wp)	-0.03 VPD	.60	28
	0.1	5.6	Pn = 0.104	-0.164 ln(Wp)	-0.01 VPD	.60	26
	0.1	3.0	Pn = 0.244	-0.078‡ ln(Wp)	-0.07 VPD	.60	24

¹Pn = mgCO₂m⁻²s⁻¹; Wp = leaf water potential, MPa; VPD = vapor pressure deficit, KPa

Daggers indicate the significant differences from 0.0 ppm ozone + pH 5.6 within drought cycles at 0.05 (†) or 0.01 (‡) levels

although slopes were not significant (Table 18).

Sweetgum exhibited no significant effect of ozone and simulated rain on the relationship between gas exchange rate and leaf W_p during both drought cycles, but showed a significant change due to the moisture stress. The slope parameters for the first cycle were more negative than the second cycle in all gas exchange rates, indicating a rapid decline of gas exchange rates with decreasing leaf W_p (Table 19).

Pollutant and moisture stress effects on root hydraulic conductivity (L_p)

Root hydraulic conductivity was greatest in loblolly pine seedlings and least in sweetgum seedlings when measured initially at the five-week exposure period (Tables 20, 21, and 22). In general, L_p decreased with both age and moisture stress in all tree species regardless of pollutant treatments. After ten weeks of ozone fumigation at 0.1 ppm, L_p in seedlings of loblolly pine and sweetgum was significantly greater than in control plants. The first and second drought cycles resulted in a further decrease in L_p , however, the effect of ozone was only evident in sweetgum after the first drought cycle (Table 22). In this case, ozone exposure enhanced the decrease in L_p compared to untreated seedlings. Although L_p of yellow-poplar seedlings was not affected by the ten-week exposure with 0.1 ppm

Table 18. Regression models and coefficients of determination for stomatal conductance (Cs) and transpiration (Ts) of one-year-old yellow-poplar seedlings during two drought cycles following exposure to ozone and simulated acidic rain¹.

Cycle	O ₃ (ppm)	pH	Model				R ²	n
First cycle	0.0	5.6	Cs = 0.604	-0.224	ln(Wp)	-0.10 VPD	.38	34
	0.0	3.0	Cs = 0.750	-0.004	ln(Wp)	-0.17 VPD	.38	33
	0.1	5.6	Cs = 0.592	-0.302	ln(Wp)	-0.08 VPD	.38	35
	0.1	3.0	Cs = 0.694	-0.036	ln(Wp)	-0.17 VPD	.38	33
Second cycle	0.0	5.6	Cs = 0.635	-0.281	ln(Wp)	-0.19 VPD	.79	27
	0.0	3.0	Cs = 1.656‡	-0.241	ln(Wp)	-0.67 VPD	.79	28
	0.1	5.6	Cs = 1.312‡	-0.183	ln(Wp)	-0.02 VPD	.79	26
	0.1	3.0	Cs = 0.931	-0.139†	ln(Wp)	-0.34 VPD	.79	24
First cycle	0.0	5.6	Ts = 32.65	-6.400	ln(Wp)	+10.4 VPD	.16	34
	0.0	3.0	Ts = 43.11	-0.738	ln(Wp)	+4.76 VPD	.16	33
	0.1	5.6	Ts = 11.84	-26.88	ln(Wp)	+22.5 VPD	.16	35
	0.1	3.0	Ts = 30.59	-2.490	ln(Wp)	+9.27 VPD	.16	29
Second cycle	0.0	5.6	Ts = 35.71	-26.71	ln(Wp)	-1.48 VPD	.60	27
	0.0	3.0	Ts = 119.7†	-20.80	ln(Wp)	-40.6 VPD	.60	28
	0.1	5.6	Ts = 91.48†	-20.93	ln(Wp)	-26.5† VPD	.60	26
	0.1	3.0	Ts = 66.06	-15.59	ln(Wp)	-16.1 VPD	.60	24

¹Cs = m/s; Ts = mgH₂O m⁻² s⁻¹; Wp = leaf water potential, MPa; VPD = vapor pressure deficit, KPa

Daggers indicate the significant differences from 0.0 ppm ozone + pH 5.6 within drought cycles at 0.05 (†) or 0.01 (‡) levels

Table 19. Regression models and coefficients of determination for net photosynthesis (Pn), stomatal conductance (Cs), and transpiration (Ts) of one-year-old sweetgum seedlings during two drought cycles following exposure to ozone and simulated acidic rain ¹.

Cycle	O ₃ (ppm)	Model			R ²	n
First cycle	0.0	Pn = 0.495	-0.152 Wp	-0.137 VPD	.73	48
	0.1	Pn = 0.337	-0.131 Wp	-0.053 VPD	.73	50
Second cycle	0.0	Pn = 0.211‡	-0.047‡ Wp	-0.023 VPD	.28	32
	0.1	Pn = 0.156‡	-0.017‡ Wp	-0.019 VPD	.28	41
First cycle	0.0	Cs = 1.448	-0.471 Wp	-0.358 VPD	.64	48
	0.1	Cs = 1.503	-0.378 Wp	-0.412 VPD	.64	52
Second cycle	0.0	Cs = 0.486‡	-0.099‡ Wp	-0.066 VPD	.53	34
	0.1	Cs = 0.388‡	-0.045‡ Wp	-0.051 VPD	.53	45
First cycle	0.0	Ts = 69.28	-38.79 Wp	-10.06 VPD	.64	48
	0.1	Ts = 108.2	-39.14 Wp	-12.61 VPD	.64	52
Second cycle	0.0	Ts = 51.14	-10.21‡ Wp	-3.82 VPD	.49	35
	0.1	Ts = 32.13	-5.24‡ Wp	-3.46 VPD	.49	45

¹Pn = mgCO₂m⁻²s⁻¹; Cs = cm/s; Ts = mgH₂Om⁻²s⁻¹; Wp = leaf water potential, MPa

Daggers indicate the significant differences from 0.0 ppm ozone of the first drought cycle at 0.01 (‡) levels

Table 20. Changes in mean root hydraulic conductivity¹ of one-year-old loblolly pine seedlings during and after 10 wks of treatment with ozone and simulated acid rain. All seedlings were submitted to two drought cycles after 10 wks of treatment.

Week	OZONE ²			RAIN pH ³			INTERACTION			
	0.0	0.1	3.0	3.0	5.6	5.6	0.0 + 3.0	0.0 + 5.6	0.1 + 3.0	0.1 + 5.6
5	338.3a	346.4a	362.4a	362.4a	322.2a		357.2a	319.4a	367.6a	325.2a
10	169.5b	231.8b†	210.9b	210.9b	190.4b		191.7b	147.3b	230.1b	233.4b
11 ⁴	141.2bc	138.3c	142.0c	142.0c	137.4c		142.2b	140.3b	141.9c	134.6c
12 ⁵	113.5c	94.91d	106.1c	106.1c	102.3c		118.4b	108.7b	93.85c	95.97c

¹ng⁻¹s⁻¹bar⁻¹

²Ozone (ppm) treatment across all rain pHs

³Rain pH across all ozone treatments

⁴First drought stress cycle

⁵Second drought stress cycle

Daggers indicate the significant differences at the 0.01 (†) level

Means within columns with common letters are not significantly different at 0.05 levels by Duncan's new multiple range test

No significant interactions between ozone and rain pH occurred

Table 21. Changes in mean root hydraulic conductivity¹ of one-year-old yellow poplar seedlings during and after 10 wks of treatment with ozone and simulated acid rain. All seedlings were submitted to two drought cycles after 10 wks of treatment.

Week	OZONE ²			RAIN pH ³			INTERACTION			
	0.0	0.1	0.1	3.0	5.6	5.6	0.0 + 3.0	0.0 + 5.6	0.1 + 3.0	0.1 + 5.6
5	198.5a	179.3a	179.7a	199.7a	178.2a	178.2a	233.6a	163.4a	165.7a	192.9a
10	91.86b	88.54b	76.70b	76.70b	103.7b†	103.7b†	81.48b	102.3b	71.92b	105.2b
11 ⁴	63.17b	39.94c	67.38b	67.38b	35.74c	35.74c	100.2b‡	26.10bc	34.52b	45.37b
12 ⁵	49.69b	30.97c	34.13b	34.13b	46.54c	46.54c	36.64b	62.75c†	31.62b	30.32b

¹nl g⁻¹ s⁻¹ bar⁻¹

²Ozone (ppm) treatment across all rain pHs

³Rain pH across all ozone treatments

⁴First drought stress cycle

⁵Second drought stress cycle

Daggers indicate the significant differences at the 0.05 (†) or 0.01 (‡) levels

Means within columns with common letters are not significantly different at 0.05 levels by Duncan's new multiple range test

Table 22. Changes in mean root hydraulic conductivity¹ of one-year-old sweetgum seedlings during and after 10 wks of treatment with ozone and simulated acid rain. All seedlings were submitted to two drought cycles after 10 wks of treatment.

Week	OZONE ²		RAIN pH ³		INTERACTION			
	0.0	0.1	3.0	5.6	0.0 + 3.0	0.0 + 5.6	0.1 + 3.0	0.1 + 5.6
5	137.5a	131.1a	136.7a	132.0a	144.4a	130.7a	128.9a	133.3a
10	75.14b	103.7b†	95.20b	83.61b	81.77b	68.51b	108.6a	98.71a
11 ⁴	33.34c‡	23.19c	29.54c	26.99c	37.48c	29.20c	21.60b	24.78b
12 ⁵	21.31c	20.14c	24.89c‡	16.57c	25.10c	17.53c	24.68b	15.60b

¹nl g⁻¹ s⁻¹ bar⁻¹

²Ozone (ppm) treatment across all rain pHs

³Rain pH across all ozone treatments

⁴First drought stress cycle

⁵Second drought stress cycle

Daggers indicate the significant differences at the 0.05 (†) or 0.01 (‡) levels

Means within columns with common letters are not significantly different at 0.05 levels by Duncan's new multiple range test

No significant interactions between ozone and rain pH occurred

ozone, drought cycles significantly reduced Lp in seedlings pre-treated with 0.1 ppm ozone.

Simulated rain had no effect on Lp in loblolly pine either before or after moisture stress (Table 20). In yellow-poplar, seedlings treated with pH 5.6 solution for ten weeks had higher Lp compared with pH 3.0 solution, however, this response was reversed after the first drought cycle. The only significant effect of rain pH on sweetgum seedlings occurred after the second drought cycle where Lp was significantly greater in seedlings treated with pH 3.0 solution.

The only significant interactions between ozone and solution pH occurred in yellow-poplar seedlings. After the first drought cycle, Lp was significantly higher in seedlings pre-exposed to 0.0 ppm ozone + pH 3.0 solution compared with any other treatment. After the second drought cycle, seedlings pre-exposed to 0.0 ppm ozone + pH 5.6 solution exhibited a significantly higher Lp than any other treatment.

DISCUSSION

The suppressive effects of ozone on photosynthesis have been reported in many plant species (Miller et al., 1969; Barnes, 1972; Pell and Brennan, 1973; Yang et al., 1983a, b; Reich et al., 1986, 1987; Reich and Amundson, 1985).

However, in this present study, P_n , measured immediately after the ten-week fumigation period, was not affected by ozone in any tree species. These results suggest that no permanent impairment of the instantaneous photosynthetic capacity occurred with ozone exposure conditions used in this studies. Barnes (1972) reported the slight decrease of P_n in loblolly, slash, pond, and white pine seedlings after fumigation with 0.15 ppm ozone for 36 days, but the inhibition was not significant, which was consistent with the loblolly pine response in this study. Currently, Taylor et al. (1986) reported no effects of ozone, acid rain, and acid mist, alone and in combination on photosynthesis and transpiration of one-year-old red spruce seedlings, and Tseng et al. (1988) also found photosynthesis, stomatal conductance, and transpiration were not altered in Fraser fir seedlings after ten weeks of fumigation with 0.05 or 0.10 ppm ozone. However, this present study showed that tree seedlings which had been previously exposed to ozone developed significant differences in gas exchange rates following moisture stress period.

At the present time, the effects of simulated acidic rain on P_n of tree seedlings are inconclusive and vary depending on species and experimental conditions. Researches have reported increased P_n (Seiler and Paganelli, 1987; Ferenbaugh, 1976; Reich et al., 1987), no effect (Neufeld et al., 1985; Reich et al., 1986; Seiler

and Paganelli, 1987), or reduced Pn (Neufeld et al., 1985). In this present study, only loblolly pine seedlings showed a stimulation effect of simulated rain at pH 3.0 compared with pH 5.6 to drought stress. Seiler and Paganelli (1987) observed no alteration in Pn of loblolly pine with pH 3.0 rain, however, the comparison was to seedlings exposed to pH 4.3 solution. Increases in both Pn and Ts in loblolly pine exposed to pH 3.0 solution in this present study indicate stimulated gas exchange and this can result from wax erosion from stomates.

Ozone fumigation did not influence gas exchange rates of yellow-poplar either before or after moisture stress in this present study. Jensen and Roberts (1986) reported that stomatal response of yellow-poplar seedlings to ozone fumigation differed with humidity. No effect of ozone on leaf diffusive resistance was found at 40% R.H., however, at 80% R.H., stomatal resistance was significantly increased by ozone treatment. The low R.H. (55 ± 5 %) within the CSTR chambers in the present study may have modified the detrimental effects of ozone on gas exchange rates of yellow-poplar. Yellow-poplar showed no change of Pn, but Cs was significantly lower in seedlings exposed to simulated rain at pH 3.0 compared with pH 5.6. Photosynthesis was significantly lower in seedlings exposed to pH 3.0 compared with pH 5.6 without changes of Cs and Ts, resulting in a significant reduction of WUE after the first drought cycle. This

indicates that a reduction in mesophyll conductance occurred in seedlings treated with pH 3.0 solution (Neufeld et al., 1985).

The exact reason for a significant shift in the response of photosynthesis to decreasing water potential, observed in this present study, is not conclusive at this present time because of the lack of studies on the effect of drought stress on plants already subjected to air pollutants. Cornic (1987) found interactions between drought stress and SO_2 . Photosynthetic oxygen evolution was decreased in five-year-old Norway spruce exposed to 0.08 ppm SO_2 compared with control plants during moisture stress. The decline of photosynthesis was mainly due to a greater dehydration of the tissue in trees exposed to SO_2 . This indicate that SO_2 can induce an alteration in stomatal mechanisms or mechanisms that control water uptake of the plant.

The different response of Pn to water potential due to air pollutant treatments, observed in this present study, may have resulted, in part, from changes in Lp. Root hydraulic conductivity is one of the major factors that is responsible for leaf water supply. The positive correlation between Lp and gas exchange rates were found in citrus rootstock species by Syvertsen and Graham (1985). Correlation between Lp and gas exchange rates were not uniform in loblolly pine, yellow-poplar, and sweetgum. The stimula-

tion effect of ozone on the L_p of loblolly pine could induce the significant increase of P_n after the first drought cycle and change the responses of P_n and T_s as water potential decreased. In yellow-poplar, L_p was significantly higher in seedlings exposed to rain pH 5.6, resulting in highly sensitive response to declining water potential during the first drought cycle. Root hydraulic conductivity of yellow-poplar was significantly decreased in all seedlings except seedlings exposed to 0.0 ppm ozone + pH 3.0 solution after the first drought cycle. The high L_p in seedlings exposed to 0.0 ppm ozone + pH 3.0 solution resulted in high sensitivity to water potential during the second drought cycle. Syvertsen and Graham (1985) also found the similar correlations between L_p and transpiration rate during the drought cycle. Carrizo citrange which has a high L_p decrease T_s more rapidly than sour orange which has 3-fold lower L_p than carrizo citrange. Furthermore, sour orange recovered more quickly than carrizo citrange after rewatering. However, in sweetgum, the increased L_p due to ozone did not show a higher sensitivity to gas exchange rate than control seedlings during the first drought cycle. This insensitivity might be due to the rapid adjustment of L_p in seedlings exposed to ozone, resulting in lower L_p than control plants after completion of the first drought cycle. As a result, during the second drought cycle, seedlings exposed to ozone had less negative slopes than con-

trol seedlings for Pn, Cs, and Ts.

A significant reduction in Lp as seedlings aged was observed in all species in this study. A similar result was reported in soybean plants (Fiscus, 1981b), and was suggested to result from an increase in the proportion of suberized roots. Sands et al. (1982) found that the average Lp was 2.5 fold higher in unsuberized roots than in the suberized roots of eight-month-old loblolly pine seedlings.

Another factor which can alter Lp is drought stress. After the first drought cycle in this study, Lp, in general, decreased 32-80% from pre-drought levels. This result is in agreement with similar findings on the reduction of Lp by drought stress reported in citrus (Ramos and Kaufman, 1979; Levy et al., 1983). The reduced Lp might be due to changes in permeability of the root cell membranes or to increased suberin deposition in the cell walls of the cortical cells (Ramos and Kaufmann, 1979).

The results from this present study indicate that ozone and/or simulated acidic rain can alter gas exchange rates and drought susceptibility of forest tree species under laboratory conditions. Although no consistent trend in the observable responses occurred between species, forest trees which are periodically exposed to ambient ozone, acidic rain and drought in natural forest ecosystems may have a response similar to the results from this studies. Additional research is necessary to determine if such effects

are occurring in natural forest ecosystems.

LITERATURE CITED

- Abouguendia, Z. M. and L. A. Baschak. 1987. Response of two western Canadian conifers to simulated acidic precipitation. *Water, Air, & Soil Pollut.* 33:15-12.
- Adams, H. S., S. L. Stephenson, T. J. Blasing, and D. N. Duvick. 1985. Growth-trend declines of spruce and fir in Appalachian subalpine forests. *Environ. Exp. Bot.* 25:315-325.
- Barnes, R. L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. *Environ. Pollut.* 3:133-8.
- Chevone, B. I., Y. S. Yang, W. E. Winner, I. Storcks-Cotter and S. J. Long. 1984. A rainfall simulator for laboratory use in acidic precipitation studies. *JAPCA* 31:355-359.
- Cornic, G. 1987. Interaction between sublethal pollution by sulphur dioxide and drought stress. The effect on photosynthetic capacity. *Physiol. Plantarum* 71:115-119.
- Dawson, P. and G. Weste. 1982. Changes in water relations associated with infection by Phytophthora cinnamomi. *Aust. J. Bot.* 30:393-340.
- Dawson, P. and G. Weste. 1984. Impact of root infection by Phytophthora cinnamomi on the water relations of two Eucalyptus species that differ in susceptibility. *Phytopathology* 74:486-490.
- Dochinger, L. S. 1976. Effects of soil applications of acidified solutions on growth and survival of forest tree species. *Proc. Amer. Phytopathol. Soc.* 3:304.
- Duchelle, S. F., J. M. Skelly and B. I. Chevone. 1982. Oxidant effects on forest tree seedling growth in the Appalachian Mountain. *Water, Air & Soil Pollut.* 12:363-373.
- Duchelle, S. F., J. M. Skelly, T. L. Sharik, B. I. Chevone, Y. S. Yang and J. E. Nellessen. 1983. Effects of ozone on the productivity of natural vegetation in a high meadow of the Shenandoah Park of Virginia. *J. Environ. Mgt.* 17:299-308.
- Evans, L. S. 1984a. Botanical aspects of acidic precipitation. *The Botanical Review* 50:449-490.

- Evans, L. S. 1984b. Acidic precipitation effects on terrestrial vegetation. *Ann. Rev. Phytopathol.* 22:397-420.
- Ferenbaugh, R. W. 1976. Effects of simulated acid rain on phaseolus vulgaris L. (Fabaceae). *Amer. J. Bot.* 63:283-288.
- Fiscus, E. L. 1975. The interaction between osmotic- and pressure-induced water flow in plant roots. *Plant Physiol.* 55:917-922.
- Fiscus, E. L. 1977. Determination of hydraulic and osmotic properties of soybean root system. *Plant Physiol.* 59:1013-1020.
- Fiscus, E. L. 1981a. Analysis of the components of area growth of bean root systems. *Crop Sci.* 21:909-913.
- Fiscus, E. L. 1981b. Effects of abscisic acid on the hydraulic conductance of and total ion transport through Phaseolus root systems. *Plant Physiol.* 68:169-174.
- Fiscus, E. L. and A. H. Markhart. 1979. Relationships between root system water transport properties and plant size in Phaseolus. *Plant Physiol.* 64:770-773.
- Glinka Z. 1973. Abscisic acid effect on root exudation related to increased permeability to water. *Plant Physiol.* 51:217-219.
- Glinka Z. 1977. Effects of abscisic acid and hydrostatic pressure gradients on water movement through excised sunflower roots. *Plant Physiol.* 59:933-935.
- Glinka Z. 1980. Abscisic acid promotes both volume flow and ion release to the xylem in sunflower roots. *Plant Physiol.* 65:537-540.
- Heck, W. W., R. B. Philbeck and J. A. Dunning. 1978. A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants. USDA-ARS Paper ARS-S-181.
- Heck, W. W., O. C. Taylor, R. Adams, G. Bingham, H. Miller, E. Perston and L. Weinstein. 1982. Assessment of crop loss from ozone. *JAPCA* 32:353-361.
- Jensen, K. F. and B. R. Roberts. 1986. Changes in yellow poplar stomatal resistance with SO₂ and O₃ fumigation. *Environ. Pollut. (ser. A)*. 41:235-245.

- Johnson, A. H. and T. G. Siccama. 1983. Acid deposition and forest decline. *Environ. Sci. Tech.* 17:294-305.
- Johnson, A. H. and T. G. Siccama. 1984. Decline of red spruce in the northern Appalachians: Assessing the possible role of acid deposition. *TAPPI* 67:68-72.
- Jones, H. G. 1983. *Plant and Microclimate: A Quantitative approach to environmental plant physiology.* Cambridge University Press, Cambridge. pp.321.
- Khatamian, H., N. O. Adedipe and D. P. Ormrod. 1973. Soil-plant-water aspects of ozone phytotoxicity in tomato plants. *Plant and Soil* 38:531-541.
- Kleinbaum, D. G., L. L. Kupper. 1978. *Applied regression analysis and other multivariable methods.* Duxbury Press, Boston, MA. pp.556.
- Lee, J. J. and D. E. Weber. 1979. The effects of simulated acid rain on seedling emergence and growth of eleven woody species. *Forest Sci.* 25:393-398.
- Levy, Y., J. P. Syvertsen, and S. Nemec. 1983. Effect of drought stress and VAM on citrus transpiration and hydraulic conductivity of roots. *New Phytol.* 93:61-66.
- Markhart, A. H. III, E. L. Fiscus, A. W. Naylor, and P. J. Kramer. 1979. Effect of temperature on water and ion transport in soybean and broccoli systems. *Plant Physiol.* 64:83-87.
- Markhart, A. H. III, M. M. Peet, N. Sionit and P. J. Kramer. 1980. Low temperature acclimation of root fatty acid composition, leaf water potential, gas exchange and growth of soybean seedlings. *Plant Cell Environ.* 3:435-441.
- Matziris, D, I. and G. Nakos. 1977. Effects of simulated acid rain on juvenile characteristics of Aleppo pine (*Pinus halepensis* Mill). *Forest ecology and management* 1:267-272.
- McColl, J. G. and R. Johnson. 1983. Effects of simulated acid rain on germination and early growth of Douglas-fir and ponderosa pine. *Plant and Soil* 74:125-129.
- McLaughlin, S. B., R. K. McConathy, D. Duvick and L. K. Mann. 1982. Effects of chronic air pollution stress on photosynthesis, carbon allocation, and growth of white pine trees. *For. Sci.* 28:60-70.

- McLaughlin, S. B. and R. K. McConathy. 1983. Effects of SO₂ and O₃ on allocation of ¹⁴C-labeled photosynthate in Phaseolus vulgaris. *Plant Physiol.* 73:630-635.
- Miller, P. R., J. R. Parmeter, Jr., B. H. Flick and C. W. Martinez. 1969. Ozone dosage response of ponderosa pine seedlings. *JAPCA* 19:435-438.
- Nelsen, C. E. and G. R. Safir. 1982. The water relations of well-watered, mycorrhizal, and non-mycorrhizal onion plants. *J. Amer. Soc. Hort. Sci.* 107:271-274.
- Neufeld, B. H., J. A. Jernstedt and B. L. Haines. 1985. Direct foliar effects of simulated acid rain: I. Damage, growth and gas exchange. *New Phytol.* 99:389-405.
- Olszyk, D. M. and T. W. Tibbitts. 1981. Stomatal response and leaf injury of Pisum sativum L. with SO₂ and O₃ exposures. II. Influence of moisture stress and time of exposure. *Plant Physiol.* 67:545-549.
- Pell, E. J. and E. Brennan. 1973. Changes in respiration, photosynthesis, adenosine 5'-triphosphate and total adenylate content of ozonated Pinto Bean foliage as they relate to symptom expression. *Plant Physiol.* 51:378-381.
- Radin, J. W. and J. S. Boyer. 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants. Role of hydraulic conductivity and turgor. *Plant Physiol.* 69:771-775.
- Radin, J. W. and M. P. Eidenbock. 1984. Hydraulic conductance as a factor limiting leaf expansion of phosphorus-deficient cotton plants. *Plant Physiol.* 75:372-377.
- Ramos, C., M. R. Kaufmann. 1979. Hydraulic resistance of rough lemon roots. *Physiol. Plant* 45:311-314.
- Raynal, D. J., J. R. Roman and W. M. Eichenlaub. 1982. Response of tree seedlings to acid precipitation: II. Effect of simulated acidified canopy throughfall on sugar maple seedling growth. *Environ. Exp. Bot.* 22:385-392.
- Reich, P. B. 1983. Effects of low concentrations of O₃ on net photosynthesis, dark respiration, and chlorophyll contents in aging hybrid poplar leaves. *Plant Physiol.* 73:291-296.
- Reich, P. B. and R. G. Amundson. 1985. Soybean yield

- and/or SO₂. Environ. Pollut. 34:345-355.
- Reich, P. B., A. W. Schoettle, and R. G. Amundson. 1986. Effects of O₃ and acidic rain on photosynthesis and growth in sugar maple and northern red oak seedlings. Environ. Pollut. (ser. A). 40:1-15.
- Reich, P. B. 1987. Quantifying plant response to ozone: a unifying theory. Tree Physiol. 3:63-91.
- Reich, P. B., A. W. Schoettle, H. F. Stroo, J. Troiano, and R. G. Amundson. 1987. Effects of ozone and acid rain on white pine (Pinus strobus) seedlings grown in five soils. I. Net photosynthesis and growth. Can. J. Bot. 65:977-987.
- Safir, G. R., J. S. Boyer and J. W. Gerdemann. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiol. 49:700-703.
- Sands, R., E. L. Fiscus and C. P. P. Reid. 1982. Hydraulic properties of pine and bean roots with varying degrees of suberization, vascular differentiation and mycorrhizal infection. Aust. J. Plant Physiol. 9:559-569.
- Scholander, P. E., H. T. Hammel, E. D. Bradstreet and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 48:339-346.
- Seiler, J. R. and D. J. Paganelli. 1987. Photosynthesis and growth response of red spruce and loblolly pine to soil-applied lead and simulated acid rain. For. Sci. 33:668-675.
- Shafer, S. R., A. S. Heagle and D. M. Camberato. 1987. Effects of chronic doses of ozone on field-grown loblolly pine: Seedling responses in the first year. JAPCA 37:1179-1184.
- Skelly, J. M., B. I. Chevone and Y. S. Yang. 1982. Effects of ambient concentrations of air pollutants on vegetation indigenous to the Blue Ridge Mountains of Virginia. In Acid Rain: A Water Resources Issue for the 80's. R. Herrmann and A. I. Johnson (eds.). American Water Resources Association, Bethesda, MD. pp. 69-74.
- Smit-spinks, B., B. Swanson and A. H. Markhart. 1983. Hardiness and water relation in root and shoot tissues of Pinus Sylvestris in response to photoperiod and thermoperiod. Plant Physiol. 72(suppl.):45.
- Syvertsen, J. P. and J. H. Graham. 1985. Hydraulic conduc-

- tivity of roots, Mineral nutrition and leaf gas exchange of citrus rootstocks. *J. Amer. Soc. Hort. Sci.* 110:865-869.
- Tal, M. and D. Imber. 1971. Abnormal stomatal behavior and hormonal imbalance in Flaca, a wilted mutant of tomato. III. Hormonal effects on the water status of the plant. *Plant Physiol.* 47:849-850.
- Taylor, G. E., Jr., R. J. Norby, S. B. McLaughlin, A. H. Johnson and R. S. Turner. 1986. Carbon dioxide assimilation and growth of red spruce (Picea rubens Sarg.) seedlings in response to ozone - precipitation chemistry and soil type. *Oecologia* 70:163-171.
- Tingey, D. T. and G. Taylor, Jr. 1982. Variation in plant response to ozone: a conceptual model of physiological events. In M. H. Unsworth and D. P. Ormrod, (eds.), *Effects of Gaseous Air Pollution in Agriculture and Horticulture*. Butterworth Scientific, London, pp. 113-138.
- Tingey, D. T. and W. E. Hogsett. 1985. Water stress reduces ozone injury via a stomatal mechanism. *Plant Physiol.* 77:944-947.
- Tseng E. C., J. R. Seiler and B. I. Chevone. 1988. Effects of ozone and water stress on greenhouse-grown Fraser fir seedling growth and physiology. *Environ. Exp. Bot.* (In Press).
- Tzeng, D. D., R. J. Wakeman and J. E. DeVay. 1985. Relationships among verticillium wilt development, leaf water potential, phenology, and lint yield in cotton. *Physiol. Plant Pathology* 26:73-81.
- Wood, T. and F. H. Bormann. 1974. Effects of an artificial acid mist upon the growth of Betula alleghaniensis Britt. *Environ. Pollut. (ser. A)*. 7:259-268.
- Wood, T. and F. H. Bormann. 1977. Short-term effects of a simulated acid rain upon the growth and nutrient relations of Pinus strobus L. *Water, Air, & Soil Pollut.* 7:479-488.
- Yang, Y. S., J. M. Skelly and B. I. Chevone. 1982. Clonal response of eastern white pine to O₃, SO₂ and NO₂ exposure singly and in combination. *Can. J. For. Res.* 12:803-808.
- Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983a. Effects of long-term ozone exposure on photosyn-

thesis and dark respiration of eastern white pine. Environ. Sci. Tech. 17:371-373.

Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983b. Effects of short-term ozone exposure on net photosynthesis, dark respiration and transpiration of three eastern white pine clones. Environ. Int'l. 9:265-269.

SUMMARY AND CONCLUSIONS

Ozone and acidic precipitation are the two most widespread airborne pollutants impacting forest ecosystems in the eastern United States, and are considered to have the greatest harmful effects on plants among the gaseous pollutants. Ambient ozone concentrations are known to change the rate of photosynthesis and photosynthate allocation of sensitive forest species, which can result in alteration of plant growth pattern and yield.

Acid rain is considered a major environmental concern throughout the northeastern United States. The recent decrease in pH is known to be largely the result of combustion of fossil fuels leading to increased emission of sulphur and nitrogen oxides.

The presence of strong acids in rain may affect the growth of individual forest plants, and subsequently alter forest ecosystems. Although a recently accelerated reduction of red spruce growth has been reported in the Appalachian Mountains, and many possible causes of this decline have been suggested, none have been established conclusively at present. However, the decline of mature trees in the northeast, beginning in the early 1960's, appeared to have been initiated by a severe drought. The similar coincidence between the initiation of growth decline and drought was reported in mid-Appalachian forests. For the

recent extensive decline of red spruce in the northeastern United States, the hypothesis has been suggested that acid deposition, heavy metal inputs, and possibly ozone stress, can impair root growth and root physiology to such an extent that affected trees become more highly drought susceptible. Although effects of ozone and water stress in combination have been studied in forest plants, the effect of drought stress on plants already subjected to ozone and acid rain has not been investigated.

The principal objective of this study was to investigate the response of forest seedlings to simulated acidic rain and ozone, particularly as these pollutant stresses influence root and shoot growth, foliar nutrient status, drought susceptibility, and plant water relations. Specific objectives were: 1) to determine root and shoot growth response of loblolly pine, red spruce, yellow-poplar, and sweetgum seedlings to simulated acidic rain and ozone, singly and in combination, 2) to determine the effect of these pollutants on foliar concentrations of N, P, K, Ca, Mg, Al, and S, 3) to determine the effect of these pollutants on seedling water status, drought susceptibility, and foliar gas exchange.

One-year-old seedlings of red spruce, loblolly pine, yellow-poplar, and sweetgum were exposed to ozone in a continuous stirred tank reactor system (CSTR). Ozone fumigations occurred for four consecutive hours, three days a

week for ten weeks at 0.00 or 0.10 ppm. All seedlings were exposed to simulated acidic rain using a rainfall simulator. Applications of simulated acidic rain were conducted for one hour, twice each week at a rainfall intensity of 0.75 cm hr^{-1} .

Rate of growth was determined by measuring seedling heights every two weeks, starting with the first day of fumigation. Fresh weight, dry weight, and root hydraulic conductivity (L_p) were measured after five and ten weeks of treatment. These dry weight data were used for determining relative growth rate (RGR) and for use in allometric growth analyses.

After the ten-week treatment with ozone and simulated acidic rain, the seedlings were submitted to two drought cycles, and water potential, net photosynthesis (P_n), stomatal conductance (C_s), transpiration (T_s) and L_p were measured.

Ozone and simulated acid rain altered certain growth patterns of red spruce seedlings. Ozone treatment significantly inhibited fresh weight increment (FWT) and total dry weight of red spruce after 10 wks exposure. Ozone also reduced shoot height growth (SHG) and increased the apparent plastochron duration (APD) of sweetgum seedlings. Treatment with simulated rain at pH 3.0 significantly increased FWT and SHG of red spruce, and stimulated FWT and RGR of loblolly pine compared to pH 5.6. The APD was sig-

nificantly reduced in sweetgum seedlings exposed to simulated acid rain at pH 3.0 compared to 5.6. Significant interactions between ozone and simulated acidic rain occurred in all species except red spruce, and these interactions were additive. The SHG was significantly lower in loblolly seedlings exposed to 0.1 ppm ozone + pH 5.6 than in seedlings exposed to either 0.1 ppm ozone + pH 3.0 or 0.0 ppm ozone + pH 5.6. The APD was significantly increased in yellow-poplar and sweetgum seedlings exposed to 0.1 ppm ozone + pH 5.6 compared to any other treatment.

Visible symptoms appeared on the adaxial leaf surface of yellow-poplar seedlings exposed to simulated rain at pH 3.0 regardless of ozone treatment, and were characterized by necrotic circular lesions. Visible foliar injury was also observed on the adaxial leaf surface of sweetgum seedlings exposed to 0.1 ppm ozone. These symptoms were characterized by red pigmentation with small brown necrotic lesions.

Foliar concentrations of P and S were significantly increased in all seedlings exposed to simulated rain at pH 3.0 compared with pH 5.6 except for red spruce which exhibited increases in K and S. In general, there was neither significant effects of ozone nor interactions between ozone and rain pH on foliar nutrient concentrations.

There were no significant effects of ozone on Pn, Cs, Ts, or water-use efficiency (WUE) prior to the drought

cycles for all species. However, after the first drought cycle, Pn and Cs were significantly changed in loblolly pine and sweetgum pre-exposed to 0.1 ppm ozone compared with controls. The ten-week treatment of simulated acidic rain at pH 3.0 significantly increased Pn and Ts of loblolly pine and reduced Cs of yellow-poplar. After the first drought cycle, yellow-poplar treated with pH 3.0 rain showed lower Pn and WUE than seedlings exposed to pH 5.6 solution.

A 0.5 MPa shift in the response of net photosynthesis (Pn) to decreasing water potential occurred in red spruce seedlings across all air pollutant treatments after the drought cycles, indicating photosynthetic acclimation to drought stress. This change might be due to an osmotic adjustment within the needle tissue, although further research is necessary to verify this hypothesis.

During the second drought cycle, Pn was more sensitive to water potential in red spruce seedlings exposed to 0.10 ppm ozone + pH 3.0 solution compared with seedlings exposed to 0.0 ppm ozone + pH 5.6 solution. In seedlings exposed to 0.1 ppm ozone + pH 3.0 rain, the predicted net photosynthetic rate at 1.0 MPa water potential (y intercept) was significantly higher, and the slope was significantly more negative than in seedlings exposed to 0.0 ppm ozone + pH 5.6. Therefore, the photosynthetic rate decreased more rapidly with decreasing branch water potential. The regres-

sion equations also indicated that the P_n of red spruce seedlings at $\ln(W_p)=0$ was the same for both drought cycles in the 0.1 ppm ozone + 3.0 pH treatment and that the slopes were also similar. The significant changes that were observed in the second drought cycle with the other treatments did not occur in the 0.1 ppm ozone + 3.0 pH treatment. Therefore, P_n was more susceptible to drought stress in seedlings exposed to ozone and low pH simulated rain. The higher L_p of red spruce observed after the first drought cycle in seedlings exposed to 0.1 ppm ozone + pH 3.0 rain might affect the ability of stomata to stabilize the plant water budget and maintain optimum water use efficiency.

In loblolly pine, pre-treatment with ozone and/or simulated rain at pH 3.0 resulted in an increase in P_n and T_s at -1.0 MPa needle water potential and also altered the physiological response of P_n and T_s to needle water potential compared to 0.0 ppm ozone + pH 5.6. The increases in both P_n and T_s indicated a stimulation of gas exchange and this could result from wax erosion from the stomata due to air pollutant treatments. No significant effect of ozone or simulated acidic rain on the relationship between gas exchange rates and water potential was observed in yellow-poplar and sweetgum seedlings.

In general, L_p was significantly affected by ozone and simulated acidic rain treatment after moisture stress.

Following the first drought cycle, Lp was decreased in yellow-poplar and sweetgum seedlings exposed to 0.10 ppm ozone. After the second drought cycle, Lp was decreased in yellow-poplar and red spruce seedlings exposed to 0.1 ppm ozone. Significant interactions between ozone and simulated acidic rain occurred in red spruce and yellow-poplar seedlings after the first drought cycle.

The results from this dissertation indicate that ozone and/or simulated acidic rain can alter the growth and drought susceptibility of forest tree species under laboratory conditions. Although no consistent trend in the observable responses occurred between species, forest trees which are periodically exposed to ambient ozone and acidic rain in natural forest ecosystems may have a response similar to the results from this studies. Additional research is necessary to determine if such effects are occurring in natural forest ecosystems.

APPENDIX

Table A1. Major ion chemistry of simulated rain^a.

Ion	Concentration
Calcium	1.60
Magnesium	0.05
Ammonium	0.45
Potassium	0.24
Chloride	0.44
Sodium	0.46
Nitrate (pH 5.6)	2.70
Nitrate (pH 3.0)	3.10
Sulfate (pH 5.6)	5.40
Sulfate (pH 3.0)	57.0

^aIon concentrations expressed in mg l⁻¹

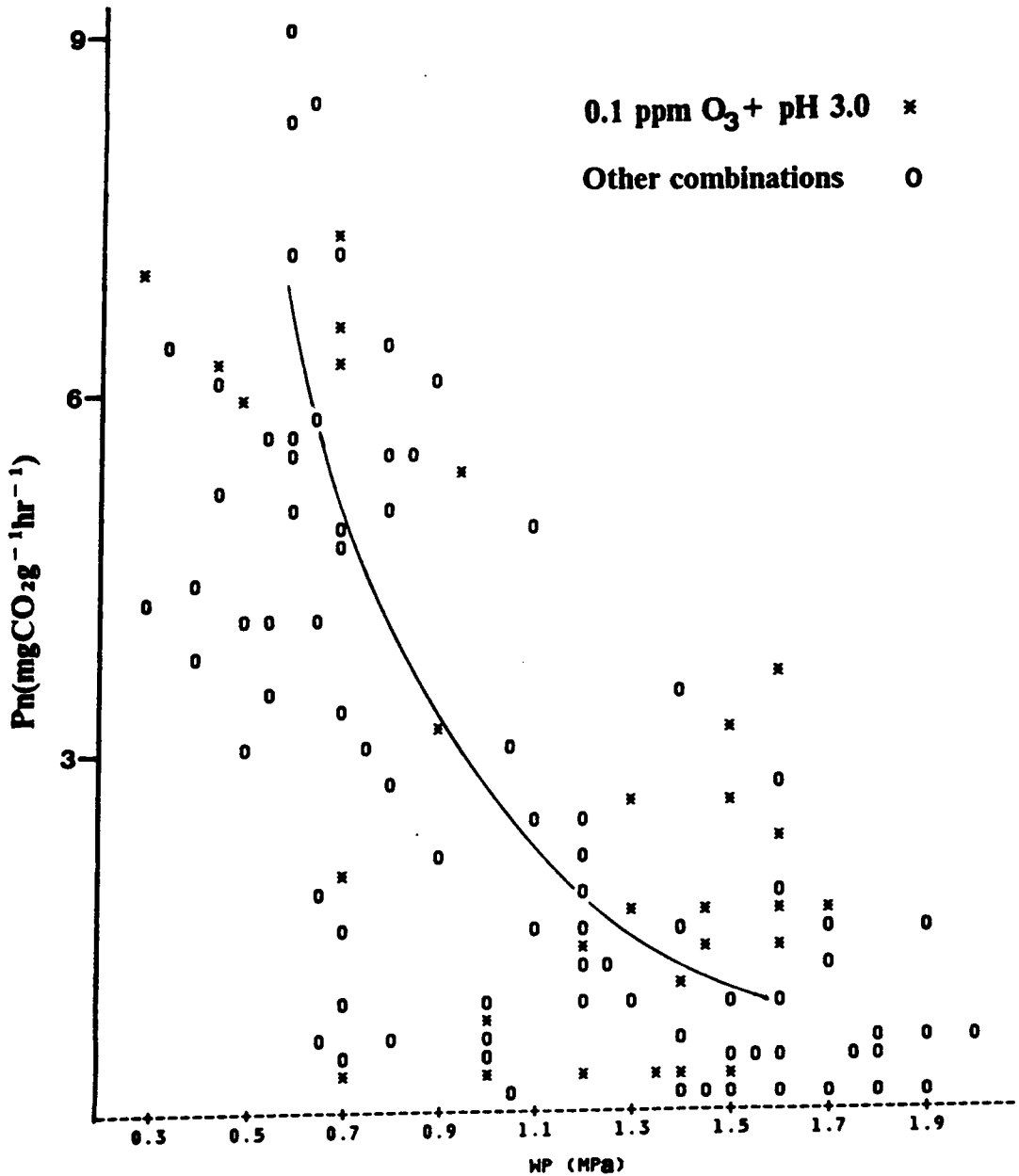


Figure A1. Photosynthesis and predicted regression response of one-year-old red spruce seedlings as affected by ozone and simulated acidic rain during the first drought cycle.

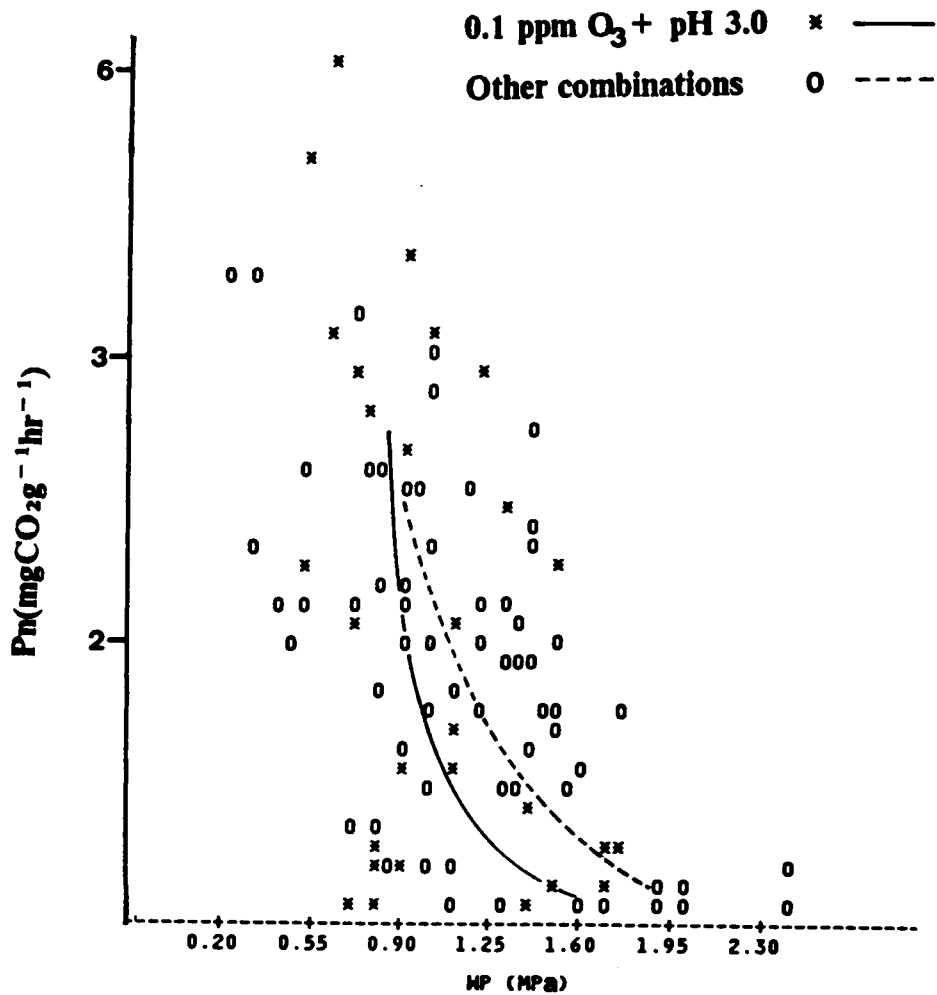


Figure A2. Photosynthesis and predicted regression response of one-year-old red spruce seedlings as affected by ozone and simulated acidic rain during the second drought cycle.

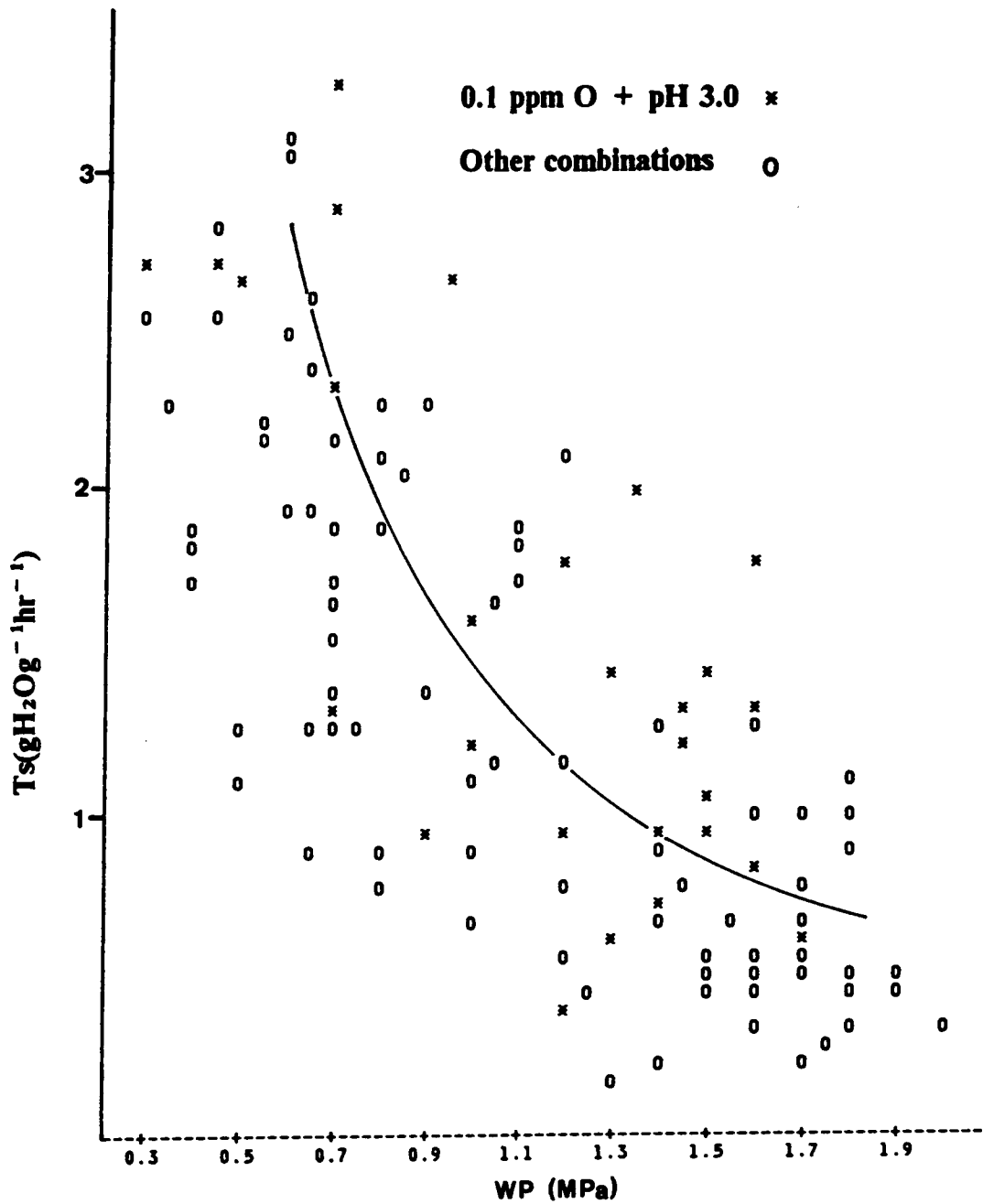


Figure A3. Transpiration and predicted regression response of one-year-old red spruce seedlings as affected by ozone and simulated acidic rain during the first drought cycle.

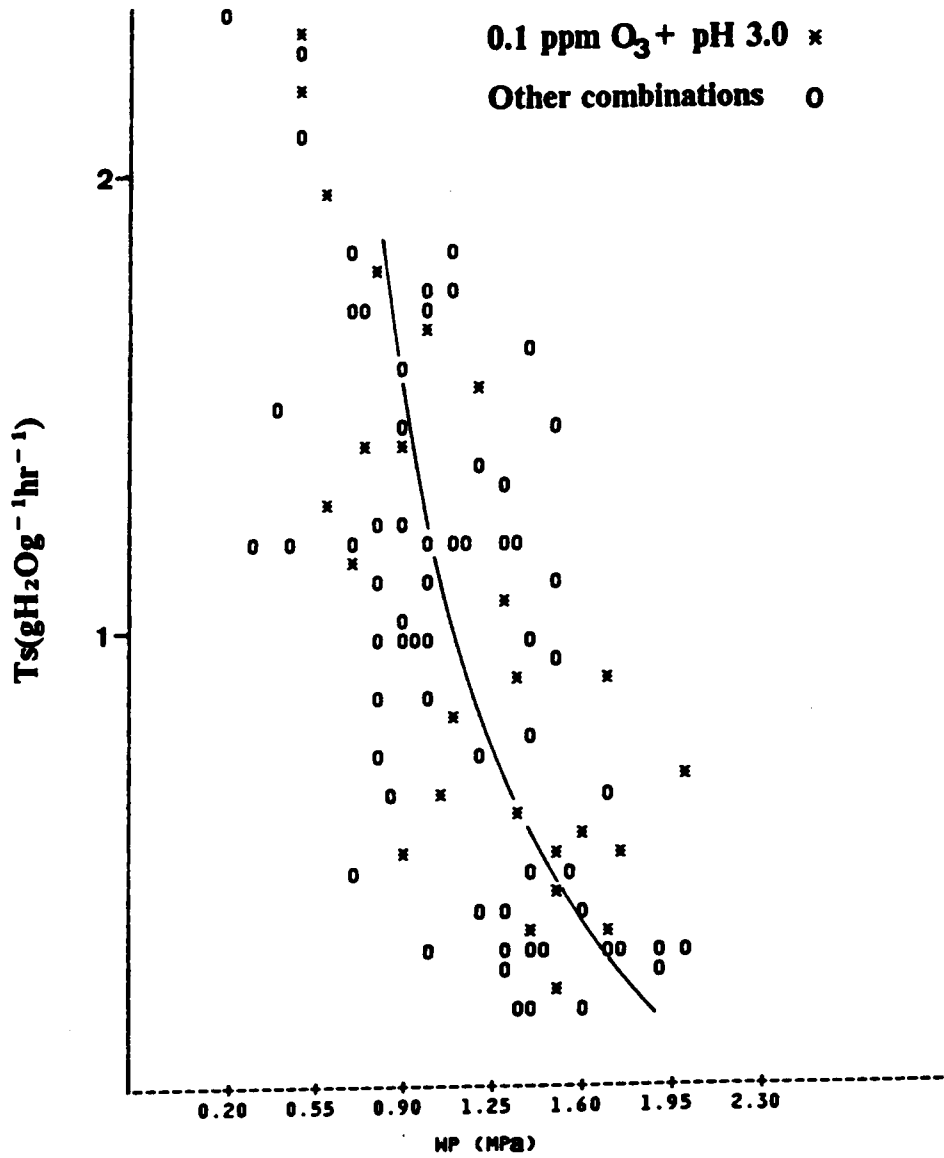


Figure A4. Transpiration and predicted regression response of one-year-old red spruce seedlings as affected by ozone and simulated acidic rain during the second drought cycle.

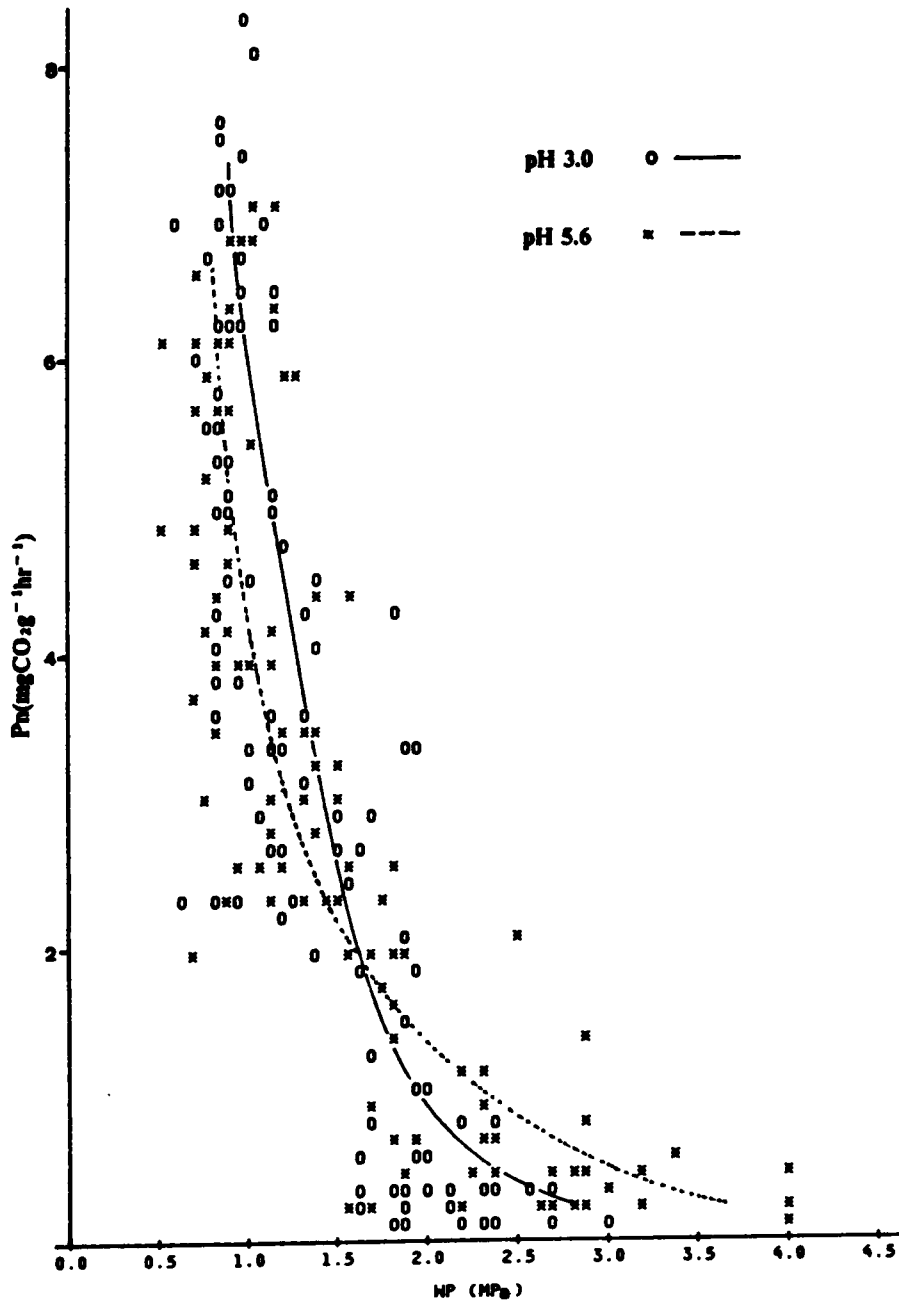


Figure A5. Photosynthesis and predicted regression response of one-year-old loblolly pine seedlings as affected by simulated acidic rain during the first drought cycle.

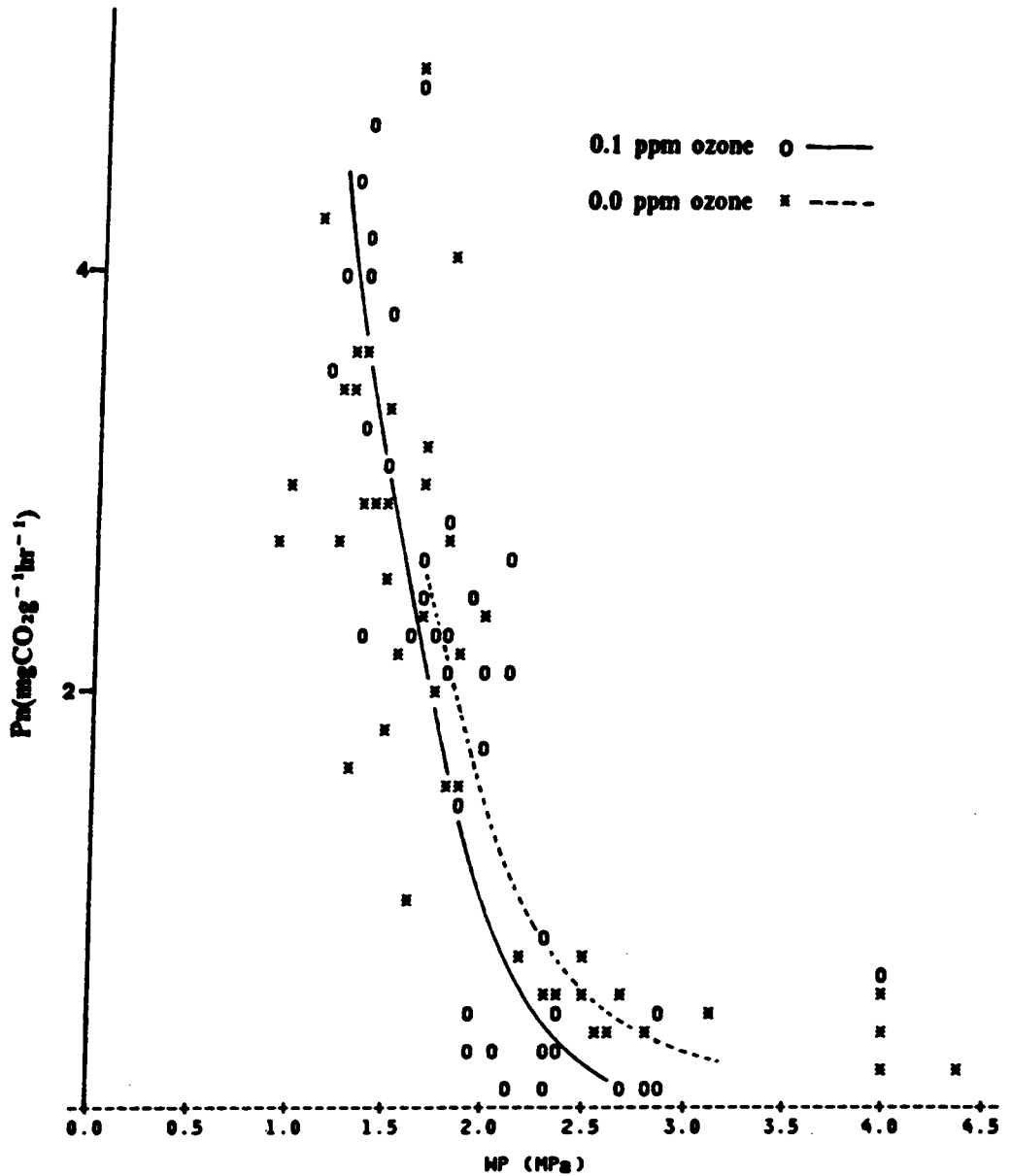


Figure A6. Photosynthesis and predicted regression response of one-year-old loblolly pine seedlings as affected by ozone during the second drought cycle.

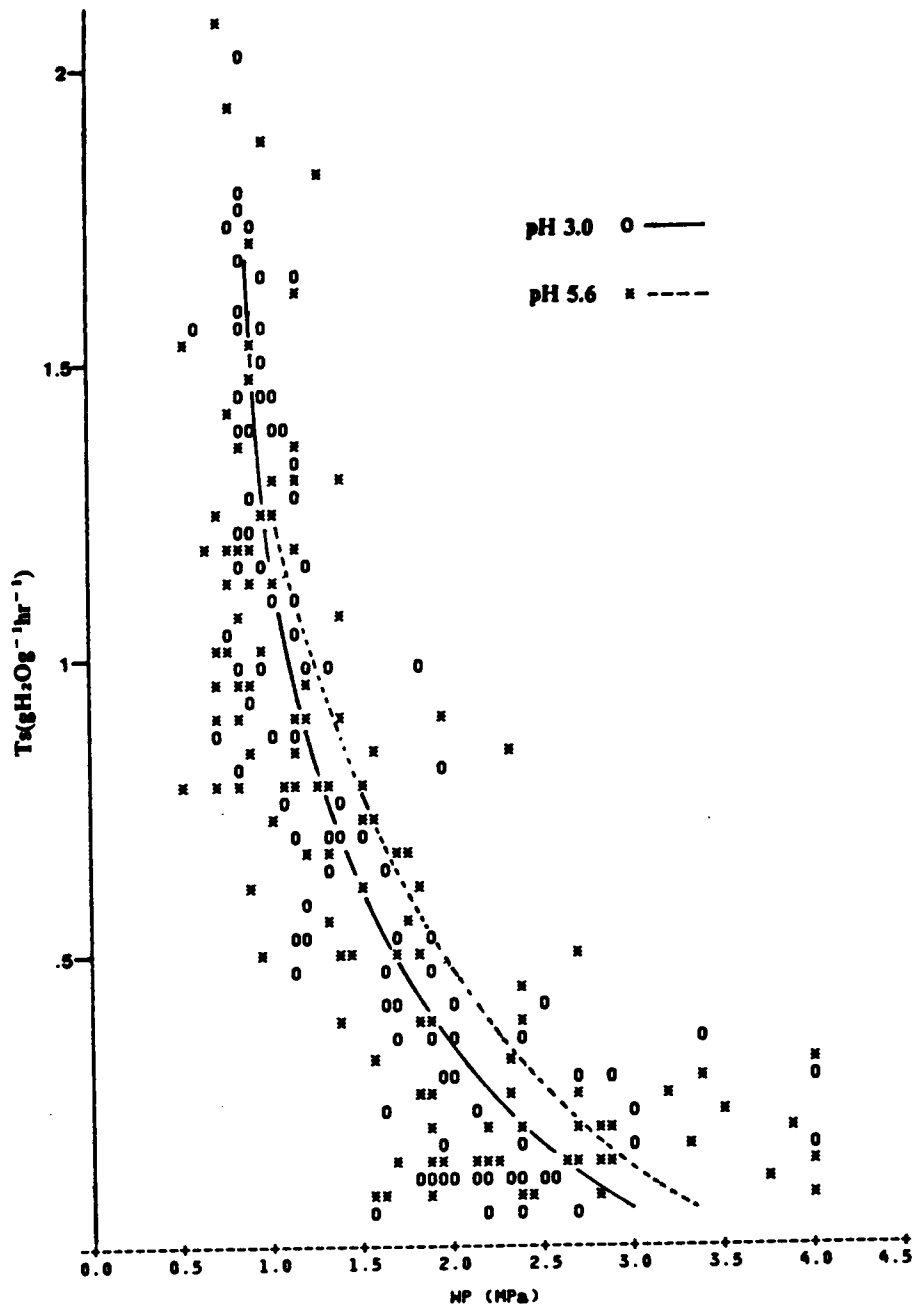


Figure A7. Transpiration and predicted regression response of one-year-old loblolly pine seedlings as affected by ozone during the first drought cycle.

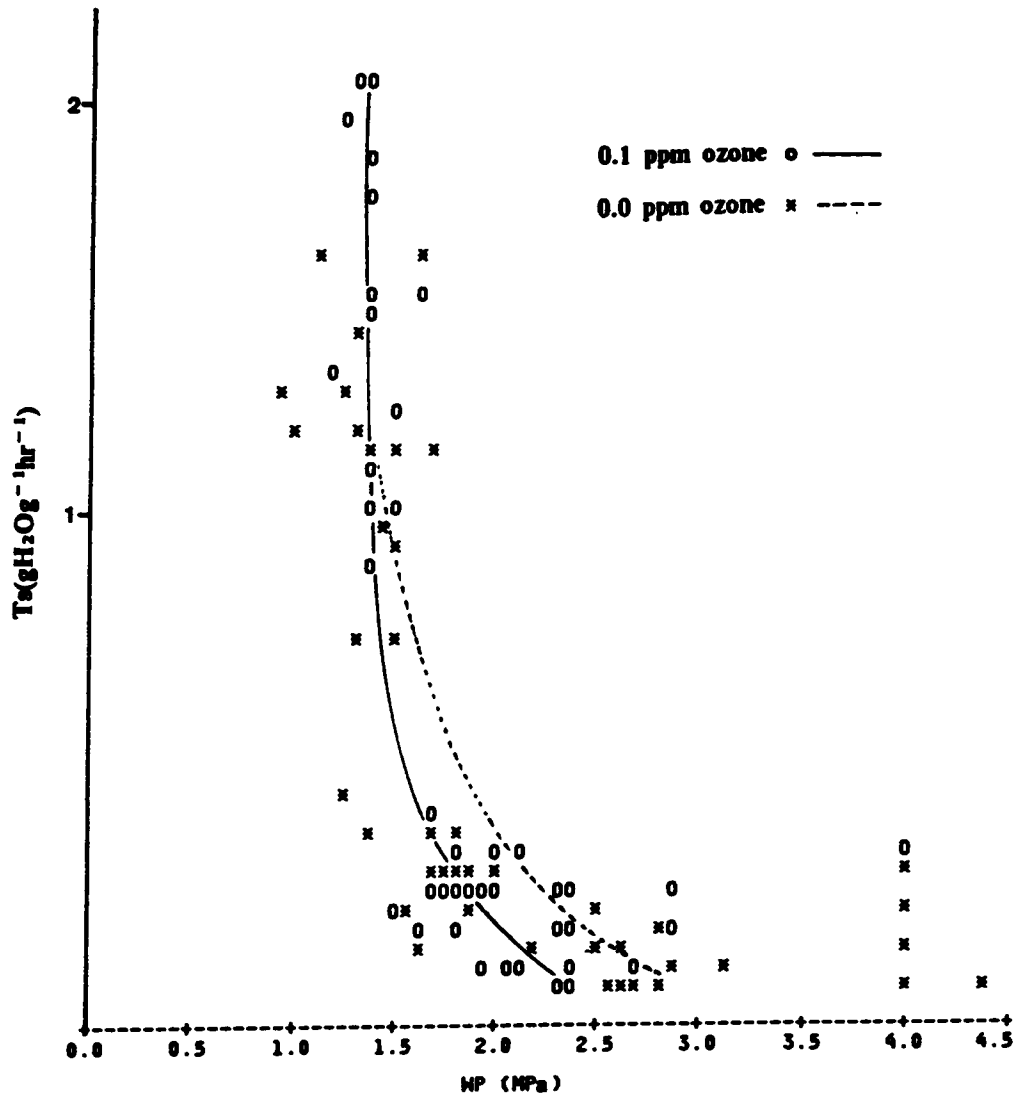


Figure A8. Transpiration and predicted regression response of one-year-old loblolly pine seedlings as affected by ozone and simulated acidic rain during the second drought cycle.

**The vita has been removed from
the scanned document**