THE COMBINED EFFECTS OF OZONE, SULFUR DIOXIDE AND SIMULATED ACID RAIN ON THE GROWTH OF THREE FOREST TREE SPECIES

by

Arthur Henry Chappelka, III

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APPROVED:

B. I. Chevone, Chairman

S. A. Alexander

T. E. Burk

L. D. Moore

J. R. Seiler

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(ABSTRACT)

Nine-week-old yellow-poplar and green and white ash were exposed to various concentrations of O_3 (0.00 to 0.15 ppm) and/or SO_2 (0.08 ppm), 4 hr/d, 5d/wk in combination with simulated rain (pHs 5.6, 4.3, 3.0), 1 hr/d, 2 d/wk, for 5 or 6 wk under controlled laboratory conditions. Pollutant exposures resulted in alterations in seedling biomass accumulation, growth rates, changes in carbon allocation among plant parts and modification in physiological processes associated with gas exchange. Ozone (0.010 ppm) and SO_2 together caused a significant decrease in height growth and biomass and an increase in leaf area ratio (LAR) in yellow-poplar. Ozone and SO_2 exposures resulted in linear decreases and increases, respectively, in root dry weight, leaf area increase, relative growth rates of all yellow-poplar plant parts and unit leaf rate with decreasing rain pH. Chlorophyll content increased in both O_3 and SO_2 treatments with increasing rain acidity. In green and white ash experiments height growth was inhibited by O_3, SO_2 and O_3 + SO_2 for green ash, whereas

only leaf dry weight was decreased by O_3 exposure in white ash. Decreasing rain pH resulted in linear decreases in root/shoot ratio (RSR) and LAR, for white ash. In green ash, a quadratic response to rain pH occurred with these growth variables. Ozone and O_3 + SO_2-treated green ash exhibited a significant quadratic response in leaf weight ratio with increasing rain acidity. Leaf area ratio and RSR exhibited linear increases and decreases, respectively, for O_3 and SO_2-treated white ash with increasing rain acidity. In white ash and yellow-poplar seedlings exposed to various O_3 concentrations and simulated rain for 5 and 6 weeks, respectively, increasing O_3 concentrations caused linear decreases in height and biomass of white ash. Linear decreases in root growth rate and biomass and RSR occurred with decreasing rain pH, across O_3 treatments. Ozone (0.05 or 0.10 ppm) caused linear decreases in these variables in combination with increasing rain acidity. For yellow-poplar, increasing O_3 concentrations caused linear increases in RSR and specific leaf area. At 0.05 and 0.10 ppm O_3, stem and leaf biomass, their relative growth rates and leaf area all decreased with decreasing rain pH. Ozone (0.10 ppm) exposure caused a decrease in stomatal conductance, and decreasing rain pH resulted in a linear decrease in this variable. A linear decrease in net photosynthesis also occurred with increasing rain acidity in O_3-treated (0.10 ppm) plants. These results demonstrate that gaseous pollutants in combination with simulated acid rain can have detrimental effects on growth of three forest tree species, under controlled laboratory conditions.

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INTRODUCTION

As the twenty-first century rapidly approaches and the world population increases, agricultural and forest lands are dwindling. In order to meet the increasing needs of the forest industry to maintain adequate amounts of fiber, lumber and fuel wood, trees must be grown at or near the maximum of their genetic potential. This goal can only be approached under optimum environmental conditions.

There is evidence to indicate that in the next twenty years coal consumption in the United States may increase by as much as sixty percent, and that the current trend of increased atmospheric loadings of sulfur dioxide (SO_2) and oxides of nitrogen (NO_x) may continue. While these pollutants can be harmful to plants by themselves they are also precursors of secondary pollutants (ozone (O_3) and acidic precipitation), and can be transported long distances from urban sources to rural or forested areas.

Ozone is considered the major phytotoxic pollutant in eastern North America and has been reported as causing significant adverse effects on plant growth, even in the absence of visible foliar injury. Increases in rainfall acidity, due to the wet deposition of sulfur and nitrogen compounds has raised concern over potential detrimental effects to terrestrial ecosystems. However, at present only limited information is available on the effects of acid rain on plant growth at hydrogen ion concentrations which approximate those occurring naturally.

Within the last twenty years several forest tree species have been observed to be in a state of decline (decreases in radial growth, dieback, mortality, etc.), both in North America and in Europe. Acid rain and gaseous pollutants (O_3,SO_2,NO_x), alone or in combination, heavy metals and drought, have been implicated as inciting or contributing to this decline. However the combined effects of these stresses on tree growth are just being investigated.

In the early work (1950-1960s) describing the phytotoxic effects of gaseous pollutants on terrestrial vegetation, plants were classified as sensitive or tolerant on the basis of visible foliar injury. In these studies, plants were exposed to high concentrations of pollutants for short periods of time (acute dosages). Since it was known however, that air pollutants could cause growth losses in the absence of visible foliar injury growth parameters, such as shoot elongation began to be used (1970s) as an indicators of the sensitivity of trees to air pollutants, regardless of the development of visible symptoms. The majority of this research has involved exposure of plants to known concentrations of a single pollutant. However, air contaminants do co-occur in the ambient atmosphere and research to determine the response of plants to pollutant mixtures is now receiving attention.

The purpose of this study was to investigate the seedling growth response of three forest tree species to simulated acid rain, O_3 and SO_2, alone or in combination. Yellow-poplar, green ash and white ash were the species chosen for this study since they are all commercial hardwood species, and are native to the southern Appalachian Mountains (including southwestern Virginia). All species have been identified as being sensitive to O_3 and/or SO_2 determined by visible foliar injury.

The long-term effects of air pollutants on trees can be reduced growth, loss of vigor, or death. Measurement of subtle changes in the physiological efficiency of a tree without visible injury may be an important indication of the prolonged effects of air pollutants on tree growth.

Recently, it has been shown that one of the primary effects of air pollutants on plants is the alteration of photosynthate translocation. A procedure now being used to gain a better understanding of these shifts in allocation patterns is growth analysis. Growth analysis is a mathematical technique which separates growth into component processes in order to study the effects of endogenous and exogenous influences.

Shifting patterns of photosynthate allocation, e.g., root/shoot ratio and biomass production, may result in trees becoming more susceptible to other abiotic and biotic stresses. By exposing tree seedlings to simulated acid rain and/or gaseous pollutants under controlled environmental conditions, shifts in biomass accumulation and allocation can be observed and mechanisms associated with these shifts explored.

The specific objectives of this research were:

1. To determine the effects of ozone, sulfur dioxide and simulated acid rain, alone or in combination on the growth of yellow-poplar seedlings, under laboratory exposure conditions.

2. To determine the effects of ozone, sulfur dioxide and simulated acid rain, alone or in combination on the growth of green and white ash seedlings, under laboratory exposure conditions.

3. To determine the effects of ozone and simulated acid rain, alone or in combination on the growth of white ash seedlings, under laboratory exposure conditions.

4. To determine any alterations of growth and

foliar gas exchange of yellow-poplar seedlings in response to ozone and simulated acid rain, alone and in combination, under laboratory exposure conditions.

The research necessary to satisfy the above objectives was undertaken in several phases. Each phase was related to the others and yet constituted a distinct study, therefore the results from each objective are presented in an individual chapter.

LITERATURE REVIEW

Introduction

Smith (60) defines air pollutants as "materials that occur in the troposphere in quantities in excess of normal amounts." These materials may be solid, liquid or gaseous in nature and may result from both natural (volcanoes, etc.) or anthropogenic (factories, automobiles, etc.) sources. This problem has intensified rapidly since the `Industrial Revolution' in the early 1900s', and during the past 10-20 years an increasing amount of data has accumulated regarding the harmful effects of various air contaminants to terrestrial vegetation.

Primary pollutants are emitted directly from an identifiable source, of which sulfur dioxide (SO_2), oxides of nitrogen (NO_x) and hydrogen fluoride (HF) are the most phytotoxic (57,60). In addition to being phytotoxic, primary pollutants are precursors of secondary pollutants. A secondary pollutant is an air contaminant which is produced by reactions in the atmosphere involving primary pollutants or other atmospheric constituents (60).

Photochemical reactions between NO_x and hydrocarbons (HC) produce secondary pollutants, of which ozone (O_3) is the most phytotoxic (61). Ozone has been reported to cause significant reductions in plant growth and productivity. Ozone and its precursors can be transported long distances from urban sources to rural and forested areas (18,51).

Acidic precipitation (acid rain) is a secondary pollutant that contains as its principal components the hydrolyzed end products from oxides of sulfur or nitrogen and halogen compounds (36,61). Recently, much concern has developed over the possible increases in acidity of precipitation caused by anthropogenic inputs into the atmosphere and its potential detrimental effects of this acidity on terrestrial ecosystems (17). Similar to O_3, precursors of acid rain can be transported long distances from urban to rural environments (3).

The growth impact of air pollutants on forest trees is potentially a very serious problem due to the perennial nature of these plants. The most potentially phytotoxic pollutants to trees include O_3, SO_2 and acid rain (57,61). This literature review will present the state of current knowledge, emphasizing the impact of the above-stated pollutants on the growth of forest trees.

Growth Losses Due To Air Pollutants: Laboratory Studies

The early literature pertaining to the effects of gaseous air pollutants on terrestrial vegetation classified plants as sensitive or tolerant on the basis of visible foliar injury (11,15,67,70,71). In these studies plants were exposed to high concentrations of pollutants for short durations and were ranked in sensitivity classes according to the percentage of the foliage that exhibited visible symptoms. Kress (30) and Smith (60) reviewed the literature on this subject in detail.

When studying the effects of long-term, low-level exposures to air pollutants, primary interest has involved the study of reductions in growth irregardless of visible symptoms. This section will concentrate on the literature pertaining to alterations in growth of forest trees caused by O_3 and SO_2.

Jensen (21) exposed one-year-old seedlings of nine deciduous forest tree species to 0.30 ppm O_3, 8 hours a day, 5 days a week, for 5 months in indoor fumigation chambers. Significant reductions in shoot elongation occurred in response to O_3 for American sycamore (_Plantanus occidentalis_ L.), sugar maple (_Acer saccaharum_ L.) and silver maple (_A. saccaharinum_ L.). Premature leaf senescence was also quite common for the majority of fumigated seedlings, irregardless of a reduction in height growth.

Kress and Skelly (31) investigated the response of two-to-four-week old seedlings of ten eastern forest tree species to O_3 applied at 0.00, 0.05, 0.10 or 0.15 ppm, 6 hours a day, for 28 consecutive days. Loblolly pine (Pinus taeda L.) exhibted a significant height growth suppression and yellow-poplar (Liriodendron tulipifera L.) exhibited a significant stimulation in height growth at 0.05 ppm O_3. Ozone applied at 0.10 ppm resulted in a decrease in shoot elongation for loblolly pine, American sycamore, sweetgum (Liquidambar styraciflua L.), green ash (Fraxinus pennsylvanica Marsh.) and pitch pine (P. rigida Mill.). All species, except white ash (F. americana L.) and Virginia pine (P. virginiana Mill.) exhibited significant suppressions in height growth when fumigated with 0.15 ppm O_3. In general, suppressions in shoot elongation were accompanied by reductions in dry weight. Root dry weight appeared to be the most sensitive variable measured regarding O_3 exposure.

The growth responses of nine western conifer species exposed to O_3 at 0.10 ppm for 6 hours a day, 7 days a week for 18 weeks, were observed by Wilhour and Neely (68). Ponderosa pine (P. ponderosa Laws.) and western white pine (P. monticola Dougl.) exhibited significant reductions in stem dry weight. In addition, ponderosa pine root dry weight and western white pine leaf dry weight were significantly reduced by O_3. No significant growth effects occurred for the other seven species studied.

Hybrid poplar (Populus deltoides X trichocarpa) was exposed to O_3 at 0.025, 0.050, 0.085 or 0.125 ppm, for 6 hours a day, for 62 days under laboratory exposure conditions by Reich and Lassoie (55). Ozone treatments did not significantly affect height or diameter growth during the first 6 weeks of fumigation. Height and diameter growth, however, were significantly reduced at harvest for the 0.125 ppm O_3-treated plants compared with controls (0.025 ppm O_3). By the end of the study, plant biomass and the number of leaves per plant decreased linearly with increasing O_3 concentrations.

Mooi (45) in a separate experiment, exposed hybrid poplar to 0.04 ppm O_3, 12 hours a day, 7 days a week, for 5.5 months, in plastic fumigation chambers in the greenhouse. The fumigated seedlings exhibited approximately 60% defoliation, 4-12% reductions in stem dry weight and 60% reductions in leaf dry weight. No significant decrease in stem elongation occurred for O_3-treated seedlings. Visible symptoms appeared on the foliage after approximately 10 days of fumigation. The findings of Mooi were similar to those of Reich and Lassoie (55) in that premature leaf drop was a primary response of hybrid poplar to O_3 exposure.

Jensen (24) investigated the response of one-year-old silver maple and eastern cottonwood (P. deltoides Batr.) to 0.00, 0.10, 0.20 or 0.30 ppm O_3, 12 hours a day, for up to 60 consecutive days. Ozone treatments resulted in decreases in leaf area and total new dry weight of both tree species and a reduction in shoot elongation for eastern cottonwood. Growth reductions increased with an increase in O_3 concentration and became evident after approximately 30 days of fumigation.

Kress and Skelly (31) investigated the response of seven eastern forest tree species to the combined effect of O_3 and nitrogen dioxide (NO_2). Seedlings were exposed 7 days a week to 6 hours of O_3 and/or NO_2 applied at 0.10 ppm, for 28 consecutive days. The combination of O_3 + NO_2 resulted in significant decreases in height growth for loblolly and Virginia pines and total biomass for sweetgum and white ash. These effects, however, were determined to be less than additive.

In another study, Kress et al. (32) exposed 1 to 3 week- old American sycamore to 0.05 ppm O_3, 0.10 ppm NO_2 and/or 0.14 ppm SO_2, 6 hours a day, for 28 consecutive days. Significant suppressions in shoot elongation occurred with O_3 + SO_2 or O_3 + SO_2 + NO_2 treatments. These pollutant effects appeared to additive in nature.

Jensen (22) fumigated one-year-old yellow-poplar, eastern cottonwood and white ash with 0.10 ppm O_3 and/or 0.20 ppm SO_2, 12 hours a day, 7 days a week, for 6 consecutive weeks. Neither O_3 or SO_2 alone or in combination had any effect on white ash growth. Ozone reduced eastern cottonwood growth more than SO_2, while SO_2 caused a greater growth reduction than O_3 for yellow-poplar. The combined effects of pollutants was less than additive for both tree species.

The growth impact of low-levels of O_3, alone and in combination with SO_2 on silver maple was investigated by Jensen (25). One-year-old silver maple were fumigated for up to 60 consecutive days with either 0.05, 0.10 or 0.20 ppm O_3, alone or in combination with 0.10 ppm SO_2 for 12 hours a day. Increasing O_3 concentrations resulted in significant reductions in height growth, leaf area and leaf dry weight. The addition of SO_2 to the O_3 treatments intensified the reductions in growth, with the most severe treatment being 0.20 ppm O_3 + 0.10 ppm SO_2. Generally, these effects appeared to be additive in nature.

Constantinidou and Kozlowski (13) investigated the effects of O_3 and/or SO_2 on the growth and visible injury of American elm (Ulmus americana L.) seedlings. Four-month-old American elm were exposed to 0.9 ppm O_3 for 5 hours, 2.0 ppm SO_2 for 6 hours, or the combination of the two pollutants for 5 hours. Leaf expansion was inhibited by all treatments. Ozone and O_3 + SO_2 treatments significantly reduced stem dry weight and SO_2 and O_3 + SO_2 treatments caused a reduction in root dry weight. Visible injury appeared on all pollutant-treated seedlings. The pollutant concentrations used in this study were high and are not typical of low-level pollutant concentrations which generally occur in the field.

The results reported in this section indicate that in general gaseous air pollutants can cause a decrease in growth of many different forest tree species. It is difficult however, to relate these studies to field situations due to several factors including, soil nutrient status, water availability, temperature and durations and fluctuations of pollutant concentrations under ambient conditions.

Growth Losses Due To Air Pollutants: Field Studies

The effects of gaseous air pollutants on tree growth have also been examined in several field studies (16,41, 47,56). Duchelle et al. (16) using open-top-chambers, observed the effect of ambient pollutant concentrations (primarily O_3) on the growth of eight different tree species in the Blue Ridge Mountains of Virginia. After two growing seasons, seedling height growth was less for all species tested, when exposed to ambient air, compared to those receiving charcoal-filtered air. Reductions in shoot elongation were 41, 66, 18, 22, 20, 12 and 23% for yellow-poplar, green ash, black locust (*Robinia pseudo-acacia* L.), Virginia pine, eastern white pine (*P. strobus* L.), table mountain pine (*P. pungens* Lamb.) and eastern hemlock (*Tsuga canadensis* L., Carr.), respectively. Visible symptoms typical of oxidant injury were also observed for green ash and yellow-poplar growing in ambient plots.

McClenahen and Dochinger (41) investigated growth of eastern white pine, white ash and black cherry (*Prunus serotina* Ehrh.) and northern red oak (*Quercus rubra* L.) seedlings to ambient air pollutants (primarily O_3), using open-top-chambers, at a site near Mansfield, Ohio. Height growth of eastern white pine and total biomass and stem diameter of black cherry were significantly greater for plants growing in non-filtered air, compared to those growing in charcoal-filtered air. Growth of the other tree species was not significantly different between chamber treatments. The reasons for species differences are not known: however, the authors' felt that shading between species protected them from pollutant damage and may have contributed to the results.

Patton (47) exposed seven hybrid poplar clones to 0.15 ppm O_3 or 0.25 ppm SO_2 plus ambient concentrations of these pollutants in open-top-chambers at Delaware, Ohio. Treatments were applied 12 hours a day, for 102 consecutive days. There was no significant difference in height growth or stem specific gravity when SO_2-treated plants were compared with non-fumigated controls. Ozone, however, caused a decrease in height growth for one clone and a decrease in specific gravity for 6 clones. These results indicate that O_3 is more detrimental to hybrid poplar growth than SO_2.

In another study with hybrid poplar, Reich et al. (56) exposed plants to O_3 and/or SO_2 using a field fumigation system. During the experiments average ambient levels of SO_2 were < 0.01 ppm and O_3 was approximately 0.04 ppm. Plants were exposed to O_3 at ambient concentrations, or 0.06 and 0.08 ppm above ambient air. Sulfur dioxide concentrations were ambient, or 0.06 and 0.11 ppm above ambient air. No significant interactions were found between pollutant treatments in this study. Exposure of plants to above ambient concentrations of O_3 and SO_2 resulted in reductions in height growth, biomass accumulation and an increase in leaf senescence. The detrimental effects of O_3 on plant growth was approximately three times greater than SO_2. These results were similar to those of Patton (47) regarding the differences in phytotoxicity of O_3 and SO_2 to hybrid poplar.

Several studies have examined the ambient air pollutant effects to mature trees in natural field conditions (6,43,49,50,59,63). Stone and Skelly (63) investigated the annual radial increment growth of eastern white pine and yellow-poplar, growing in the vicinity of a large ammunition factory in southwestern Virginia. This plant periodically emitted large amounts of SO_2 and NO_x to the ambient atmosphere. Radial increments of these trees were compared with annual factory production levels from 1941-1971. A significant, linear, inverse relationship between annual radial growth and plant production levels were observed for both species. With a large increase in factory production, such as occurred in times of war, radial growth dramatically decreased.

Phillips et al. (50) in an effort to relate the intensity of visible foliar symptoms of air pollution to growth suppressions of eastern white pine, re-evaluated the stand studied by Stone and Skelly (63). Four levels of symptom expression were used to categorize the trees for analyses. Analyses of regression correlations indicated no significant difference in growth rate between symptom classes during peak years of factory production; growth of asymptomatic trees was reduced as much as symptomatic trees. Skelly et al. (59) found, however, that height growth of trees in a 13-year-old eastern white pine stand that exhibited severe visible air pollution injury (excluding `chlorotic dwarfs') were on an average, 33% as tall as asymptomatic trees in the same stand.

The growth response of three young (15 to 18-year-old) loblolly pine stands was also examined by Phillips et al. (49), near the vicinity of the same ammunition factory. In two of the stands a significant inverse relationship was demonstrated between annual production levels and radial increment growth. Radial growth reduction was not correlated with visible foliar injury in these stands.

Benoit et al. (6) evaluated the effect of oxidant air pollution (primarily O_3) on the radial increment growth of eastern white pine, differing in sensitivity to O_3 in the vicinity of the Blue Ridge Parkway in Virginia. A general decrease in radial growth was recorded throughout the growth period 1955-1978 for trees in all sensitivity classes. Mean annual radial growth however, was significantly less for the O_3-sensitive trees than the tolerant trees during the same period of growth.

Radial growth of ponderosa pine was compared for periods of low air pollution (1910-1940) and high air pollution (1941-1971), primarily O_3, in the San Bernardino National Forest in California by Miller (43). Average annual radial growth was 0.20 mm less for the period of high pollution compared with the period of low pollution. A 30-year-old tree growing in the period of low pollution was calculated to have 38% and 23% greater diameter and height growth, respectively, than a tree growing in the period of high air pollution.

Effects of Acid Rain on Tree Growth: Lab Studies

Studies to determine the effects of acid rain on tree growth are limited and results inconclusive, since the majority of this research has been initiated within the last 10 years. These findings are presented in the following sections.

Wood and Bormann (72) exposed 2 to 6 week-old yellow birch (*Betula aleghaniensis* Britt.) with H_2SO_4 acidified distilled water applied as a mist at pHs 4.7, 4.0, 3.3, 3.0 and 2.3. Misting was conducted for a 6 hour period weekly for 11 to 15 weeks. Differences in growth were only observed at the pH 2.3 level. The majority of the younger seedlings (2 weeks-old) to which pH 2.3 mist solutions were applied died, indicating that yellow birch in the cotyledonary stage of development was very sensitive to pH 2.3 acidic mists.

In a later study, Wood and Bormann (73) investigated the short term effect of simulated acid rain on the growth and nutrient relations of 2-week-old eastern white pine. Seedlings were subjected to 1, 6 hour application of simulated rain, per week for 20 weeks. Solutions were composed of H_2SO_4, HNO_3 and HCL mixed in distilled water and pH adjusted to 5.6, 4.0, 3.3, 3.0 or 2.3. Total dry weights and needle lengths were significantly increased by the pH 2.3 treatment after 20 weeks. This growth increase occurred despite the incidence of visible injury on all pH 2.3-treated seedlings. Increases in foliar nitrogen content indicated that N fertilization, added as HNO_3, may have contributed to the observed increase in growth.

Matziris and Nakos (39) examined the growth response of two-year-old half-sib Aleppo pine (P. halepensis Mill.) irrigated with simulated rain solutions for 5 months. Solutions consisted of H_2SO_4 diluted in distilled water and pHs adjusted to 5.1 (control), 3.5 and 3.1. After one growing season seedlings irrigated with pH 3.1 solutions exhibited an 8% reduction in height growth compared with controls.

Neufeld et al. (46) reported on the effects of foliar applications of simulated acid rain to seedlings of four deciduous tree species native to the eastern United States. Rain solutions consisted of ionic components characteristic of rainfall at Coweeta, North Carolina and were amended with a mixture of H_2SO_4, HNO_3 and HCL to produce solutions of pHs 5.6, 4.0, 3.0 or 2.0. Simulated rain was applied every 3 days for 48 days (16 exposures). Significant reductions in growth were only evident with the pH 2.0-treated seedlings, for only American sycamore, black locust and sweetgum. These results demonstrate a strong tolerance to pH in these tree species.

The effects of simulated acid rain on germination and seedling growth of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) and ponderosa pine were investigated by McColl and Johnson (40). In the germination experiment, Douglas-fir seeds were planted in sterile sand and sprayed with solutions simulating the average ionic composition of rain in northern California. Solutions were adjusted to pHs 5.6, 4.0, 3.0 and 2.0 using a 2:3 mix (v/v) of H_2SO_4 and HNO_3 diluted in distilled water. There was no difference in percent germination between treatments, except at pH 2.0. Germination decreased from about 90% in all other treatments to 60% with pH 2.0. In the growth study, two-year-old Douglas-fir and ponderosa pine were exposed to the same solutions as previously described. There was no significant differences in needle length and dry weight between treatments, for both species tested.

Lee and Weber (35) examined the effect of simulated acid rain on seedling emergence and growth of eleven woody plant species. The rain solutions contained ionic constituents approximating ambient rain at Hubbard Brook New Hampshire, and were acidified with H_2SO_4 to pHs 5.6, 4.0, 3.5 or 3.0. Solutions were applied 3 days a week for 5-7 months. The percent germination of eastern white pine, yellow birch and eastern red cedar (*Juniperus virginiana* L.) was significantly increased as the acidity in rain increased. Simulated acid rain inhibited seedling germination of staghorn sumac (*Rhus typhina* L.). No species showed a significant decrease in top growth at any pH level. Douglas-fir, however, exhibited a significant increase in top dry weight with increasing rain acidity. Staghorn sumac was the only species which exhibited a significant decrease in root dry weight with the acid treatments (pHs 3.5 and 3.0). The results in this study demonstrate a broad range in pH effects among tree species.

The effect of substrate acidity on seed germination of five tree seedlings was investigated by Raynal et al. (52). Seeds were placed on fiberglass cloth saturated with 100 ml treatment solutions of pHs 5.6, 4.0 or 3.0 composed of dilute H_2SO_4. Solutions were applied 3 times a week. Germination inhibition was observed at pHs 4.0 and 3.0 for red maple and at 3.0 for yellow birch, compared with seeds treated with pH 5.6. Silver maple and eastern hemlock were not affected by any pH treatment. Percent germination of eastern white pine increased with increasing rain acidity with maximum germination observed at pH 3.0. The authors' concluded that a wide range of sensitivities to solution pH exist among these five tree species.

Effects of Acid Rain on Tree Growth: Field Studies

In a field study in Norway to determine the effects of acid rain on forest tree growth, Abrahamsen et al. (1) examined radial growth increments of various tree species in regions known to have different inputs of acid rain. No consistent trends were found to indicate that acid rain may be directly affecting tree growth.

Cogbill (12) using tree ring analysis, compared patterns and historical trends of acid rain deposition with growth trends from mature forest stands in New Hampshire and Tennessee. The residual variation of tree growth responses, after removal of variation due to other climatic variables, demonstrated no synchronized decreases in radial growth for any tree species observed, and there was no significant correlation between historical trends of acid rain and tree growth at these sites.

In an early study to determine the effects of artificial acid rain on forest productivity, Tamm et al. (64) treated the forest floor with various combinations of H_2SO_4, N-P-K fertilizer, lime, micronutrients and irrigation water. Nitrogen was found to be the limiting growth factor at this site. Nitrogen fertilization increased basal area of trees more than height growth. The application of H_2SO_4 at 100 kg/ha killed ground flora of lichens, mosses and scrubs, but had no negative effect on tree growth.

Tveite and Abrahamsen (66) irrigated Norway spruce (Picea abies L., Karst), Scots pine (P. sylvestris L.) and lodgepole pine (P. contorta Dougl.) with artificial applications of H_2SO_4 in the field for up to five years. They observed slight height growth stimulations for all tree species, following application of pHs less than 4.0. The authors' attributed these increases to improved N and S nutrition with the acidic treatments.

Results from these studies would tend to indicate that acid rain has little or no deleterious effect on tree growth. In fact, in some cases, growth was enhanced by the application of acid solutions (66). The results from these acid rain studies are in contrast with those examining gaseous pollutants (6, 43, 49, 50, 59, 63) where growth losses have been directly correlated with increases in concentrations of gaseous air contaminants.

Combined Effects of Acid Rain X Gaseous Pollutants

Since both acidic precipitation, in the form of rain, mists, fogs or aerosols, and gaseous pollutants, primarily O_3, can impact the same area (61) it is important to understand the combined effects of these pollutants on tree growth. At the present time there are only two reports in the literature pertaining to this subject, and they are reviewed in this section.

In a series of experiments Krause et al. (29) examined the combined effects of O_3 and acid fogs on foliar nutrient leaching of Norway spruce in West Germany. Five-year-old trees were fumigated with 0.10 or 0.30 ppm O_3, 5 days a week, for 68 days. Twice a week, plants were fogged with an acid solution (pH 3.5). At the termination of the experiment significant amounts of Mg, NO_3, SO_4 and Ca were leached from the foliage of trees treated with O_3 and acid fog, compared with those treated with O_3 or acid fog alone. They concluded that these nutrient losses would affect photosynthesis and cause eventual reductions in growth, although growth alterations were not examined.

Skeffington and Roberts (58) examined the combined effects of O_3 and acid mists on growth and nutrient leaching of Scots pine. Three-year-old trees were exposed to 0.00, 0.05, 0.10 and 0.16 ppm O_3 and/or pH 3.0 acid mists for 2 months. Ozone alone at 0.10 ppm or greater caused a significant reduction in dry weight of fine roots per plant. Both O_3 and acid mist alone caused a reduction in dry weight of old needles. There were no significant interactions observed for O_3 X acid mist in seedling biomass accumulation. They found that increasing concentrations of O_3 resulted in increased foliar concentrations of most ions in new needles as well as a decrease only in P content in older needles. Increased O_3 concentrations did however, also cause a decrease in foliar uptake of NO_3 from the acid mist solution.

Effects of Air Pollutants on Yellow-Poplar

Yellow-poplar is one of the major commercial hardwood species in the eastern United States (5). It is among the tallest of all hardwood trees, has excellent form, early natural pruning and good wood working quality. Several studies, both in the laboratory and field have examined the effects of various air pollutants on yellow-poplar.

Chrenk and Spaulding (11) reported in 1909 on the sensitivity of several tree species to sulfur emissions growing near a copper smelting factory. Based on visible injury, yellow-poplar was reported as being intermediate in sensitivity. Hedgecock (19) in 1914, in a similar study, reported on the sensitivity (based on visible symptom expression) of ten forest tree species to high concentrations of SO_2 near two copper smelting factories in Tennessee. Yellow-poplar was also reported as being intermediate in sensitivity to SO_2. Davis and Wilhour (15), however, reported yellow-poplar as sensitive to acute dosages of SO_2 in the laboratory.

Wood and Davis (71) investigated the relative sensitivity of 34 species of woody plants, based on visible foliar injury, to acute dosages of O_3. Yellow-poplar, green and white ash and white oak (<u>Q. alba</u> L.) were reported as the most sensitive hardwood species tested. In a separate study, Wood and Coppolino (70) examined the foliar response of 21 deciduous tree species to O_3. Yellow-poplar, white ash, American sycamore, hybrid poplar and honey locust (<u>Gleditsia triacanthos</u> L.) were reported as the most sensitive tree species to O_3.

The effect of O_3 on height growth and leaf drop of yellow-poplar was investigated by Jensen (21). Jensen fumigated nine forest tree species with O_3 at 0.30 ppm, 8 hours a day, 5 days a week, for 5 months. Yellow-poplar seedling height growth was not significantly different between fumigated and non-fumigated seedlings. Premature leaf drop was 60% greater for the O_3-treated seedlings than controls. Since yellow-poplar has previously been reported as being sensitive to O_3, based on foliar injury (70,71), this large percentage of defoliation would be expected with this exposure regime. The O_3 dose used in this study, however, was unrealistic compared to a field situation.

Jensen (22), in another study, examined the relative rates of growth rather than final biomass accumulation of three hardwood tree species, including yellow-poplar exposed to O_3 and/or SO_2. Plants were fumigated for 12 hours a day, for up to 50 consecutive days, to no pollutants, 0.10 ppm O_3 and/or 0.2 ppm SO_2. Yellow-poplar relative growth rates, based on the amount of new growth produced during the study were decreased 7, 19 and 53% for

O_3 + SO_2-, O_3- and SO_2-treated seedlings, respectively. Leaf area ratio (leaf area/plant dry weight) was not altered by O_3, but was decreased 25 and 20% by exposure to SO_2 and O_3 + SO_2, respectively. Sulfur dioxide and O_3 treatments suppressed unit leaf rate, i.e., net assimilation rate (relative growth rate/leaf area ratio) by 37 and 15%, respectively. The combined effect of these pollutants, however, resulted in a 10% increase in unit leaf rate. Jensen concluded that O_3 and SO_2 do reduce the physiological efficiency (growth rates) of yellow-poplar and that SO_2 was more phytotoxic than O_3 to this tree species. He further noted that the combined effect of O_3 + SO_2 was less than additive.

In a later study, Jensen (26) examined the response of one-year-old yellow-poplar to intermittent air pollution fumigation. Yellow-poplar seedlings were treated with clean air, 0.10 ppm O_3, 0.10 ppm O_3 + 0.20 ppm SO_2 or 0.10 ppm O_3 + 0.20 ppm NO_2, for 1 or 2, 12 hour fumigations per week, for up to 20 weeks. Ozone and O_3 + SO_2 caused a suppression in relative growth rate after 5 months of fumigation. In addition, O_3-treated plants had a reduced unit leaf rate compared with controls. When pollutants were applied twice weekly O_3 + SO_2 and O_3 + NO_2 caused a greater decrease in relative growth rate and unit leaf rate than O_3 alone. In fact, O_3-treated plants exhibited an increase in unit leaf rate after 20 weeks fumigation. He found that there were significant differences in growth between control seedlings which were moved from the greenhouse to the fumigation chambers, once or twice a week;seedlings which were moved only once a week exhibited the greatest growth. No reasons were given for these differences in results, but they are probably related to differences in environmental conditions (lighting, etc.) between the chambers and greenhouse. These results also contradict his earlier findings (22) that O_3 and SO_2 alone have a greater effect on yellow-poplar seedling growth than the combined effects of the two pollutants.

Mahoney et al. (38) examined the effects of various pollutant combinations at low concentrations, on yellow-poplar seedling growth. Five-week-old, half-sib, yellow-poplar were fumigated 6 hours a day, for 35 consecutive days with 0.07 ppm O_3, 0.06 ppm SO_2 and 0.10 ppm NO_2, in all possible combinations. Ozone and SO_2 alone did not suppress total dry weight or height growth when compared with controls. The combination of O_3 + SO_2, SO_2 + NO_2, and O_3 + SO_2 + NO_2 caused a significant decrease in shoot elongation of 51, 64 and 51%, respectively, and total dry weights were decreased 45, 50 and 34%, respectively. Differences in results between Jensens' studies (22,26) and Mahoneys' et al. (38) could be due to several factors including seed source, tree age, different environmental conditions, pollutant concentrations and duration of exposures.

Kress and Skelly (31) fumigated ten, 2 to 4 week-old forest tree species, including yellow-poplar, to 0.00, 0.05, 0.10 or 0.15 ppm O_3, 6 hours a day, for 28 consecutive days. Yellow-poplar seedling height growth and biomass accumulation were not significantly decreased by O_3 at any concentration, in fact, 0.05 ppm O_3 caused a significant stimulation in yellow-poplar height growth when compared with controls.

Results from the previous studies (22,26,31,38), in the laboratory, indicate that O_3 and SO_2 may or may not have an effect on yellow-poplar seedling growth. Discrepancies in these results make interpretation related to growth losses under field conditions difficult. Several field studies involving air pollution effects on yellow-poplar growth have been conducted, however, and are reported in this section.

Stone and Skelly (63) studied the relationship between annual radial increment growth and yearly factory production levels of mature yellow-poplar and eastern white pine growing in the vicinity of a large ammunition factory. A significant, inverse, linear correlation was observed between decreases in radial tree growth, for both species, and increases in factory production levels. Near the same source of periodic air pollution emissions, Phillips (48) exposed yellow-poplar seedlings to ambient and charcoal-filtered air using open-top-chambers, for one growing season. Height growth was decreased approximately 33% for seedlings in ambient air, compared with those receiving charcoal-filtered air.

Duchelle et al. (16) investigated the response of seven tree species, including yellow-poplar, to ambient concentrations of O_3, for two years, in the Blue Ridge Mountains of Virginia. Height growth of seedlings growing in non-charcoal-filtered chambers was 41% less than those receiving charcoal-filtered air. Typical oxidant injury was observed on yellow-poplar receiving ambient air.

Results from these studies (16,48,63) indicate that ambient concentrations of air pollutants can cause decreases in yellow-poplar height and radial growth under field conditions. Discrepancies in field and laboratory studies regarding yellow-poplar growth may be the result of several factors, such as, nutrient status, water availability, temperature, and durations and fluctuations of pollutant concentrations under ambient conditions.

The effects of acid rain on yellow-poplar growth has not been extensively investigated and literature pertaining to this subject is reviewed in this section. Lee and Weber (35) examined the effect of simulated acid rain on seedling emergence and growth of eleven woody plant species, including yellow-poplar. There was no effect of pH at any treatment on yellow-poplar seedling emergence. The production of above ground biomass was stimulated in the pH 4.0 treatment. No other significant effects were observed for this species.

Neufeld et al. (46) investigated the direct foliar effects of acid rain on growth and foliar gas exchange of five deciduous tree species, including yellow-poplar. No significant differences in height growth, biomass accumulation or foliar gas exchange characteristics were observed between any pH treatment. Yellow-poplar seedlings in this study were collected in the field, transplanted and then the experiments were initiated immediately. These seedlings were probably in transplant shock, as reflected by the lack of height growth with any pH treatment, and therefore, these results should be interpreted with respect to experimental shortcomings.

Effects of Air Pollutants on Green and White Ash

Green and white ash are two commercial hardwood species and are also commonly planted landscape trees in North America (2). Since these species are morphologically very similar; studies to examine the effects of air pollutants on green and white ash generally include both species for comparison.

Wilhour (67) investigated the influence of O_3 on the sensitivity of white ash. Seedlings were grown in both controlled and ambient environments before and after fumigation with 0.10 or 0.25 ppm O_3 for from 0.5 to 8.0 hours. White ash was found to be sensitive to O_3, based on visible foliar injury, with symptoms appearing after only one hour of exposure to 0.25 ppm. Sensitivity to O_3 increased with an increase in relative humidity during fumigation.

In an effort to determine if genetic variation to O_3 exists among green and white ash families, Steiner and Davis (62) exposed 10 open-pollinated families of green ash and 2 families of white ash to 0.25 ppm O_3 for 6 hours. Both the percentage of leaf surface injury and the intensity of symptoms varied among families of the same and different species, indicating that genetic variability to O_3 was present in these species. The sample size was so small, however, that differences among provenances could not be detected.

Karnosky and Steiner (27), in a later study, investigated the provenance and family variation of green and white ash to O_3 and SO_2. Two-year-old seedlings from 16 provenances and 59 half-sib families of green ash and white ash from 10 provenances and 50 families were exposed to 0.5 ppm O_3 or 1.0 ppm SO_2 for 8 hours. White ash were exposed to O_3 for a second day to ensure maximum symptom expression. Trees from near-coastal provenances of white ash were more tolerant to O_3 than those from interior provenances, and green ash from southern locations were generally more tolerant to both O_3 and SO_2 than trees from northern locations. Mean visible SO_2 injury to white ash exhibited no distinct geographic pattern. Green ash, as a species, was considerably more sensitive to O_3 than white ash. In contrast, white ash was more sensitive to SO_2 than green ash.

Jensen (21) examined the effect of 0.30 ppm O_3 applied 8 hours a day, 5 days a week , for 5 months, on leaf drop and height growth of nine forest tree species, including green and white ash. White ash was reported as being tolerant to O_3, regarding height growth, however, both the O_3-treated and non-treated seedlings ceased height growth after one month of fumigation. Green ash treated with O_3 exhibited a 45% decrease in height growth after 5 months of fumigation and was reported as being intermediate in sensitivity. Ozone-treated green and white ash dropped 2 and 57%, respectively, of their leaves prematurely, compared with controls, after 20 weeks of fumigation. These results are unusual since green ash has been reported as being more sensitive to O_3 than white ash, based on visible foliar injury (27).

Kress and Skelly (31) exposed green and white ash and eight other tree species to 0.00, 0.05, 0.10 and 0.15 ppm O_3, 6 hours a day, for 28 consecutive days. Green ash exhibited a significant decrease in height growth with increasing O_3 concentrations. No difference in height growth between treatment occurred with white ash. These findings were similar to those of Jensens' (21). Green ash top dry weight for 0.15 ppm-treated seedlings was significantly less than the other O_3 treatments. White ash, however, exhibited a significant decrease in top and root dry weight for seedlings treated with 0.10 or 0.15 ppm O_3, compared with those treated with 0.00 or 0.05 ppm O_3. These results indicate that on the basis of biomass accumulation, white ash is more sensitive to O_3 than green ash, however, green ash has been reported as being more sensitive than white ash, based on visible injury (27) and height growth (21,31).

In the only known report regarding the effect of pollutant mixtures on white ash seedling growth, Jensen (22) exposed seedlings to 0.10 ppm O_3 and/or 0.20 ppm SO_2, 12 hours a day for up to 42 consecutive days. No significant differences in growth of white ash occurred for any variable measured.

Duchelle et al. (16) exposed 7, 1-year-old tree species, including green ash to ambient O_3 concentrations in the Blue Ridge Mountains of Virginia using open-top-chambers. Green ash seedling height growth was suppressed by approximately 66% after two years growing in non-charcoal-filtered chambers, compared with trees receiving charcoal-filtered air. Green ash receiving ambient air exhibited typical foliar O_3 symptoms. These results support laboratory studies (21,31) regarding the sensitivity of green ash to O_3, based on height growth.

McClenahan and Dochinger (41) investigated the seedling growth of four tree species, including white ash to ambient air pollutant concentrations (primarily O_3), using open-top-chambers. After two years there was no significant difference in white ash seedling height growth and above ground biomass between chamber treatments.

The relative response of leaf photosynthesis of three forest tree species, including white ash to O_3 and/or SO_2 was investigated by Carlson (10). Eight-ten-year-old saplings were fumigated with 0.5 ppm O_3 and/or SO_2 7 to 11 hours a day, for up to 10 days and net photosynthesis measured during each fumigation period. Net photosynthesis was not significantly reduced by the O_3 or SO_2 treatments after 3 weeks of fumigation. The combined effect of O_3 + SO_2 caused a significant 38% reduction in net photosynthesis of white ash after 1 week of fumigation. The pollutant concentrations and durations of exposure used in this experiment, however, were extremely high making interpretation of these results to a field situation difficult.

Jensen (23) investigated the root carbohydrate content and above ground biomass of green ash seedlings exposed to 0.50 ppm O_3, 8 hours a day, 5 days a week, for up to 6 weeks. After 6 weeks of exposure O_3-treated seedlings exhibited reduced levels of starch and reducing sugars in the roots and decreased leaf and stem dry weights, compared with the controls. However, as with Carlsons' study (10), the pollutant dose used was extremely high making extrapolation to a field situation unrealistic.

Growth Analysis and Application to Air Pollution Studies

Growth analysis is a technique which separates growth of a plant into component processes in order to study the effects of endogenous and exogenous influences on plant growth (20,34). This procedure focuses attention on rates of growth and patterns of allocation, instead of final biomass accumulation (20). Information provided by growth analysis can be used to calculate relative growth relationships, which can be used further to compare growth rates of various plant parts (stems, leaves, roots). Growth analysis was originally developed by Blackman (7) and Briggs et al. (8,9) to study the ontogenetic and environmental influences on growth of agronomic crops.

Jensen has found this technique useful in studying the effects of air pollutants on tree growth (22,24,25, 26). Jensen (22) used growth analysis to determine the response of eastern cottonwood, yellow-poplar and white ash to O_3 and/or SO_2. One-year-old seedlings were fumigated with 0.10 ppm O_3 and/or 0.20 ppm SO_2, 12 hours a day for 5 months. Relative growth rates and unit leaf rates were suppressed in cottonwood seedlings in all fumigation treatments and in yellow-poplar with the O_3 or SO_2 exposures. Leaf area ratio of the cottonwood seedlings fumigated with O_3 + SO_2 and yellow-poplar fumigated with SO or O_3 + SO_2 was dramatically decreased. There was no effect of any treatment on white ash growth, for any variable measured. In this study, Jensen suggested that growth of eastern cottonwood and yellow-poplar exposed to O_3 and/or SO_2 was altered by reducing the physiological efficiency of these seedlings.

In a later study, Jensen (24) investigated the growth of eastern cottonwood and silver maple exposed to O_3 by using growth analysis. One-year-old seedlings were exposed to 0.00, 0.10, 0.20 or 0.30 ppm O_3, 12 hours a day, for up to 60 consecutive days. Relative growth rates decreased with an increase in O_3 concentration, for both species. Unit leaf rate also exhibited a similar pattern with silver maple. Unit leaf rate decreased with eastern cottonwood treated with 0.10 ppm O_3, but increased thereafter with increasing O_3 concentrations. Leaf area ratio exhibited no consistent trend for either species tested.

Jensen (25) further evaluated the growth response of silver maple to O_3 alone or in combination with SO_2. Seedlings were exposed up to 60 consecutive days with 0.05, 0.10 or 0.20 ppm O_3, alone or in combination with 0.10 ppm SO_2, for 12 hours a day. Ozone applied at 0.20 ppm caused a significant reduction in relative growth rate and unit leaf rate, however, the addition of SO_2 increased the intensity of this response.

In a final experiment, Jensen (26) evaluated the response of yellow-poplar seedlings to intermittent air pollution exposures. Seedlings were treated with no pollutants, 0.10 ppm O_3, 0.10 ppm O_3 + 0.20 ppm SO_2, or 0.10 ppm O_3 + 0.20 ppm NO_2, applied once or twice a week, 12 hours a day, for up to 20 weeks. Seedling response was different when plants exposed once a week were compared with those treated twice weekly. Relative growth rates decreased with all pollutant treatments, however, unit leaf rate decreased for O_3-treated plants fumigated once a week and increased with plants to which O_3 was applied twice a week. The combined effects of these pollutants caused significant reductions in unit leaf rate for plants treated once or twice a week.

These findings (26) and the results from Jensen's previous experiments (22,24,25) demonstrate the effective use of growth analysis in determining tree response to air pollutants. Since trees are perennial in nature, shifts in rates of growth in response to air pollutants, reflected by growth analysis, may be more important than the determination of final biomass accumulation after a short-term fumigation experiment.

Air Pollution Effects on Foliar Gas Exchange in Trees

This section reviews the literature pertaining to the effects of air pollutants on foliar gas exchange in trees. Carlson (10) investigated the effect of high concentrations (0.50ppm) of O_3 and/or SO_2 on photosynthesis of sugar maple, white ash and black oak (*Q. velutina* Lam.). After 3 weeks fumigation SO_2 caused a 74, 43 and 7% reduction in photosynthesis in black oak, sugar maple and white ash, respectively. Ozone reduced photosynthesis 43, 55 and 6%, respectively, in black oak, sugar maple and white ash. The combined pollutant effect resulted in a decrease in photosynthesis of 44, 41 and 38%, respectively, in black oak, sugar maple and white ash after one week of fumigation. The combined effect of O_3 + SO_2 had a less than additive effect on net photosynthesis of black oak and sugar maple, but a greater than additive effect for white ash.

Lamoreaux and Chaney (33) investigated the effect of SO_2 on photosynthesis in silver maple. After fumigation with 1.0 ppm or 2.0 ppm SO_2 for 45 hours the net photosynthetic rate of excised silver maple leaves was reduced by approximately 50% for both pollutant treatments, compared with controls. The pollutant dose used in this experiment, however, was extremely high, making comparisons to a field situation difficult.

Keller (28) exposed grafts of Norway spruce to continuous SO_2 fumigation at 0.05, 0.10 or 0.20 ppm for 10 weeks. There was no reduction in net photosynthesis observed with 0.05 ppm SO_2, but continuous exposures to 0.10 or 0.20 ppm SO_2 resulted in significant depressions in net photosynthesis, with the intensity of this decrease increasing with increases in SO_2 concentration.

Barnes (4) examined the impact of O_3 on photosynthesis and respiration of eastern white pine, loblolly pine, slash pine (P. elliottii Var. elliottii Englem.) and pond pine (P. serotina Michx.). Seedlings were continuously exposed to either 0.05 or 0.15 ppm O_3 for up to 18 weeks. Net photosynthesis was significantly reduced in two-year-old eastern white pine by 0.15 ppm O_3. There were no effects of O_3 on net photosynthesis of loblolly, slash and pond pines. Dark respiration was however, significantly greater than controls in eastern white, slash and loblolly pines after 36 days of fumigation with 0.15 ppm O_3.

The effect of O_3 on photosynthesis of three-year-old ponderosa pine was investigated by Miller et al. (44). After 60 days of continuous fumigation with 0.15 ppm O_3 photosynthesis was suppressed by 25%. A 30 day exposure to 0.30 ppm resulted in a 67% reduction in photosynthesis. The results of this experiment and the previous two studies (4, 28) must however, be interpreted with caution since continuous fumigation with air pollutants for as many as 20 weeks is unrealistic to a field situation.

Yang et al. investigated the effects of short- (74) and long-term (75) exposures of O_3 on net photosynthesis, dark respiration and transpiration of three eastern white pine clones differing in sensitivity to this pollutant. In the short-term study (74), tolerant, intermediate and sensitive eastern white pine clones were exposed to 0.00, 0.10, 0.20 or 0.30 ppm O_3 for 4 hours. Photosynthesis was significantly depressed for O_3-treated plants in all three clones and this response was intensified with increasing O_3 concentrations. The reduction in photosynthesis was greatest for the sensitive clone and the least for the tolerant clone. The sensitive clone exhibited a significant decrease and significant increase in transpiration and dark respiration, respectively for O_3-treated plants. These results support the findings of Barnes (4) regarding an increase in dark respiration with O_3 fumigation.
The effects of long-term O_3 exposures on net photosynthesis and dark respiration in eastern white pine

were examined by Yang et al. (75). Tolerant, intermediate and sensitive clones were fumigated 4 hours a day, for 50 consecutive days with 0.00, 0.10, 0.20 or 0.30 ppm O_3. Net photosynthesis was significantly reduced by O_3 applied at all concentrations in the sensitive clone. Ozone applied at 0.20 or 0.30 ppm significantly suppressed photosynthesis for the intermediate clone, however, this effect was not statistically significant. Dark respiration was significantly inhibited by O_3 in the sensitive clone and the initiation of this response was coincident with the production of visible symptoms. These results regarding dark respiration were different than previously reported studies (4, 74); however, Yang et al. (74) and Barnes (4) did not observe visible foliar symptoms, which may account for the reported differences.

Reich (53) investigated the impact of low concentrations of O_3 on net photosynthesis, dark respiration and chlorphyll content in hybrid polar leaves. One hybrid poplar clone (207) was exposed to 0.03, 0.08 or 0.12 ppm O_3 for 6 hours, for 62 consecutive days. Net photosynthesis and leaf chlorophyll content decreased and dark respiration increased with increasing O_3 concentration. Response of these variables became apparent after 20 days exposure. These results are similar to previous reported studies (4, 74) regarding an increase in dark respiration in response to air pollution exposure, however, Reich did not report whether or not visible symptoms occurred in his study.

Reich and Amundson (54) studied the effects of O_3 and simulated acid rain on the photosynthetic response of four tree species and three crop species. Only the photosynthetic response of trees will be discussed in this review. Ozone was applied for 7 hours at variable concentrations, ranging from 0.02 to 0.14 ppm, depending upon the tree species, 5 to 7 days a week, for 7 to 12 weeks. In all four tree species examined, eastern white pine, sugar maple, hybrid poplar and northern red oak, O_3 caused a linear reduction in net photosynthesis with increasing concentrations. No visible injury was observed for any tree species tested. A linear decrease in stomatal conductance occurred with increasing O_3 concentration only for hybrid poplar. Intermittent exposures for up to 10 weeks to simulated acid rain (pHs between 5.6 to 3.0) resulted in no significant effect on net photosynthesis for northern red oak, hybrid poplar or sugar maple. A significant linear increase in photosynthesis occurred with increasing rain acidity for eastern white pine. These results re-enforce those of others (35,73) pertaining to increases in eastern white pine growth with increasing rain acidity. Reich and Amundson (54) observed no significant interaction between acid rain and O_3 related to the photosynthetic response, for any tree species examined.

Neufeld et al. (46) investigated the effects of simulated acid rain on the foliar gas exchange of American sycamore, black locust, yellow-poplar and sweetgum seedlings. After 16 exposures to simulated rain ranging in pHs from 5.6-2.0 American sycamore exhibited a suppression of photosynthesis, which only occurred with the pH 2.0-treated plants. This was the only species examined which exhibited an alteration in photosynthesis related to rain pH. Stomatal conductance was significantly reduced by the pH 2.0 treatment in sweetgum. These results demonstrate that net photosynthesis and stomatal conductance were altered only at extremely low pHs and that these responses varied among species.

Effects of Air Pollutants on Photosynthate Allocation

Shifts in photosynthate allocation may be a more reliable indicator of air pollutant effects on tree growth than a change in biomass accumulation (22,24,25,26,-37,41). Research pertaining to this subject is presented in this section.

Constantindou and Kozlowski (14) investigated the metabolite content of four-month-old American elm fumigated with 0.90 ppm O_3 for 5 hours, 2.00 ppm SO_2 for 6 hours or a combination of the two pollutants for 5 hours. Exposure to all pollutant treatments resulted in a significant reduction in total non-structural carbohydrates and protein content of all plant parts, 24 hours after fumigation. Five weeks after fumigation, carbohydrate and protein content of new leaves (< 1 cm in length at time of fumigation) were comparable with controls indicating that their functional capacity had returned to normal. Carbohydrate and protein content of mature leaves of fumigated seedlings were not restored to normal levels five weeks after exposure. Although the combined effect of $O_3 + SO_2$ caused greater reductions in metabolites than single pollutants, their effect was less than additive.

Jensen (23) studied the root carbohydrate content and above ground dry weight of one-year-old green ash exposed to 0.50 ppm O_3, 8 hours a day, 5 days a week, for up to 6 weeks. Ozone-treated seedlings exhibited significant decreases in stem and leaf dry weight, and root starch and reducing sugar contents. He postulated that the change in root carbohydrate content and decreases in above ground biomass were directly related. During the first two weeks of the study, starch reserves for all seedlings were reduced, however, after this initial period,starch content in roots of non-fumigated seedlings remained consistent, but continued to be depleted in the O_3-treated plants. These decreases in starch reserves in the roots may have contributed to the observed decreases in biomass.

Tingey et al. (65) exposed one-week-old ponderosa pine to 0.00 or 0.10 ppm O_3, 6 hours a day, for 20 weeks to determine the metabolite levels in tops and roots. Ozone-treated plants contained high amounts of nitrogen and free amino acids in roots. Levels of soluble sugars, starch and phenols increased in tops and decreased in roots of plants to which O_3 was applied. The authors' concluded that the increase in carbohydrate and phenolic contents in the tops and coincident decreases of these metabolites in roots resulted from the retention of metabolites in foliage and subsequent unavailability for transport to the roots.

Wilkinson and Barnes (69) exposed eastern white and loblolly pine branches to 0.00, 0.10 or 0.20 ppm O_3 continuously, for up to 21 days to study the ^{14}C fixation patterns in these trees in response to air pollutants. The primary changes in ^{14}C distribution in branches, as a result of O_3 exposure, were a reduction in soluble sugars, an increase in sugar phosphates and an increase in free amino acids. These results indicate that O_3 affects the photosynthate distribution in branches of loblolly and eastern white pines, however, the duration of exposure was unrealistic compared to a field situation.

Lorenc-Pluncinska (37) exposed two-year-old SO_2 tolerant and susceptible families of Scots pine to 0.50 ppm SO_2, 6 hours a day, for 5 days. Directly after fumigation radioactively labelled $^{14}CO_2$ was applied and its uptake and translocation observed. Sulfur dioxide inhibited $^{14}CO_2$ uptake in sensitive and tolerant families by 24 and 8%, respectively. Transport of ^{14}C was inhibited by 100% in both groups of plants, as indicated by the lack of radioactively labelled carbon in the stems and roots. The amount of ^{14}C retained in the foliage was 27 and 38% greater for the SO_2-treated tolerant and sensitive families, respectively. The amount of ^{14}C transported to the stems and roots was decreased dramatically, especially for the sensitive family where only 5% of the ^{14}C was translocated to the stems and roots in fumigated seedlings.

McLaughlin et al. (41) found similar results with oxidant stressed eastern white pines growing near Oak

Ridge, Tennessee. Sensitive, intermediate and tolerant 25-year-old trees were used in this study. The fate of radioactively labelled ^{14}C was followed after applying $^{14}CO_2$ to foliage four times during the growing season. The foliage and branches of oxidant sensitive trees retained significantly more photosynthate than intermediate or tolerant trees indicating that export of photosynthate to the stems and roots of sensitive trees was reduced.

LITERATURE CITED

1. Abrahamsen, G., R. Horntvendt and B. Tveite. 1976. Impacts of acid precipitation on coniferous forest ecosystems. pp. 991-1009, In 1st Internat. Symp. Acid Precip. and the For. Ecosyst., L. S. Dochinger and T. A. Seliga eds, USDA For. Serv., Upper Darby, PA.

2. Anonymous. 1965. Silvics of Forest Trees of the United States. USDA Agric. Handb. No. 271.

3. Altshuller, A. P. 1976. Regional transport and transformation of sulfur dioxide to sulfates in the U. S. J. Air Pollut. Contr. Ass. 26:318-324.

4. Barnes, R. L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut. 3:123-138.

5. Beck, D. E. and Della-Bianca. 1981. Yellow-poplar: characteristics and management. USDA For. Serv. Agric. Handb. No. 583.

6. Benoit, L. F., J. M. Skelly, L. D. Moore and L. S. Dochinger. 1983. Radial growth reduction in 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.

7. Blackman, V. H. 1919. The compound interest law and plant growth. Ann. Bot. 33:353-360.

8. Briggs, G. E., F. Kidd and C. West. 1920. A quanti tative analysis of plant growth; part I. Ann. Appl. Biol. 7:353-360.

9. Briggs, G. E., F. Kidd and C. West. 1920. A quantit ative analysis of plant growth; part II. Ann. Appl. Biol. 202-223.

10. Carlson, R. W. 1979. Reduction in the photosynthetic rate of Acer, Quercus and Fraxinus species caused by sulphur dioxide and ozone. Envron. Pollut. 18:159-170.

11. Chrenk, H. V. and P. Spaulding. 1909. Diseases of deciduous forest trees. USDA Bureau of Plant Indus. 149, 85 pp.

12. Cogbill, C. V. 1977. The effect of acid precipitation on tree growth in eastern North America. Water, Air, Soil Pollut. 8:89-93.

13. Constantinidou, H. A. and T. T. Kozlowski. 1979. Effects of sulfur dioxide and ozone on Ulmus americana seedlings. I. Visible injury and growth. Can. J. Bot. 57:170-175.

14. 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.

15. Davis, D. D. and R. G. Wilhour. 1976. Susceptibility of woody plants to sulfur dioxide and photochemical oxidants. USEPA Publ. No. EPA-600/3-76-102, Corvalis, OR.

16. Duchelle, S. F., J. M. Skelly and B. I. Chevone. 1982. Oxidant effects on forest tree seedlings in the Appalachian Mountains. Water, Air, Soil Pollut. 16:363-373.

17. Evans, L. S., G. R. Hendrey, G. L. Stensland, D. W. Johnson and A. J. Francis. 1981. Acidic precipitation: considerations for an air quality standard. Water, Air, Soil Pollut. 16:469-509.

18. Hayes, E. M. and J. M. Skelly. 1977. Transport of ozone from the northeastern U. S. into Virginia and its effects on eastern white pines. Plant Dis. Rep. 61:778-782.

19. Hedgecock, G. G. 1914. Injury by smelter smoke in southern Tennessee. J. Wash. Acad. Sci. 4:70-71.

20. Hunt, R. 1982. Plant Growth Curves. Univ. Park Press, Baltimore, MD.

21. Jensen, K. F. 1973. Response of nine forest tree species to chronic ozone fumigation. Plant Dis. Rep. 57:914-917.

22. Jensen, K. F. 1981. Air pollutants affect the relative growth rate of hardwood seedlings. USDA For. Serv. Res. Pap. NE-470.

23. Jensen, K. F. 1981. Ozone fumigation decreased the root carbohydrate content and dry weight of green ash seedlings. Environ. Pollut. 26:147-152.

24. Jensen, K. F. 1982. An analysis of the growth of silver maple and eastern cottonwood exposed to ozone. Can. J. For. Res. 12:420-424.

25. Jensen, K. F. 1983. Growth relationship in silver maple fumigated with O_3 and SO_2. Can. J. For. Res. 13:298-302.

26. Jensen, K. F. 1985. Response of yellow poplar seedlings to intermittent fumigation. Environ. Pollut. (series A) 38:183-191.

27. Karnosky, D. F. and K. C. Steiner. 1981. Provenance and family variation in response of _Fraxinus americana_ and _F. pennsylvanica_ to ozone and sulfur dioxide. Phytopathology 71:804-807.

28. Keller, T. The effect of a continuous springtime fumigation with SO_2 on CO_2 uptake and structure of annual ring in spruce. Can. J. For. Res. 10:1-6.

29. Krause, G. H. M., K. D. Jung and B. Prinz. 1984. Forest effects in West Germany. pp. 297-332. _In_: Sym posium on Air Pollution and the Productivity of the Forest. Izzak Walton Leaque of America, Arlington, VA.

30. Kress, L. W. 1978. Growth impact of O_3, SO_2 and NO_2 singly and in combination on loblolly pine (_Pinus taeda_ L.) and American sycamore (_Plantanus occidentalis_ L.). Ph.D. Diss. Virginia Polytechnic Institute and State University, Blacksburg, VA, 201pp.

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

32. Kress, L. W., J. M. Skelly and K. H. Hinkelmann. 1982. Growth impact of O_3, NO_2 and/or SO_2 on _Plantanus occidentalis_. Agric. and Environ. 7:265-274.

33. Lamoreaux, R. J. and W. R. Chaney. 1978. Photosyn thesis and transpiration of excised silver maple leaves exposed to cadmium and sulphur dioxide. Environ. Pollut. 17:259-268.

34. Ledig, F. T. 1974. Concepts of growth analysis, pp. 166-182. In Proceed. 3rd North American For. Biol. Workshop, C. P. P. Reid and G. H. Fechmer, eds., Colorado State Univ, Fort Collins, CO.

35. Lee, J. J. and D. E. Weber. 1979. The effect of simulated acid rain on seedling emergence and growth of eleven woody species. For. Sci. 25:393-398.

36. Likens, G. E. and T. J. Butler. 1982. Recent acidification of precipitation in North America. Atmos. Environ. 15:1103-1109.

37. Lorenc-Plucinska, G. 1984. The uptake and translocation of ^{14}C in Scots pine seedlings of differing susceptibility to sulphur dioxide. Eur. J. For. Path. 14:310-318.

38. 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 O_3, SO_2 and NO_2. Can. J. For. Res. 14:150-153.

39. Matziris, D. I. and G. Nakos. 1978. Effect of simulated "acid rain" on juvenile characteristics of Aleppo pine (Pinus halepensis Mill.). For. Ecol. Mange. 1:267-272.

40. 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.

41. McClenahen, J. R. and L. S. Dochinger. 1981. Tree seedling growth and leaf element accumulation in open-top-chambers near and urban-industrial area. Can. J. For. Res. 11:274-280.

42. McLaughlin, S. B., R. K. McConthy, 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.

43. Miller, P. R. (ed.). 1977. Photochemical oxidant air pollutant effects on a mixed conifer forest eco system. USEPA Publ. No. EPA-600/3-77-104, Corvalis, OR, 338 pp.

44. Miller, P. R., J. R. Parmeter, Jr., B. H. Flick and C. W. Marting. 1969. Ozone dosage response of ponderosa pine seedlings. J. Air Pollut. Cont. Ass. 19:435-438.

45. Mooi, J. 1980. Influence of ozone on growth of two poplar cultivars. Plant Dis. 64:772-773.

46. Neufeld, H. S., 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.

47. Patton, R. L. 1981. Effects of ozone and sulfur dioxide on height and stem specific gravity of *Populus* hybrids. USDA For. Serv. Res. Pap. NE-471.

48. Phillips, S. O. 1975. Growth loss of loblolly pine (*Pinus taeda* L.) white pine (*P. strobus* L.) and sycamore (*Plantus occidentalis* L.) proximal to a periodic source of air pollution. M. S. Thesis. Virginia Polytechnic Institute and State University. Blacksburg, VA, 109 pp.

49. Phillips, S. O., J. M. Skelly, and Burkhart. 1977. Growth flucuations of loblolly pine (*Pinus taeda* L.) proximal to a periodic source of air pollution. Phytopathology 67:716-720.

50. Phillips, S. O., J. M. Skelly and H. E. Burkhart. 1977. Inhibition of growth in asymptomatic white pine exposed to fluctuating levels of air pollution. Phytopathology 67:721-725.

51. Pratt, G. C., R. C. Hendrickson, B. I. Chevone, D. A. Christopherson, M. V. O'Brien and S. V. Krupa. 1983. Ozone and oxides of nitrogen in the rural upper-midwestern U. S. A. Atmos. Environ. 17:2013-2023.

52. Raynal, D. J., J. R. Roman and W. M. Eichenlaub. 1982. Response of tree seedlings to acid precipitation. I. Effect of substrate acidity on seed germina

tion. Environ. Expt. Bot. 22:377-383.

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

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

55. 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. (Series A) 39:39-51.

56. Reich, P. B., J. P. Lassoie and R. G. Amundson. 1984. Reduction in growth of hybrid poplar following field exposure to low levels of O_3 and (or) SO_2. Can. J. Bot. 62:2835-2841.

57. Roberts, T. M. 1985. Effects of air pollutants on agriculture and forestry. Atmos. Environ. 18:629-652.

58. Skeffinton, R. A. and T. M. Roberts. 1985. The effects of ozone and acid mists on Scots pine sap lings. Oecologia 65:201-206.

59. Skelly, J. M., L. D. Moore and L. L. Stone. 1972. Symptom expression of eastern white pine located near a source of oxides of nitrogen and sulfur dioxide. Plant Dis. Rep. 56:3-6.

60. Smith, W. H. 1981. Air Pollution and Forests. Springer-Verlag, New York, 379 pp.

61. Smith, W. H. 1985. Forests and air quality. J. For. 83:84-92.

62. Steiner, K. C. and D. D. Davis. 1979. Variation among Fraxinus families in foliar response to ozone. Can. J. For. Res. 9:106-109.

63. Stone, L. L. and J. M. Skelly. 1974. The growth of two forest tree species adjacent to a periodic source of air pollutants. Phytopathology 64:773-778.

64. Tamm, C. O., G. Wiklander and B. Popovic. 1977.

Effects of application of sulphuric acid to poor pine forests. Water, Air, Soil Pollut. 8:75-87.

65. Tingey, D. T., R. G. Wilhour and C. Standley. 1976. The effect of chronic ozone exposures on the metabolite content of ponderosa pine seedlings. For. Sci. 22:234-241.

66. Tveite, B. and G. Abrahamsen. 1980. Effects of artificial acid rain on the growth and nutrient status of trees. pp. 305-318, In Effects of Acid Precipitation on Terrestrial Ecosystems. T. C. Huchinson and M. Havas, eds. Plenum Press, New York.

67. Wilhour, R. G. 1971. The influence of ozone on white ash (Fraxinus americana L.). Penn. St. Univ. Cent. Environ. Stud. No. 188-71.

68. Wilhour, R. G. and G. E. Neely. 1976. Growth response of conifer seedlings to low ozone concentrations. pp. 635-645, In Proc. Int'l Conf. Photo. Oxidant Pollut. and its Cont. USEPA Rep. No. 300/377-010b. 1169 pp.

69. Wilkinson, T. G. and R. L. Barnes. 1973. Effects of ozone on $^{14}CO_2$ fixation patterns in pines. Can. J. Bot. 51:1573-1578.

70. Wood, F. A. and J. B. Coppolino. 1972. The influence of ozone on deciduous tree species. Mitt. Forstl. Bundesversuchanst., Win. 97:233-253.

71. Wood, F. A. and D. D. Davis. 1969. Sensitivity to ozone determined for trees. Sci. Agric. 17:4-5.

72. Wood, T. and F. H. Bormann. 1974. The effects of an artificial acid mist upon the growth of Betula alleghaniensis Britt. Environ. Pollut. 7:259-268.

73. 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.

74. Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983. Effects of short-term ozone exposure on net photosynthesis, dark respiration and transpiration of three eastern white pine clones. Environ. Intn't.

9:265-269.

75. Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983. Effects of long-term exposure on photosynthesis and dark respiration of eastern white pine. Environ. Sci. Tech. 17:371-373.

CHAPTER I

GROWTH RESPONSE OF YELLOW-POPLAR (LIRIODENDRON TULIPIFERA L.) SEEDLINGS TO OZONE, SULFUR DIOXIDE, AND SIMULATED ACIDIC PRECIPITATION, ALONE AND IN COMBINATION

INTRODUCTION

Ozone (O_3) has been considered as the most important phytotoxic air pollutant in the eastern United States (32), and has been reported to cause significant reductions in plant growth and productivity, even in the absence of macroscopic foliar symptoms (8,9). Sulfur dioxide (SO_2) and oxides of nitrogen (NO_x) can be harmful to plants but are also precursors to secondary pollutants (O_3 and acid rain), and can be transported long distances from urban to rural or forested areas (1,13). Recently much concern has developed over the possible increase in acidity of precipitation due to anthropogenic inputs into the atmosphere and the potential detrimental effects of this acidity on terrestrial ecosystems (11,26).

Within the last 10-20 years several forest tree species have been observed to be in rapid state of decline (decreases in radial growth, dieback, mortality, etc.) both in Europe (31,35) and North America (20). Acid rain, alone or in combination with gaseous pollutants (O_3, SO_2, NO_x), heavy metals and drought, have been implicated as possible contributing factors to this decline (2,21,28,38). However, the combined effects of these stresses on tree growth have not yet been investigated.

The purpose of this study was to examine the effects of O_3, SO_2 and simulated acid rain, alone and in combination, on the growth and productivity of yellow-poplar (Liriodendron tulipifera L.) seedlings under laboratory exposure conditions. The effects of O_3 and SO_2 were also compared when these pollutants were applied before or after a rain event.

Yellow-poplar was selected for this study since it is one of the major commercial hardwood species in eastern North America, ranging from southern New England, west through southern Ontario and Michigan and south to Louisiana and Florida (4). This species has been reported as being sensitive to O_3 (7,8,18), SO_2 (19) and mixtures of O_3 and SO_2 (27) and slightly sensitive to acid rain (25).

MATERIALS AND METHODS

Plant Material

To minimize variability in any genotypically controlled response to air pollution stress, yellow-poplar seed was collected from a single tree on the campus of Virginia Polytechnic Institute and State University.

Seedlings collected from this source are reported as being intermediate in sensitivity to O_3 and SO_2 singly, and sensitive to mixtures of these pollutants (27).

Stratified seeds were surface sterilized with 0.2% sodium hypochlorite, rinsed in tap water and sown in flats containing vermiculite. Seedlings at 5 wk postemergence were transfered to 10-cm diameter plastic pots containing a 2:2:1 (v/v/v) mixture of steam pasturized Weblite ^{R} (Weblite Corp., Inc., Roanoke, VA 24061), vermiculite and peat moss with 1 g 14-14-14 Osmocote (Sierra Chemical Co., Milpitas, CA 95035) per pot. Peter's water soluble general purpose 20-20-20 (N-P-K) fertilizer (W. R. Grace & Co., Fogelsville, PA 18051) was added to the planting medium at a rate of 12g/1000 ml deionized H_2O (approx. 100 ml solution/pot) during week 8 postemergence to ensure adequate plant nutrition. Seedlings were grown in a greenhouse filtered with charcoal to reduce ambient O_3 concentrations, under a temperature range of $20-35^{o}C$, and supplemented with 21-24 Klux illumination (approx. 400 $umol/m^2/sec$ photon flux density) to maintain a 14-hr photoperiod. Pollutant and rain exposures began when the plants were 9 weeks old.

Simulated Rain and Gaseous Pollutant Exposures

Simulated acid rain was applied to the plants using a simulator developed on the principle of droplet formation from needle tips (5). Rain solutions with ionic concentrations similar to the average ambient rainfall in southwestern Virginia (32) were prepared as previously decribed by Chevone et al. (5). See Table A-1 for ion concentrations. The pH's of solutions were adjusted to 5.6, 4.3 or 3.0 by the addition of 1M NaOH or 1M H_2SO_4. Rain was applied to the seedlings at a rate of 0.75 cm/hr for 1 hr, 2 times weekly for 6 wk, either just before or just after fumigation with gaseous pollutants.

Seedlings were exposed to O_3 and/or SO_2, 4hr daily (1200-1600), 5 days a week, for 6 wk in 12 continuously stirred tank reactors (CSTRs) (13). Pollutant treatments included charcoal-filtered air (controls), 0.10 ppm O_3, 0.08 ppm SO_2, and 0.10 ppm O_3 + 0.08 ppm SO_2. Environmental conditions within the CSTRs were maintained at 30 $\pm$ 2^oC, 70 $\pm$ 5% RH, and 350-400 umol/m^2/sec photon flux density.

The concentrations of O_3 and SO_2 were realistic since O_3 episodes commonly occur in the eastern United States having durations of 1-3 days, with peak hourly means often exceeding 0.10 ppm (29). Concentrations of SO_2 often exceed 0.10 ppm for several hours in the vicinity of coal-fired power plants during atmospheric inversions. While elevated levels of $O_3 + SO_2$ do not commonly occur naturally they represent the effect of pollutant mixtures on plant growth and in several cases have been shown to have a more deleterious effect on tree growth than O_3 or SO_2 alone (23,27).

Ozone was generated with a Model T-408 Welsbach Laboratory Ozonator (Welsbach Ozone Systems Corp., Philadelphia, PA 19129) and monitored with a Bendix Model 8002 Chemiluminescent Ozone Analyzer (Bendix Corp., Lewisburg, WV 24901). Periodically, the monitor was calibrated with a Photocal 3000 Automated Calibration System (Columbia Scientific Industries, Austin, TX 78776), according to USEPA quality assurance methods.

Sulfur dioxide was delivered to the CSTRs from commercial bottled SO_2 diluted in nitrogen (1.03% SO_2) and monitored using a Model 43 Pulsed Fluorescent Ambient Sulfur Dioxide Analyzer (Thermo Electron Corp., Hopkinton, MA 01748), and calibrated with a Bendix Dynamic Calibration System utilizing a SO_2 permeation tube.

Measurement of Plant Response

Every 7 days, starting with the first day of fumigation, seedling height was measured from the cotyledonary node to the base of the terminal bud. Cumulative increases, measured at 1-6 wk, were calculated for each treatment. Seedlings were also examined periodically for symptoms of foliar injury.

Leaf area (ALA) was determined by measuring the length and width (LW) of each leaf at 2-wk intervals, begining with the first day of fumigation. One hundred leaves in all ontogenilogical stages were selected, LW determined, and ALA measured using a Li-Cor Model LI-3000 Portable Area Meter (Li-Cor, Ltd., Lincoln, NE 68504). A regression equation relating ALA to LW was then developed: $Y = 1.21 + 0.71X$, $R^2 = 0.98$, where $Y =$ ALA and $X =$ LW. Using this equation we estimated plant leaf area at each time of measurement. Cumulative increases, measured at 2, 4 and 6 wk, were then calculated for each treatment.

Ten seedlings were harvested initially (9 wk postemergence) and dried at $60^{o}C$ for 36 hr and mean root, stem, and leaf dry weights were determined (0.08, 0.08, 0.14 g, respectively). At the end of the experiment, all seedlings were harvested and dry weights determined as before. Chlorophyll content of the leaf at the 6th node from the cotylendenary node was determined at the termination of the study for a pooled sample of one leaf from each of two trees (3 samples per treatment combination) using the ethanol extraction procedure of Knudson et al. (22).

Relative growth responses were calculated using classical growth analysis (14). The following growth parameters were calculated: mean relative growth rate (RGR) for each plant component= [ln(final dry wt.)-ln(mean initial dry wt.)]/wk; leaf area ratio (LAR)= leaf area/plant dry wt; leaf wt ratio (LWR)= leafwt/plant dry wt; specific leaf area (SLA)=leaf area/leaf dry wt; root/shoot ratio (RSR)= root dry wt/(stem + leaf dry wt); and mean unit leaf rate (ULR), i.e., net assimilation rate = [(final plant dry wt- initial mean dry wt)] X [ln(final leaf area)- ln(initial leaf area)]/ (final leaf area-initial leaf area).

Experimental Design and Data Analysis

The overall design for fumigation exposures was a modified split-plot, replicated 3 times (3 blocks of 4 CSTRs each). Three blocks were used to determine the variation between CSTR's due to differences in temperature, RH, and light intensity. Whole plot treatments were gaseous pollutants, with one pollutant treatment/CSTR. Sub-plot treatments were a 3 X 2 factorial combination of three simulated rain solutions and two application times of rain. Treatments were replicated 2 times/CSTR (12 trees/CSTR, 36 trees/pollutant treatment, 48 trees/rain pH, 72 trees/time of rain application, 144 total trees).

Data were analyzed by analysis of variance (ANOVA). Analysis of covariance (COANOVA) was performed to determine if the the data were influenced by a significant covariate (initial seedling height or initial leaf area), and the data were adjusted where neccessary. Whole plot treatments were partitioned into the following three orthogonal constrasts (32): 1) (O_3 - control) + (SO_2 - control) vs (O_3 + SO_2) - control; 2) O_3 + SO_2 vs control; 3) O_3 vs SO_2. The first contrast is a test of the additivity of O_3 + SO_2 exposure on plant response. If the constrasts were equal (no significant differences), the combined effects were additive. If the constrasts were significantly different, then inspection of the sign of this difference indicated whether the combined effects were greater or less than additive. Treatment variance for simulated rain treatments was partitioned into linear and quadratic components. Significant interactions were also partitioned into orthogonal contrasts.

RESULTS

Individual Treatment Effects

The mean squares for dry matter production, height increase, leaf area increase, chlorophyll content, and selected plant growth responses (RGR, ULR, etc.) are presented in Tables 1 and 2. A significant difference

Table 1. Mean squares and levels of significance of analysis of variance for dry matter, height increase leaf area increase and leaf chlorophyll content of 15-week-old yellow-poplar exposed to gaseous air pollutants and simulated acid rain under laboratory conditions.

		Mean squares[a]					
		Dry matter production					
Source of variation	df	stem	root	leaf	HTI	LAI	CHLOR[b]
Block	2	0.019	0.006	0.016	546.035	5135.204	46.374
Pollutant	3						
Additive[c]	1	0.123	0.337	0.133	6142.225	24520.768	56.747
Control vs. O_3 + SO_2	1	1.299**	3.029**	2.030**	25878.090*	131775.509	11.266
O_3 vs. SO_2	1	0.000	0.054	0.004	971.017	2183.085	69.353
Block x pollutant	6	0.036	0.068	0.102	12716.905	10079.167	10.749
Acidity of simulated rain	2						
Linear	1	0.016	0.144	0.005	1196.088	771.634	12.970
Quadratic	1	0.044	0.002	0.389**	1151.805	1502.062	6.129
Timing of rain application	1	0.400**	0.513**	0.343	11134.338**	25418.067**	1.402
Pollutant x acidity	6	0.030	0.151**	0.130	2504.189	11413.833**	40.458**
Pollutant x time	3	0.026	0.043	0.075	2295.543	8762.755	6.390
Acidity x time	2	0.006	0.002	0.055	530.972	5129.500	0.019
Pollutant x acidity x time	6	0.014	0.049	0.057	842.443	5936.862	1.488
Covariate[d]	1	1.297**	2.259**	1.616**	32606.033**	101070.081**	NA
Error	111	0.032	0.014	0.098	1298.976	5806.523	12.364

[a] Calculated F-values significant at the 0.05 or 0.01 levels are denoted by * or **, respectively. Pollutant x block interaction used as error to test for block and pollutant effects; HTI = total height increase (cm), LAI = total leaf area increase (cm^2), CHLOR = total chlorophyll content (µg/mg dry weight); total dry weight mean squares have same levels of significance as those for stem dry matter production.

[b] Represents pooled values of one leaf from each of two plants per block, error df = 40; no covariate applied.

[c] Additive = (O_3 - control) + SO_2 - control) vs. (O_3 + SO_2) - control.

[d] Covariate for dry matter production and height increase was initial tree height; for leaf area increase covariate was initial leaf area.

Table 2. Mean squares and levels of significance of analysis of variance for selected plant growth responses of 15-week-old yellow-poplar exposed to gaseous air pollutants and simulated acid rain under laboratory conditions.

		Mean squares[a]					
Source of variation	df	RSR	LAR	LWR	SLA	$\overline{RGR}$	$\overline{ULR}$
Block	2	0.011	851.320*	0.004	1224.424	0.877*	5.908*
Pollutant	3						
Additive[b]	1	0.015	51.034	0.008*	2635.470	0.190	20.440**
Control vs. $O_3 + SO_2$	1	0.119*	3826.208**	0.059**	1752.864	3.519**	0.243
O_3 vs. SO_2	1	0.019	6.692	0.003	93.285	0.003	0.751
Acidity of simulated rain	2						
Linear	1	0.023	6.442	0.001	1282.567	0.003	0.068
Quadratic	1	0.041	247.077	0.001	7813.976	0.291	1.198
Timing of rain application	1	0.003	21.283	0.004	4566.505	0.390*	3.670*
Block x pollutant	6	0.008	171.305	0.001	2584.864	0.107	0.751
Pollutant x acidity	6	0.018	202.740	0.003	5406.256	0.328**	1.997*
Pollutant x time	3	0.267	646.491	0.005	2711.017	0.037	0.318
Acidity x time	2	0.004	914.596	0.003	1144.455	0.087	0.453
Pollutant x acidity x time	6	0.248	244.221	0.006	4581.235	0.051	0.147
Error	112	0.012	358.414	0.003	5451.396	0.102	0.680

[a] Calculated F-values significant at the 0.05 or 0.01 levels are denoted by * or **, respectively; block x pollutant interaction is used as error to test for block and pollutant effects; RSR = root/shoot (above ground biomass) ratio (w/w); LAR = leaf area ratio (cm^2/g); LWR = leaf weight/total weight (w/w); SLA = specific leaf area (cm^2/g); RGR = mean relative growth rate (per week); ULR = mean unit leaf rate ($mg/cm^2/wk$).

[b] Additive = (O_3 - control) + (SO_2 - control) vs. ($O_3 + SO_2$) - control.

between the combined effects of O_3 + SO_2 vs control treatments across rain pHs occurred for all variables measured, except chlorophyll content and SLA. The combined effect of O_3 and SO_2 was additive in nature for all variables measured, except for root dry weight and LWR, where a greater than additive effect was observed (Tables 1 and 2).

Initial seedling height was found to be a significant covariate ($p= 0.01$) for dry matter production and total stem elongation and initial leaf area a significant covariate ($p= 0.01$) for leaf area increase (Table 1). These growth variables were adjusted for the appropriate covariate (Table 3 and Figs. 1 and 2).

Ozone + SO_2 caused a 40, 40 and 26% reduction in stem, root and leaf dry weights, respectively, compared with controls (Table 3). A similar reduction (34%) in biomass was observed for total plant dry weight. A 12% increase in LAR and LWR resulted from O_3 + SO_2 fumigation compared with the controls.

The cumulative shoot height growth curve of yellow-poplar seedlings exposed to either 0.10 ppm O_3 or 0.08 ppm SO_2 was similar to non-fumigated seedlings (Fig. 1). However, a 26% reduction in total height increased occurred with the O_3 + SO_2 treatment compared with

Table 3. Total dry weight, relative growth rate and leaf characteristics of 15-week-old yellow-poplar for pollutant, acidity of rain and timing of rain application, across all other treatments.[a]

Characteristic[b]	Pollutant				Rain pH			Time of rain application	
	Control	O_3	SO_2	$O_3 + SO_2$	5.6	4.3	3.0	Before	After
Stem dry wt (g)	0.66	0.58	0.59	0.39	0.56	0.58	0.53	0.50	0.61
Root dry wt (g)	1.00	0.94	0.88	0.60	0.90	0.85	0.82	0.80	0.92
Leaf dry wt (g)	1.31	1.19	1.20	0.97	1.13	1.24	1.12	1.12	1.21
RSR	0.52	0.52	0.48	0.44	0.52	0.47	0.49	0.49	0.50
LAR (cm^2/g)	108.35	116.52	117.13	122.93	116.90	114.38	117.42	116.62	115.85
LWR	0.44	0.45	0.46	0.50	0.46	0.47	0.46	0.47	0.46
SLA (cm^2/g)	257.21	261.97	259.70	247.34	265.42	246.14	258.11	262.19	250.92

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[a] Statistical analyses and significant responses as reported in Tables 1 and 2. Dry weight for individual plant parts is not included due to a significant pollutant x rain pH interaction for root dry weight.

[b] RSR = root/above ground biomass; LAR = leaf area ratio; LWR = leaf weight ratio; SLA = specific leaf area; dry weights adjusted for initial height as a covariate.

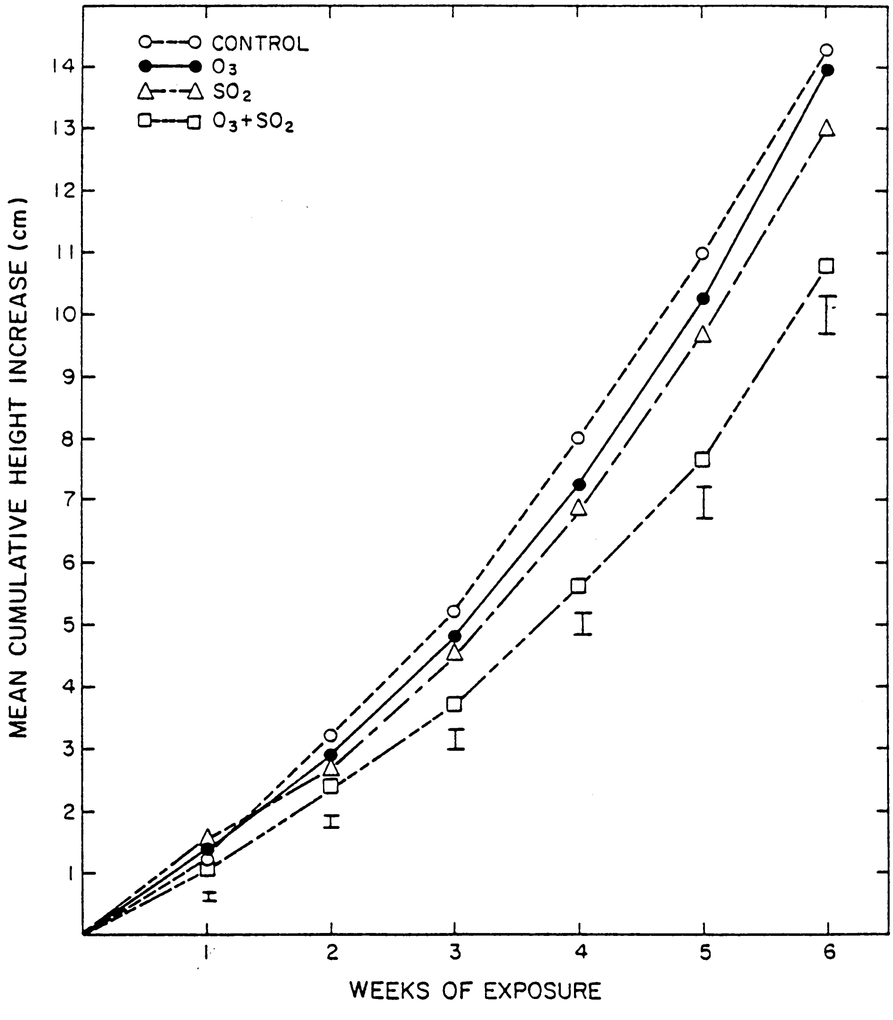

Figure 1. Cumulative shoot height increase (cm) of yellow-poplar seedlings during 6 wk. of fumigation with ozone and sulfur dioxide. Vertical bars represent standard errors. Data are adjusted for initial tree height.

controls. A significant difference in shoot elongation between O_3 + SO_2 and all other treatments became apparent after 3 wk of exposure.

A significant quadratic effect was observed in leaf dry weight for rain pH treatments across all other treatments (Table 1); the mean values for leaf dry weight were 1.13, 1.24 and 1.12 g for pH's 5.6, 4.3 and 3.0, respectively (Table 3). No other significant rain pH treatment effects were observed.

Visible foliar symptoms developed on plants exposed to O_3 and O_3 + SO_2, but not to SO_2 alone. Symptoms first appeared on the oldest leaves 3 wk after treatment initiation, and were characterized by purple stippling on the adaxial leaf surface. Foliar symptoms were observed on 14 and 75% of the plants (n= 36) in the O_3 and O_3 + SO_2 treatments, respectively. There were no visible foliar symptoms attributable to the acidity of the rain solutions at any pH level.

The timing of the rain application relative to gaseous pollutant exposure significantly affected dry matter production, shoot elongation, leaf area increase, RGR and ULR (Tables 1 and 2). Regardless of pollutant and rain pH treatment, there was approximately a 10-15% reduction for these growth variables if rain was applied to seedlings immediately before fumigation with gaseous pollutants (wet leaf surface) compared with plants to which rain was applied after fumigation (Table 3).

Combined Effects of Rain and Gaseous Pollutants

A significant pollutant X rain pH interaction was observed for root dry weight, leaf area increase, chlorophyll content, RGR and ULR (Tables 1 and 2). To determine where the interaction occurred among treatments, the sums of squares were partitioned into the following six orthogonal contrasts using fixed levels of gaseous pollutants across either linear or quadratic trends of rain pH: 1) control (0.00 ppm) vs linear; 2) O_3 vs linear; 3) SO_2 vs linear; 4) O_3 + SO_2 vs quadratic; 5) O_3 vs quadratic; and 6) SO_2 vs quadratic.

Mean root dry weight exhibited a significant linear decrease as rain pH decreased from 5.6 to 3.0 for controls and O_3 treatments (Table 4). Dry weights were reduced 20 and 25% for controls and O_3 treatments, respectively, at pH 3.0 compared with pH 5.6. No other contrast was significant for this variable.

A significant linear decrease and significant linear increase in RGR and ULR was observed for the O_3 and SO_2 treatments, respectively, as rain pH decreased (Table 4). Relative growth rates decreased 32% and increased 19% for

Table 4. Mean root dry weight, relative growth rate ($\overline{\text{RGR}}$), unit leaf rate ($\overline{\text{ULR}}$), and total chlorophyll content of 15-week-old yellow-poplar for pollutant treatment by rain pH.

	Root dry weight (g)[a]			$\overline{\text{RGR}}$ (per week)			$\overline{\text{ULR}}$ (mg/cm^2/wk)			Total chlorophyll content (µg/mg/dry wt)		
	Rain pH											
Pollutant	5.6	4.3	3.0	5.6	4.3	3.0	5.6	4.3	3.0	5.6	4.3	3.0
Control	1.12	0.99	0.90*	0.38	0.39	0.37	2.47	2.62	2.29	16.1	16.7	13.6
O_3	1.10	0.88	0.82**	0.39	0.36	0.34**	2.74	2.12	1.65**	16.2	16.9	21.1*
SO_2	0.80	0.88	0.96	0.35	0.37	0.38*	1.60	2.21	2.35*	13.0	14.4	18.4*
$O_3 + SO_2$	0.55	0.65	0.60	0.30	0.32	0.31	1.12	1.65	1.43	16.6	13.5	12.9

[a] Dry weights adjusted for initial height as a covariate; Calculated F-values significant at the 0.05 or 0.01 levels are denoted by * or **, respectively.

the O_3 and SO_2 treatments, respectively, as rain pH decreased. There was a 40% decrease and 32% increase in ULR as rain pH decreased from 5.6 to 3.0 for O_3 and SO_2 treatments, respectively.

The mean cumulative leaf area increase at each rain pH within a gaseous pollutant treatment is graphically represented in Fig. 2. A linear decrease and linear increase was observed for the O_3 ($p= 0.01$) and SO_2 ($p= 0.05$) treatments, respectively, with decreasing rain pH. The total cumulative leaf area increase was reduced by 26% in the O_3 treatment at pH 3.0 compared with pH 5.6, and increased 23% in the SO_2 treatment between these pH's.

As the rain pH decreased from 5.6 to 3.0, there was a significant linear increase observed in total chlorophyll content for both the O_3 and SO_2 treatments (Table 4). Chlorophyll content increased from 16.2 (pH 5.6) to 21.1 (pH 3.0) ug/mg dry wt for the O_3 treatment, and increased from 13.0 (pH 5.6) to 18.4 (pH 3.0) ug/mg dry wt for the SO_2 treatment. Results were similar for chlorophyll a and b concentrations.

DISCUSSION

The results of this study demonstrate that the effect of pollutant combinations, whether O_3, SO_2 or acid rain, is generally more deleterious to yellow-poplar

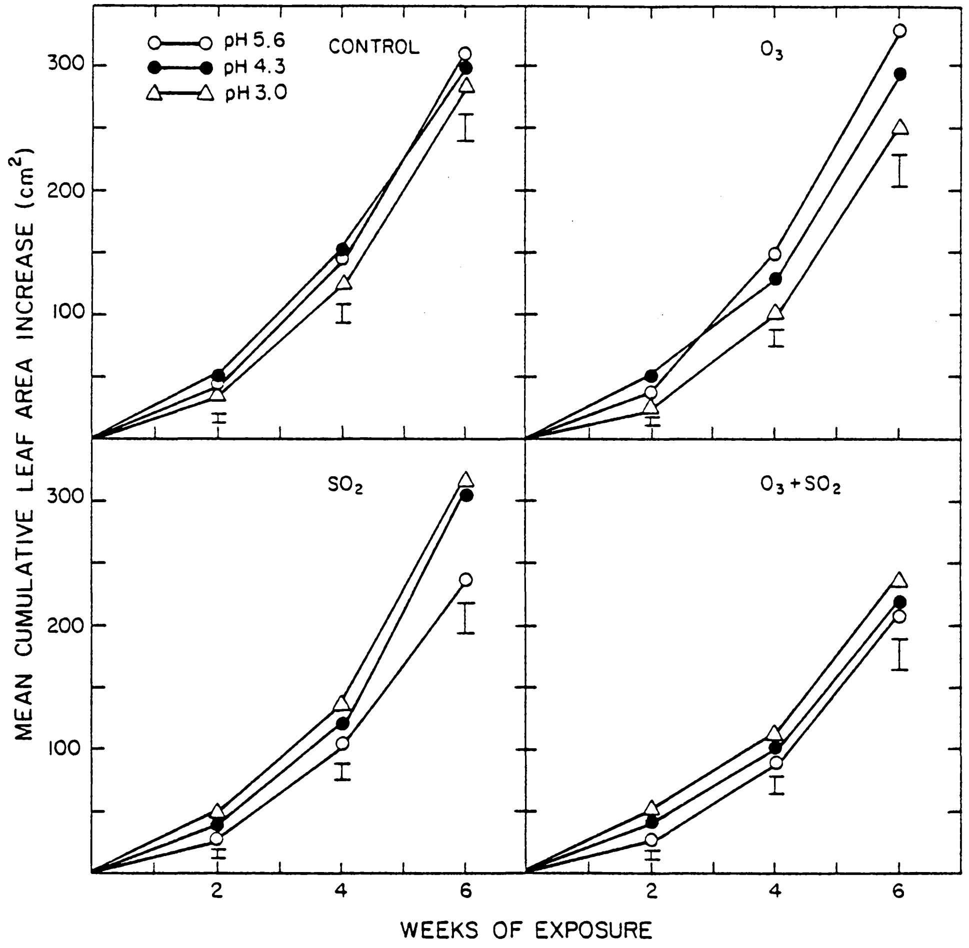

Figure 2. Cumulative leaf area increase (cm^2) of yellow-poplar seedlings during 6 wk. of fumigation for rain pH treatments by gaseous pollutant treatment. Vertical bars represent standard errors. Data are adjusted for initial area.

growth than single pollutant exposures. Mahoney et al. (27) reported that O_3 + SO_2 significantly reduced shoot elongation and dry matter production of yellow-poplar compared with plants fumigated with O_3 and SO_2 alone, or non-fumigated plants. Using the same half-sib family we conclude our data confirm the results of Mahoney et al. (27).

The combined effects of O_3 + SO_2, across all other treatments, for the majority of variables measure resulted in the most deleterious effect on plant growth and was additive in nature. Mahoney et al. (27) report a greater than additive response with O_3 + SO_2 for yellow-poplar growth, and Jensen (19) observed the effect of O_3 + SO_2 to be less than additive and reported yellow-poplar as being more sensitive to O_3 or SO_2 alone than in combination. Differences in seed source, duration of exposure and pollutant concentration, or tree age may have caused these discrepancies in results.

To demonstrate if air pollutants affected the growth and development of yellow-poplar seedlings, relative growth relationships, which can be used to compare plant growth rates, were calculated using classical plant growth analysis (15,24). Growth analyses divides growth into component processes and is a technique that is becoming more widely applied in air pollution research (19,30).

Root/shoot ratio (RSR), a measure of the change in root weight compared with shoot (above ground biomass) weight is very sensitive to environmental stresses (15). A significant decrease in RSR was observed for $O_3 + SO_2$ treated plants when compared with all other gaseous pollutant treatments. This indicates a disproportionate decrease in root dry matter compared with above ground biomass. Similar trends were observed when RSR was determined for root/stem and root/leaf dry weights. This reduction in RSR for $O_3 + SO_2$ treated plants may be the result of reduced photosynthesis, increased respiration or reduced translocation of photosynthate (3,34).

Leaf area ratio (LAR), an index of leafiness, is an important measure of plant growth (14). It expresses the proportion of assimilatory surface to respiratory mass and is composed of leaf weight ratio (LWR) and specific leaf area (SLA). Although leaf area, leaf weight, total dry weight and total height increase were significantly less for $O_3 + SO_2$ treated plants, LAR was significantly greater for $O_3 + SO_2$ treatments when compared with the controls. These results are contrary to most reports in the literature (19,30). Jensen (19) reported that LAR of yellow-poplar fumigated with SO_2 and O_3 + SO_2 was reduced significantly compared with controls, and concluded that the reduction in LAR was caused by early leaf senescence or early bud set. Leaf area ratio of soybeans [<u>Glycine max</u> (L.) Merr. cv. Davis] treated with pH 2.6 rain compared with pH's 3.4, 4.2 or 5.6 was significantly reduced and attributed to a reduction in SLA caused by leaf deformation (30). In our study, there were no significant differences in SLA between any treatments, but a significant increase in LWR for O_3 + SO_2 treated plants (Tables 2 and 3). These results indicate that O_3 + SO_2 fumigated plants were small (reduced height growth and leaf area), but maintained a full complement of leaves. No unusual amount of premature senescence was observed for these plants. These factors probably account for the difference in response between our study and those previously reported (19,30).

Relative growth rate (RGR) is a measure of plant productivity and expresses the efficiency of the plant as a producer of new material (15). The change in RGR among treatments reflects an influence of these multiple pollutant components on physiological processes in the plant. Relative growth rates were calculated for individual plant parts and the results were similar to those for total RGR. The linear decrease in RGR as rain acidity increased for O_3-treated plants was reflected by similar linear decreases in root growth and leaf area. This reduction in RGR indicated that the rate of dry matter production was reduced for the O_3-treated plants as the rain pH decreased and could result from a decrease in the photosynthetic rate or an increase in respiration (3), or a decrease in the translocation of photosynthate from the leaves to the roots (34). Tingey et al. (34) found that in _Pinus ponderosa_ Laws. ssedlings, exposed to low concentrations of O_3 for several months, assimilated substances remained in the needles and were not translocated to the roots. They concluded that an increase in assimilate retention in the needles resulted from the formation of glycosides that rendered the sugars unavailable for translocation to the roots.

Relative growth rate is a complex function and is equal to ULR X LAR. Unit leaf rate (ULR) is a measure of the efficiency of photosynthate that is used or stored and is a function not only of the rate of photosynthesis, but also the respiration rate (14,23). The reduction in leaf area that occurred with O_3 and rain treatments, as reflected by a decrease in RGR and ULR, would result in a subsequent decrease in root growth.

The linear increase in chlorophyll content for O_3 X acid rain exposure has not been reported previously in the literature. It appears from these results that chlorophyll biosynthesis is unaffected by this treatment.

The increased effect of O_3 exposure at low rainfall pH may be related to the solubility of O_3 in water under different conditions of acidity (29). In alkaline solutions O_3 is broken down rapidly, and is more soluble than O_2; however, in acidic solutions O_3 is considerably more stable.

The RGR for SO_2 treated plants increased linearly as the acidity of rain decreased and was reflected by a subsequent linear increase in root dry weight, chlorophyll content and leaf area. Root dry weight, RGR, ULR, leaf area and chlorophyll content of seedlings treated with SO_2 and pH 5.6 rain, however, were lower than controls and similar to seedlings treated with O_3 + pH 3.0 rain, suggesting an adverse effect of SO_2 at high pH.

The formation of HSO_3^- and SO_3^{2-} as pH inreases may account for the reduction in growth observed at high pH in our study. Although it is not known if SO_3^{2-} and HSO_3^- formation is similar in both plant cytoplasm and aqueous solution, this may be a possible explanation for the different effects observed with the three pH treatments.

The timing of rain application, irrespective of gaseous pollutant or rain pH treatment, had a significant effect on dry matter production, height increase, leaf area, RGR and ULR. These growth components were significantly less for plants that were treated with rain before fumigation with gaseous pollutants (wet leaf surface during fumigation) compared to plants to which rain was applied after fumigation. Elkiey and Ormrod (10) reported the severity of injury in Poa pratensis L. exposed to gaseous pollutants (O_3, SO_2, NO_2) was increased significantly by misting the plants twice daily with deionized H_2O for 5 min during the fumigation period. They determined that stomatal conductance increased in misted plants compared to untreated plants. Pinus virginiana Mill (6) and Fraxinus americana L. (39), preconditioned at high high humidity were more sensitive to O_3 than those preconditioned at a lower humidity. Relative humidity during fumigation was directly related to injury in both species; the higher the RH the greater the percent injury. These results suggest that water itself can enhance gaseous pollutant effects, probably through increased humidity at the leaf surface and subsequently, increased stomatal conductance.

This work represents one of only a few studies in the literature investigating the combined effect of gaseous pollutants and acid rain on plant growth (16,17, 30,36,37), and the only report concerning the effects of O_3, SO_2 and acid rain alone and in combination on tree growth. Although O_3 + SO_2 depressed plant growth more than any other treatment, the response to O_3 and SO_2 singly was depressed or stimulated as the acidity of rain increased.

The alteration of plant response to O_3 + acid rain is cause for concern. Ozone at 0.10 ppm alone in the laboratory has been shown to have little effect on yellow-poplar growth (19,23,27). In contrast to these laboratory studies, yellow-poplar seedlings growing under field conditions in Virginia, where O_3 was the predominant gaseous pollutant, had reduced growth rates (7). The average pH of ambient rainfall in the study area was 4.2 (32), but the pH of rain in Virginia has been reported as low as 3.30 for a single event.

The response of other plant species to gaseous air pollutants and acidic precipitation have also been observed to be different between field and laboratory experiments (6,8,11). Factors such as nutrient status, water availability, temperature and duration and fluctuations of pollutant concentrations under ambient conditions may also contribute to the discrepancy between field and laboratory results.

The potential exists for the interactive effects of acidic precipitation and gaseous pollutants (primarily O_3 in the eastern United States) to incite a reduction in yellow-poplar growth and productivity, as indicated by this study. Additional research using different seed sources, soil types, pollutant concentrations, tree ages, and field tests are neccessary, however, to determine if such effects are occurring in the natural forest ecosystem.

LITERATURE CITED

1. Altshuller, A. P. 1976. Regional transport and transformation of sulfur dioxide to sulfates in the U. S. J. Air Pollut. Contr. Assoc. 26:318-324.

2. Arnt, U., G. Seufert and W. Nobel. 1982. The contribution of ozone to the silver fir (Abies alba Mill.) disease complex-a hypothesis worth examining. Staub-Reinhalt. Luft. 42:243-247. (English trans.).

3. Barnes, R. L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut. 3:123-138.

4. Beck, D. E. and L. Della-Bianca. 1981. Yellow-poplar: characteristics and management. USDA For. Serv. Agric. Handb. 583, 91pp.

5. Chevone, B. I., Y. S. Yang, W. E. Winner, I. Storks-Cotter and S. J. Long. 1984. A rainfall simulator for laboratory use in acidic precipitation studies. J. Air Pollut. Contr. Assoc. 34:355-359.

6. Davis, D. D. and F. A. Wood. 1973. The influence of environmental factors on the sensitivity of Virginia pine to ozone. Phytopathology 63:371-376.

7. Davis, D. D., D. M. Umbach and J. B. Coppolino. 1981. Susceptibility of tree and shrub species and response of black cherry foliage to ozone. Plant Dis. 65:904-907.

8. Duchelle, S. F., J. M. Skelly and B. I. Chevone. 1982. Oxidant effects on forest tree seedlings in the Appalachian Mountains. Water, Air and Soil Pollut. 16:363-373.

9. Duchelle, S. F., J. M. Skelly, T. L. Sharick, B. I. Chevone, Y. S. Yang and J. E. Nellessen. 1983. Effects of ozone on the productivity of natural vegetation in a high medow of theShenandoah National Park of Virginia. J. Environ. Manag. 17:294-308.

10. Elkiey, T. and D. P. Ormrod. 1981. Sulphur and nitrogen nutrition and misting effects on the response of bluegrass to ozone, sulphur dioxide,

nitrogen dioxide or their mixture. Water, Air and Soil Pollut. 16:177-186.

11. Evans, L. S., G. R. Hendrey, G. L. Stensland, D. W. Johnson and A. J. Francis. 1981. Acidic precipitation: considerations for an air quality standard. Water, Air and Soil Pollut. 16:469-509.

12. Gravehorst, G., S. Beilke, M. Betz and H. W. Georgii. 1981. Sulfur dioxide absorbed in rain water. pp. 41-55. In Effects of Acid Precipitation on Terestrial Ecosystems, T. Hutchinson and M. Havas eds. Plenum Press, New York.

13. Hayes, E. M. and J. M. Skelly. 1977. Transport of ozone from the northeast U. S. into Virginia and its effects on eastern white pine. Plant Dis. Rep. 61: 778-782.

14. Heck, W. W., R. B. Philbeck and J. A. Dunning. 1978. A continuous stirred tank reactor system for exposing plants to gaseous air contaminants. USDA-ARS Paper ARS-S-181.

15. Hunt, R. 1982. Plant Growth Curves Unviv. Park Press, Baltimore, 248 pp.

16. Irving, P. M. and J. E. Miller. 1981. Productivity of field-grown soybeans exposed to acid rain and sulfur dioxide alone and in combination. J. Environ. Qual. 10:473-478.

17. Jacobson, J. S., J. Troiano, L. J. Colavitto, L. I. Heller and D. C. McCune. 1980. Polluted rain and plant growth. pp 291-305, In Polluted Rain T. Y. Toribara, M. W. Miller and P. E. Morrow. 12 th Rochester Internat. Conf. on Environ. Tox., Plenum Publ. Co., New York.

18. Jensen, K. F. 1973. Response of nine forest tree species to chronic ozone fumigation. Plant Dis. Rep. 57:914-917.

19. Jensen, K. F. 1981. Air pollutants affect the relative growth rate of hardwood seedlings. USDA For. Serv. Res. Pap. NE-470.

20. Johnson, A. H. and T. G. Siccama. 1983. Evaluating

the relationship between acid deposition and forest decline. Environ. Sci. Technol. 17:294-305A.

21. Johnson, A. H. and T. G. Siccama. 1984. Decline of red spruce in the northern Appalachins: assessing the possible role of acid deposition. Tappi 67:68-72.

22. Knudson, L. L., T. W. Tibbitts and G. E. Edwards. 1977. Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Phsiol. 60:606-608.

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

24. Ledig, F. T. 1974. Concepts of growth analysis. pp. 166-182. _In_ Proceed. of 3rd Noth American For. Biol. Workshop. C. P. P. Reid and G. H. Fechmer eds. Colorado State Univ., Fort Collins.

25. Lee, J. J. and D. E. Weber. 1979. The effect of simulated acid rain on seedling emergence and growth of eleven woody species. For. Sci. 25:393-398.

26. Likens, G. E. and T. J. Butler. 1982. Recent acidification of precipitation in North America. Atmos. Environ. 15:1103-1109.

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 O_3, SO_2, and NO_2. Can. J. For. Res. 14:150-153.

28. McLaughlin, S. B., T. J. Blasing, L. K. Mann and D. N. Duvick. 1983. Effects of acid rain and gaseous pollutants on forest productivity: a regional scale approach. J. Air Pollut. Contr. Assoc. 33: 1042-1048.

29. Mudd, J. B. and T. T. Kozlowski. 1975. _Response of Plants to Air Pollution_, pp. 9-51. Academic Press, New York.

30. Norby, R. J. and R. J. Luxmoore. 1983. Growth analysis of soybean to simulated acid rain and

gaseous air pollutants. New Phytol. 95:277-287.

31. Pinkerton, J. E. 1984. Acidic deposition and its relationship to forest productivity: an update on the current state of knoweledge. Tappi 67:36-39.

32. 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. pp. 69-73, In Internt. Sympos. in Hydro. Amer. Wat. Res., Bethesda, MD.

33. Steele, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics McGraw Hill, New York, 481 pp.

34. Tingey, D. T., R. G. Wilhour and C. Standley. 1976. The effect of chronic ozone exposures on the metabolic content of ponderosa pine seedlings. For. Sci. 22:234-241.

35. Tomlinson, G. I., III. 1983. Air Pollutants and forest decline. Environ. Sci. Technol. 17:246-256A.

36. Troiano, J., L. Colavito, L. Heller and D. C. McCune. 1982. Observations on the viability of seed harvested from two soybean cultivars exposed to acidic simulated rain and photochemical oxidants. Agric. Environ. 7:275-283.

37. Troiano, J., L. Colavito, L. Heller, D. C. McCune and J. S. Jacobson. 1983. Effects of acidity of simulated rain and its joint action with ambient ozone on measures of biomass and yield in soybeans. Environ. Expt. Bot. 23:113-119.

38. Ulrich, B. 1984. Effects of air pollution on forest ecosytems and waters-the principles demonstrated at a case study in central Europe. Atmos. Environ. 18:621-628.

39. Wilhour, R. G. 1971. The influence of ozone on white ash (Fraxinus americana L.). Penn. State Univ. Center Air Environ. Stud. Publ. No. 188-71.

CHAPTER II

GROWTH RESPONSE OF GREEN AND WHITE ASH SEEDLINGS TO OZONE, SULFUR DIOXIDE AND SIMULATED ACID RAIN

INTRODUCTION

The harmful effects of air pollutants on numerous plant species have been documented over the past several decades. The majority of this research has involved exposure of plants to known concentrations of a single pollutant. However, air contaminants do co-occur in the ambient atmosphere (18,20), and research to determine the response of plants to pollutant mixtures is now receiving more attention (15,16,19,23).

White and green ash (*Fraxinus americana* L. and *F. pennsylvanica* Marsh., respectively) are two commercial hardwood species and are also commonly planted landscape trees in North America (1). Morphologically, these species are very difficult to differentiate (1), but their responses to air pollutants have been reported as being different (11,13,15).

Karnosky and Steiner (13) observed that, as a species, green ash was more sensitive to ozone (O_3), whereas white ash was more sensitive to sulfur dioxide (SO_2), on the basis of visible foliar injury. Both white ash and green ash have been reported as being tolerant (11) to intermediate in sensitivity (15) to O_3 with respect to height growth and dry matter accumulation.

Little is known about the growth response of white and green ash exposed to pollutant mixtures. In the only reported study, Jensen (12) found no significant effect of O_3, SO_2 or O_3 + SO_2 in one-yr-old white ash for any growth variable measured. The direct effect of acid rain on seedling tree growth has not been reported for these two species.

The purpose of this study was to determine the effect of O_3, SO_2 and simulated acid rain, alone and in combination, on the growth of white and green ash seedlings, under laboratory exposure conditions. The effects of O_3 and SO_2 were also compared when the pollutants were applied before or after a simulated rain event.

METHODS AND MATERIALS

Plant Material

Open-pollinated, white and green ash seed obtained from a wild collection (Herbst Brothers Seedmen, Inc., Brewster, NY 10509), were surface sterilized with 0.2% sodium hypochlorite, rinsed in tap water, stratified at 4°C for 12 weeks, then sown in flats containing vermiculite. Seedlings at five weeks postemergence were transferred to 10-cm diameter plastic pots containing a 2:2:1 (v/v/v) mixture of steam pasteurized Weblite^R (Weblite Corp., Inc., Roanoke, VA 24061), vermiculite and peat moss with 1g, 14-14-14 (N-P-K) Osmocote (Sierra Chemical Co., Milpitas, CA 95035) per pot. Peters' water soluble general purpose 20-20-20 (N-P-K) fertilizer (W.R. Grace & Co., Fogelsville, PA 18051) was added to the planting medium at a rate of 12 g/1000 ml deionized H_2O (approx. 100 ml solution/pot) during week 8 postemergence to ensure adequate plant nutrition.

Seedlings were grown in a greenhouse filtered with charcoal to reduce ambient O_3 concentrations to < 0.025 ppm. Greenhouse temperature ranged from 30-35^{0}C and supplemental lighting was used (approx. 400 umol/m^2/s photon flux density) to maintain a 14 hr photoperiod. Pollutant and rain exposures were initiated when the plants were nine weeks old postemergence.

Simulated Rain and Pollutant Exposures

Artificial rain was applied to the seedlings using a simulator developed on the principle of droplet formation from needle tips (5). Solutions, with ionic concentrations similar to the average ambient rainfall in southwestern Virginia (25), were prepared as previously described (5). Ionic concentrations are shown in Table

A-1. The pH of the solution was adjusted to either 5.6, 4.3 or 3.0 by the addition of 1M NaOH or H_2SO_4. Simulated rain was applied to the seedlings at a rate of 0.75 cm/hr for 1 hr, 2 times a week for 5 weeks, either just before or after fumigation with gaseous pollutants.

Seedlings were exposed to O_3 and/or SO_2, 4 hr daily (1200-1600), 5 days a week, for 6 weeks in 12 continuously stirred tank reactors (CSTRs) (9). Pollutant treatments included charcoal-filtered air (controls <0.025 ppm O_3 and <0.001 ppm SO_2), 0.10 ppm O_3, 0.08 ppm SO_2, and 0.10 ppm O_3 + 0.08 ppm SO_2. Environmental conditions within the CSTRs were maintained at 32 ± 2^{0}C, 70 ± 5% RH and 350-400 umol/m^2/s photon flux density. Pollutant concentrations were dispensed and monitored as previously reported (3).

Measurement of Plant Response

Every seven days, beginning with the first day of fumigation, seedling height was measured from the cotyledonary node to the base of the terminal bud. Cumulative increases, measured at weeks one through six were calculated for each treatment. Seedlings were examined periodically for visible foliar injury. At the termination of the experiment, all seedlings were harvested, dried at 60^{0}C for 36 hrs, and root, leaf and stem dry weights determined.

Relative growth responses were calculated using growth analysis (10). The following growth variables were calculated: Mean relative growth rate (RGR) for total (TRGR), stem (RGSTEM), root (RGROOT) and leaves (RGLEAF); leaf area ratio (LAR)= leaf area/plant dry wt; Leaf wt ratio (LWR)=leaf wt/plant dry wt; specific leaf area (SLA)=leaf area/leaf dry wt; root/shoot ratio (RSR)=root dry wt/(stem+leaf dry wts); and mean unit leaf rate (ULR), (net assimilation rate)=[(final plant dry wt-initial dry wt)/wk X ln (final leaf area)-ln(initial leaf area)]/ (final leaf area-initial leaf area).

Total leaf area (ALA) was determined by measuring the length and width (LW) of each leaf/plant at the initiation and termination of the study. Seventy-eight leaves, combined from both species (regression equations were similar for both tree species), in all ontogenilogical stages were selected, LW determined, and ALA measured using a Li-Cor Model 3000 Portable Area Meter (Li-Cor, Ltd., Lincoln, NE 68504). A regression equation relating ALA to LW was then developed: $Y=0.49 + 0.50X$, $R^2= 0.99$, where Y=ALA and X=LW. Using this equation total leaf area increase was then estimated.

Experimental Design and Data Analysis

A split-plot, replicated 3 times was used. Three blocks (4 CSTRs each) were used to remove any variation between CSTRs due to differences in temperature, RH and light intensity. Whole plot treatments were gaseous pollutants, with one pollutant treatment/CSTR. Sub-plot treatments were a 3 X 2 factorial combination of three simulated rain solutions and two application times of rain. Each treatment combination was replicated 2 times/tree species/CSTR (288 total trees, 144 trees/species).

Data were analyzed by analysis of variance (ANOVA). Analysis of covariance (COANOVA) was preformed where necessary, to determine if the data were influenced by a significant covariate, and the data were adjusted where appropriate.

Whole plot treatments were partitioned into the following three orthogonal contrasts (26): 1) (O_3- control)+(SO_2-control) vs (O_3+SO_2-control); 2) O_3+SO_2 vs control; 3) O_3 vs SO_2. The first contrast is a test of the additivity of O_3+SO_2 exposure on seedling growth. If the contrasts were equal, the combined pollutant effect was additive. If the contrasts were significantly different, then inspection of the sign of this difference indicated whether the combined effects of O_3 + SO_2 were greater or less than additive. Variance for simulated rain treatments was partitioned into linear and quadratic components, using orthogonal polynomial contrasts.

RESULTS

Mean dry weights, RGR and total height increase (HTI) for green and white ash, across all gaseous pollutant and rain treatments are shown in Table 5. Green ash exhibited 19,12,13, 10 and 33% greater stem, leaf and total dry weights, RGR and HTI, respectively, compared with white ash. Because of these significant differences in growth and numerous species X pollutant or rain pH treatment interactions observed in an initial analysis of variance, data were analyzed separately, by species.

The mean squares for dry matter production and selected growth components are presented in Tables 6 and 7, for green and white ash, respectively. Initial seedling height and leaf area were found to be a significant covariate for the majority of the variables measured, and the data were adjusted appropriately.

Individual Treatment Effects: Green Ash

Across all other treatments, significant effects of gaseous pollutants were observed only for HTI, RGR and LWR for green ash (Table 6). The combined effect of O_3 + SO_2 was additive in nature for HTI, but less

Table 5. Mean dry weights, mean relative growth rate (RGR) and height increase (HTI, cm) for 15-week-old green and white ash, by species, across all other treatments.[a]

	Biomass (g)					
Species	stem	root	leaf	total	RGR	HTI
green ash	1.97	2.09	3.09	7.15	0.31	27.7
white ash	1.73	2.00	2.49	6.22	0.28	18.3

[a] Means for all variables, except root dry wt. are significantly different at the 0.05 level (LSD), between tree species.

Table 6. Mean squares and levels of significance of analysis of variance for selected plant growth responses of 15-week-old green ash exposed to gaseous air pollutants and simulated acid rain, under laboratory exposure conditions.

Source of variation	df	Mean squares[a]											
		Biomass											
		stem	root	leaf	total	HTI	RSR	LAR	LWR	SLA	$\overline{\mathrm{RGR}}$	$\overline{\mathrm{ULR}}$	LAI
Block	2	0.008	0.815	0.377	1.615	1878.9	0.025	1560.1	0.000	7339.3	0.001	0.950	38646.0
Pollutant	3	1.770	1.940	1.433	12.888	41775.5*	0.028	3897.0	0.017	10456.5	0.017	3.528	58089.3
Additive[c]	1	0.792	1.632	0.259	7.167	8321.8	0.015	3737.6	0.016*	9224.1	0.014*	2.615	53372.5
Control vs O_3+SO_2	1	0.833	0.166	1.130	5.678	33323.4*	0.009	153.9	0.001	1045.4	0.002	0.617	1202.6
O_3 vs SO_2	1	0.145	0.142	0.044	0.043	130.3	0.004	5.5	0.000	187.0	0.001	0.296	3514.2
Block x Pollutant	6	0.288	0.588	0.481	2.658	4033.5	0.166	1421.0	0.001	8223.4	0.002	0.715	18259.0
Rain pH	2												
Linear	1	0.257	0.030	0.429	1.473	1237.0	0.006	291.3	0.001	1126.9	0.003	1.230	11022.6
Quadratic	1	0.065	0.970	0.001	0.491	24532.5*	0.049*	6804.8*	0.004	28295.2	0.001	2.066	20219.4
Timing of rain	1	0.071	2.254*	0.034	2.510	147.9	0.109*	238.3	0.022*	11357.5	0.001	4.540	8.6
Pollutant x pH	6	0.493	0.595	0.540	2.459	4938.7	0.020	1474.3	0.011*	8651.9	0.002	1.555	10284.6
Pollutant x time	3	0.230	0.713	0.494	3.328	11780.7	0.006	2080.9	0.002	6623.5	0.002	0.963	13525.2
pH x time	2	0.569	0.113	0.316	1.794	32335.8	0.022	3439.6	0.003	13708.4	0.004	1.375	33822.4
Poll x pH x time	6	0.441	0.378	0.415	3.366	8586.9	0.005	1331.0	0.003	7991.6	0.002	0.382	37852.9
Covariate[d]	1	7.107**	5.260**	1.212	36.733**	16114.3	0.039	1809.0	0.023*	602.4	0.029**	11.657**	40578.0
Error	111	0.293	0.477	0.581	2.860	5634.9	0.012	1680.0	0.004	9186.0	0.002	1.620	26539.3

[a] Calculated F-values significant at the 0.05 or 0.01 levels are denoted by * or **, respectively. Pollutant x block interaction used as error to test for pollutant and block effects; HTI = total height increase (cm); RSR = root/stem + leaf dry weight; LAR = leaf area/total plant dry wt. (cm^2/g); LWR = leaf dry wt/total plant dry wt:SLA = leaf area/leaf dry wt. (cm^2/g); RGR = mean relative growth rate (per wk); ULR = mean unit leaf rate (mg/cm^2)/wk); LAI = total leaf area expan. (cm^2).

[b] Additive = (O_3 - control) + SO_2 - control) vs. (O_3 + SO_2) - control.

[c] Covariate for biomass and RSR and HTI was initial tree height; For all other variables covariate was initial leaf area.

Table 7. Mean squares and levels of significance of analysis of variance for selected plant growth responses of 15-week-old white ash exposed to gaseous air pollutants and simulated acid rain, under laboratory exposure conditions.

Source of variation	df	Mean squares[a]											
		Biomass											
		stem	root	leaf	total	HTI	RSR	LAR	LWR	SLA	$\overline{RGR}$	$\overline{ULR}$	LAI
Block	2	1.030	2.760	0.062	8.013	3640.0	0.066	764.2	0.022	5623.8	0.005	3.365*	1707.0
Pollutant	3	0.841	1.249	4.172*	14.526	19423.6	0.047	202.5	0.007	19215.5	0.006	1.349	56770.6
Additive[c]	1	0.068	1.160	2.389	8.306	4164.6	0.003	12.8	0.002	967.5	0.003	0.502	35137.3
Control vs O_3+SO_2	1	0.093	0.027	0.592	1.531	7397.5	0.015	34.8	0.002	10807.4	0.001	0.032	20162.1
O_3 vs SO_2	1	0.680	0.062	1.191	4.689	7861.5	0.029	154.9	0.003	7440.6	0.002	1.267	1471.2
Block x pollutant	6	0.623	1.420	0.893	6.157	15755.6	0.560	571.5	0.016	15226.4	0.004	1.080	42580.2
Rain pH	2												
Linear	1	0.210	1.060	0.968	0.170	11795.8	0.143**	968.4*	0.019	312.1	0.000	0.931	42782.6
Quadratic	1	0.102	0.489	0.131	0.432	1029.3	0.078	2.0	0.014	24366.4	0.000	0.220	497.5
Timing of rain	1	1.315*	1.215	2.460*	14.576*	182.0	0.003	238.8	0.000	1763.7	0.009**	3.240	35479.9
Pollutant x pH	6	0.200	0.783	0.325	2.288	7525.9	0.056*	447.0*	0.008	5674.0	0.002	1.327	24894.1
Pollutant x time	3	0.266	0.172	0.432	2.170	458.9	0.006	430.2	0.006	5585.9	0.001	0.840	21103.4
pH x time	2	0.785	0.147	0.748	3.795	3143.2	0.025	337.6	0.001	4689.4	0.002	0.380	16504.5
Poll x pH x time	6	0.376	0.388	0.146	0.878	11405.0	0.033	183.9	0.007	8104.6	0.003	0.233	20910.8
Covariate[d]	1	3.795**	2.058*	0.000	11.584*	1113.6	0.005	394.2	0.010	13819.0	0.007	2.980	57.8
Error	111	0.256	0.379	0.479	2.213	3884.5	0.021	2081.5	0.006	9498.8	0.002	0.819	14730.8

[a] Calculated F-values significant at the 0.05 or 0.01 levels are denoted by * or **, respectively. Pollutant x block interaction used as error to test for pollutant and block effects; HTI = total height increase (cm); RSR = root/stem + leaf dry wt; LAR = leaf area/total plant dry wt (cm^2/g); LWR = leaf dry wt/total plant dry wt; SLA = leaf area/leaf dry wt (cm^2/g); RGR = mean relative growth rate (per wk); ULR = mean unit leaf rate (mg/cm^2/wk); LAI = total leaf area expan. (cm^2).

[b] Additive = (O_3 - control) + (SO_2 - control) vs. (O_3 + SO_2) - control.

[c] Covariate for biomass, RSR and HTI was initial tree height; For all other variables covariate was initial leaf area.

than additive for RGR and LWR. Relative growth rates were 0.33, 0.30, 0.30 and 0.32 (per week) and LWRs were 0.43, 0.45, 0.45 and 0.42 for controls, O_3, SO_2 and O_3 + SO_2 treated plants, respectively. Although not reported, results were similar for RGRs for individual plant parts. The cumulative shoot height increase curves of green ash seedlings exposed to O_3, SO_2 and O_3 + SO_2 were less than controls after 6 wks (Fig. 3A). Total height increase was 14,14 and 17% less for plants exposed to O_3, SO_2 and O_3 + SO_2, respectively, compared with controls. A significant difference in shoot elongation between gaseous pollutant treatments and controls became evident after 3 wks of exposure.

Visible symptoms were observed on 14, 17 and 37% of the O_3, SO_2 or O_3 + SO_2 treated plants, respectively (n=36 plants/species/treatment). Symptoms first appeared on the older leaves, beginning with the third week of fumigation. Ozone and O_3 + SO_2 symptoms were characterized initially by purple-white stipples on the adaxial leaf surface. Stipples generally coalesced and eventually resulted in tan-brown necrotic lesions. Symptoms of SO_2 were interveinal, white, amorphous patches approximately 1 mm in diameter and were observed first on the older leaves

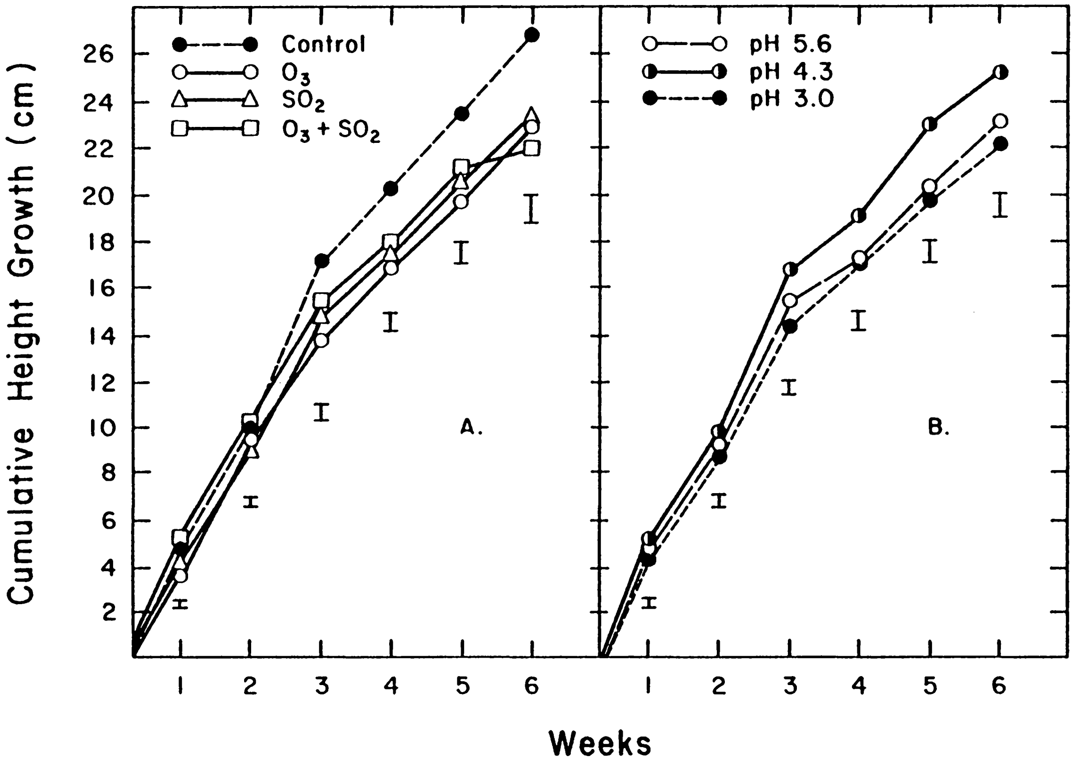

Figure 3. Cumulative shoot height increase (cm) of green ash during 6 weeks of exposure to (a) gaseous pollutants, and (b) simulated rain solutions. Vertical bars represent least significant difference (LSD, p = 0.05).

three weeks after the initiation of fumigation.

The cumulative shoot height increase curves of green ash seedlings exposed to simulated rain are shown in Fig. 3B. Seedlings exposed to pH 4.3 solutions exhibited the greatest increase in shoot elongation. Significant differences between treatments became apparent after three weeks of rain exposure, and HTI was 9 and 12% greater for seedlings treated with pH 4.3 rain solutions than pH 5.6 or 3.0 rain solutions, respectively.

Significant quadratic responses to rain pH, across all other treatments occurred for HTI, LAR and RSR (Table 6). Leaf area ratio (LAR) was the greatest (Fig. 4A) and RSR (Fig. 5A) the least for plants treated with pH 4.3 rain solutions when compared with plants exposed to either pH 5.6 or 3.0 rain solutions. There were no visible foliar symptoms attributable to the acidity of rain solutions at any pH level.

The timing of rain application relative to gaseous pollutant exposure significantly affected root dry weight, RSR and LWR (Tables 6 and 8). For these variables, biomass was greatest when rain was applied before fumigation with gaseous pollutants. Mean root dry weight, RSR and LWR were 11, 11 and 7% greater, respectively for plants exposed to rain before fumigation than after fumigation

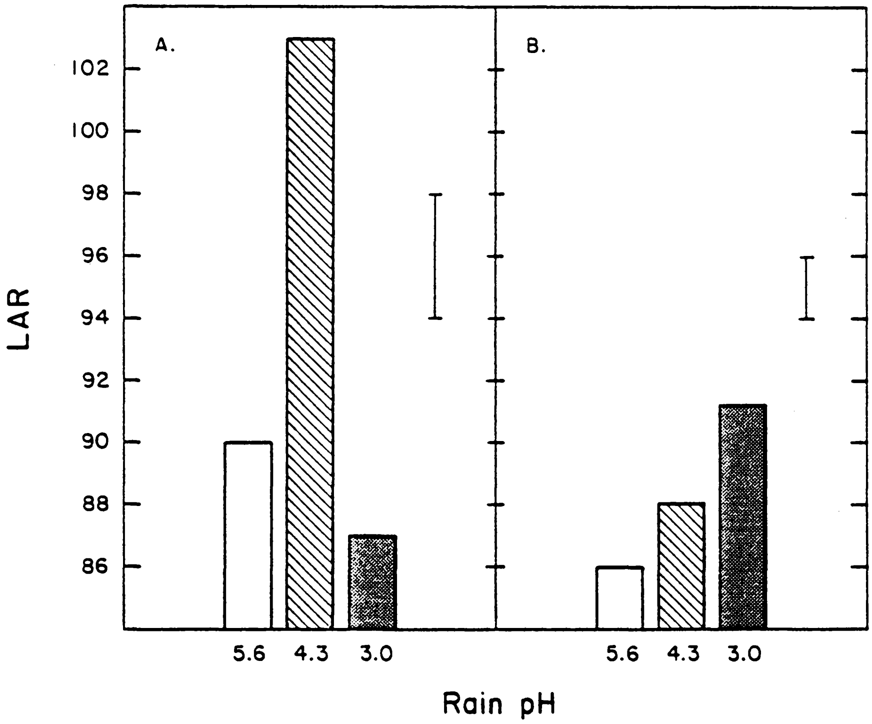

Figure 4. Mean LAR (total leaf area/total plant dry wt., cm^2/g) of 15-wk-old (a) green ash and (b) white ash, exposed to simulated rain for 6 weeks. Vertical bars represent least significant difference (LSD, $p = 0.05$).

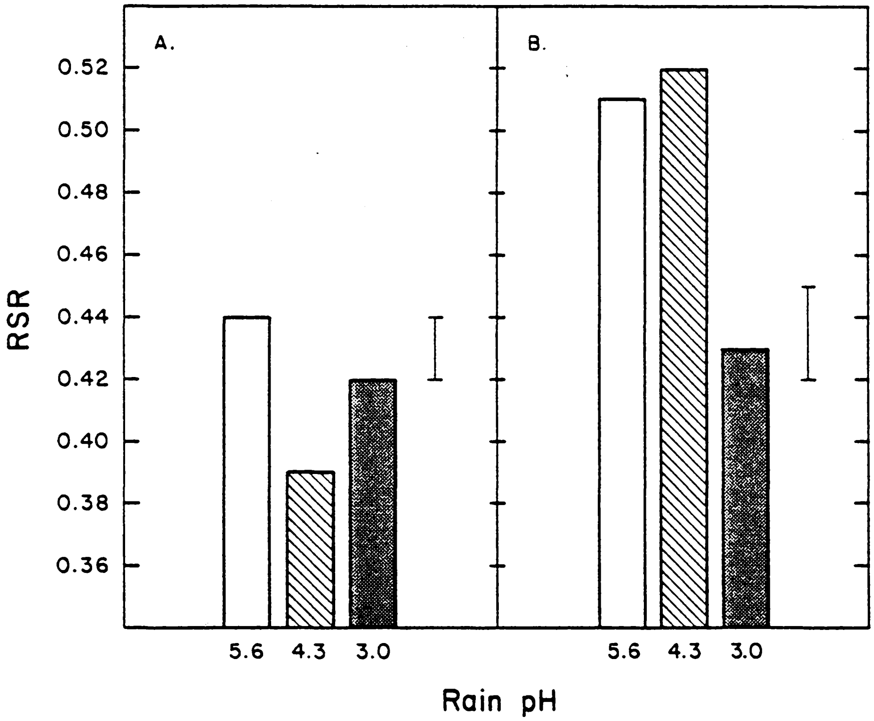

Figure 5. Mean RSR (root dry wt/above ground dry wt., g/g) of 15-wk-old (a) green ash and (b) white ash, exposed to simulated rain for 6 weeks. Vertical bars represent least significant difference (LSD, $p = 0.05$).

Table 8. Mean dry weights (g), LWR, RGR and RSR or 15-week-old green and white ash exposed for 6 weeks, to simulated rain before or after fumigation with O_3 and SO_2.[a]

	Tree Species			
	Green ash		White ash	
	time		time	
Biomass	before	after	before	after
stem	1.99	1.95	1.83	1.64*
root	2.22	1.97*	2.08	1.89
leaf	3.08	3.11	2.59	2.33*
total	7.29	7.03	6.50	5.86*
LWR	0.45	0.42*	0.40	0.40
RGR	0.31	0.31	0.28	0.30*
RSR	0.44	0.39*	0.49	0.48

[a] Before = rain applied before fumigation; after = rain applied after fumigation; LWR = leaf weight ratio (leaf wt/total plant wt), RGR = relative growth rate (per wk), RSR = root/shoot (above ground biomass) ratio (w/w); Numbers in a row followed by a * are significantly different at the 0.05 level.

with gaseous pollutants.

Combined Effects of Rain and Gaseous Pollutants: Green Ash

A significant pollutant X rain pH interaction occurred for LWR (Table 6). To determine where the interaction occurred, treatment variation was partitioned into the following six orthogonal contrasts using fixed levels of gaseous pollutants across either linear or quadratic components of rain pH: 1) control (0.00 ppm) vs linear; 2) O_3 vs linear; 3) SO_2 vs linear; 4) O_3 + SO_2 vs quadratic; 5) O_3 vs quadratic and 6) SO_2 vs quadratic. Mean LWR exhibited a significant ($p=0.01$) linear decrease as rain pH decreased from 5.6 to 3.0 for controls (Fig. 6). Leaf weight ratio decreased 12% at pH 3.0 compared with pH 5.6. Ozone and O_3 + SO_2 treated plants showed a significant ($p=0.05$) quadratic effect with decreasing rain pH. For O_3 treated plants, LWR was 16 and 8% greater for seedlings treated pH 4.3 rain solutions than pH 5.6 or 3.0 solutions, respectively. Leaf weight ratio was 9% greater for pH 4.3 treated seedlings than plants treated with either pH 5.6 or 3.0 rain solutions, respectively, for O_3 + SO_2 treated green ash. No other contrasts were significant for this variable.

Individual Treatment Effects: White Ash

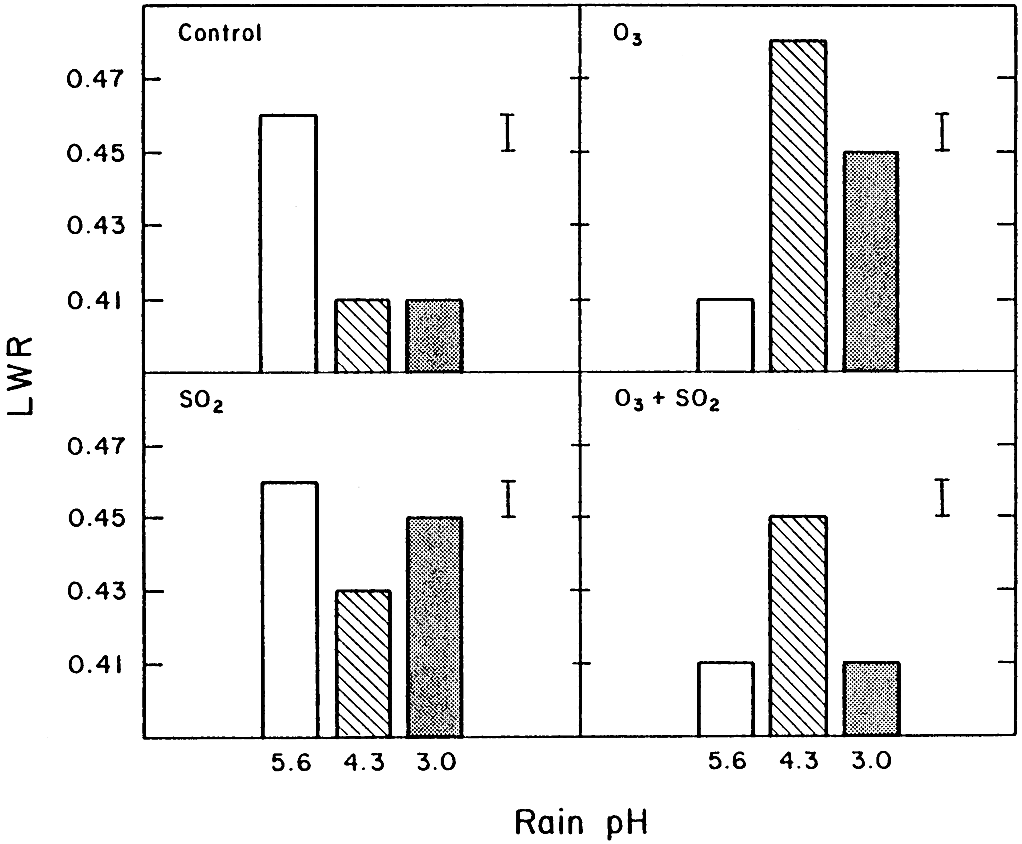

Figure 6. Mean LWR (leaf dry wt./total plant dry wt., g/g) of 15-wk-old green ash, exposed to simulated rain and O_3, SO_2 or $O_3 + SO_2$ for 6 weeks. Vertical bars represent least significant difference (LSD, $p = 0.05$).

No significant effects of gaseous pollutants on white ash were observed, across all other treatments for all variables, except leaf dry weight (Table 7). Ozone-treated plants had significantly less leaf dry weight compared to controls (LSD, p=0.05). Mean leaf dry weights were 2.68, 2.50, 2.46 and 2.20 g, for controls, O_3 + SO_2, SO_2 and O_3 treatments, respectively. Visible symptoms occurred on 22, 66 and 36% of the O_3, SO_2 or O_3 + SO_2 treated plants, respectively. Symptom expression was similar to green ash, which has been previously described. There were no visible foliar symptoms attributable to the acidity of rain solutions at any pH level.

The timing of rain application relative to gaseous pollutant exposure significantly affected stem, leaf and total dry weights and RGR (Tables 7 and 8). Growth was greatest, for these variables when rain was applied before fumigation with gaseous pollutants. Mean dry weights of plant parts were approximately 10% greater and RGR 7% greater for seedlings exposed to simulated rain before fumigation than after fumigation with gaseous pollutants.

Combined Effects of Rain and Gaseous Pollutants: White Ash

A significant linear response (p=0.05) to rain pH treatments occurred for LAR (Fig. 4B) and RSR (Fig. 5B) for rain pH; however, these variables exhibited significant pollutant X rain pH interactions (Table 7). The variation associated with this interaction was partitioned into orthogonal comparisons, as previously described for green ash.

Mean LAR exhibited a significant (p=0.05) linear increase as rain pH decreased from 5.6 to 3.0 for SO_2- and O_3-treated plants (Fig. 7). Leaf area ratios were 14 and 11% greater, respectively, for plants exposed to SO_2 and O_3 to which pH 3.0 rain was applied compared with pH 5.6 treated seedlings. Plants exposed to O_3 + SO_2 demonstrated a significant (p=0.05) quadratic response with decreasing rain pH; LAR was 12 and 15% less for seedlings treated with pH 4.3 rain solutions than pH 5.6 or 3.0 solutions, respectively. No other contrasts were significant for this variable. Significant linear decreases (p=0.05) occurred in RSR for O_3- and SO_2-treated plants with decreasing rain pH (Fig. 8). Root/shoot ratios were 25 and 19% less, respectively, for plants exposed to SO_2 and O_3 and treated with pH 3.0 simulated rain solutions compared with pH 5.6 treated seedlings. A significant quadratic response (p=0.05) also was observed in RSR for O_3 + SO_2 treated plants;

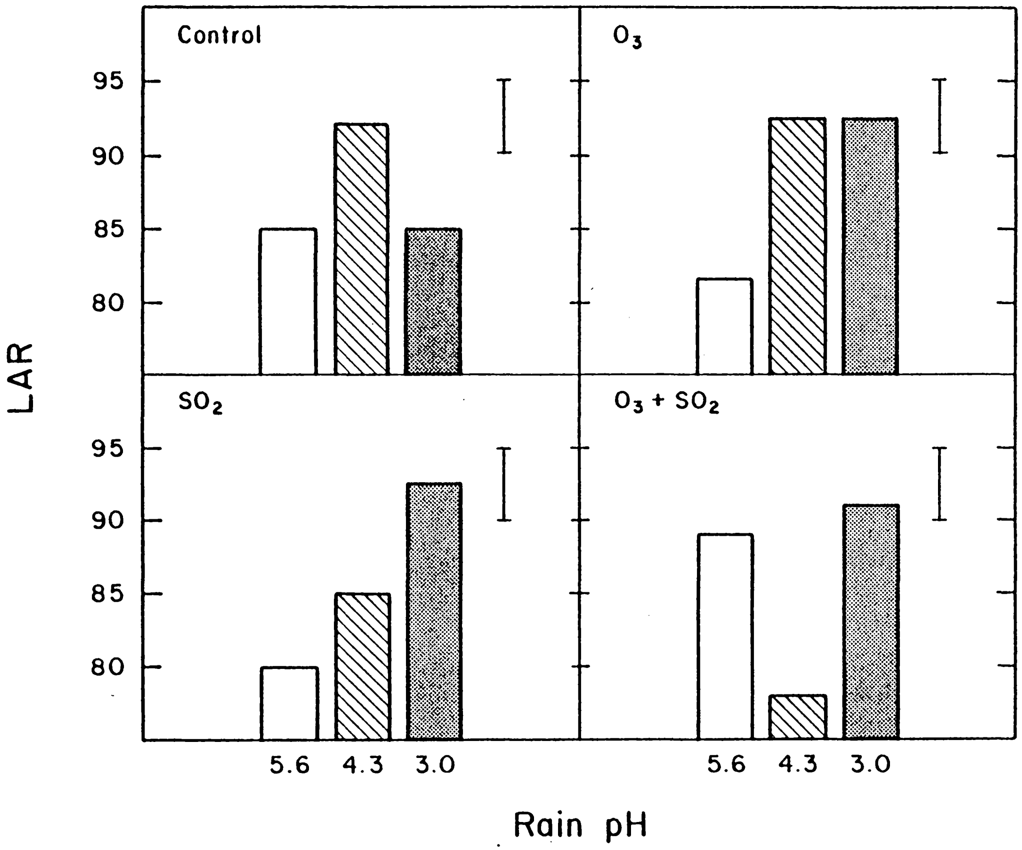

Figure 7. Mean LAR (total leaf area/total plant dry wt., cm^2/g of 15-wk-old white ash, exposed to simulated rain and O_3, SO_2 or O_3 + SO_2 for 6 weeks. Vertical bars represent least significant difference (LSD, p = 0.05).

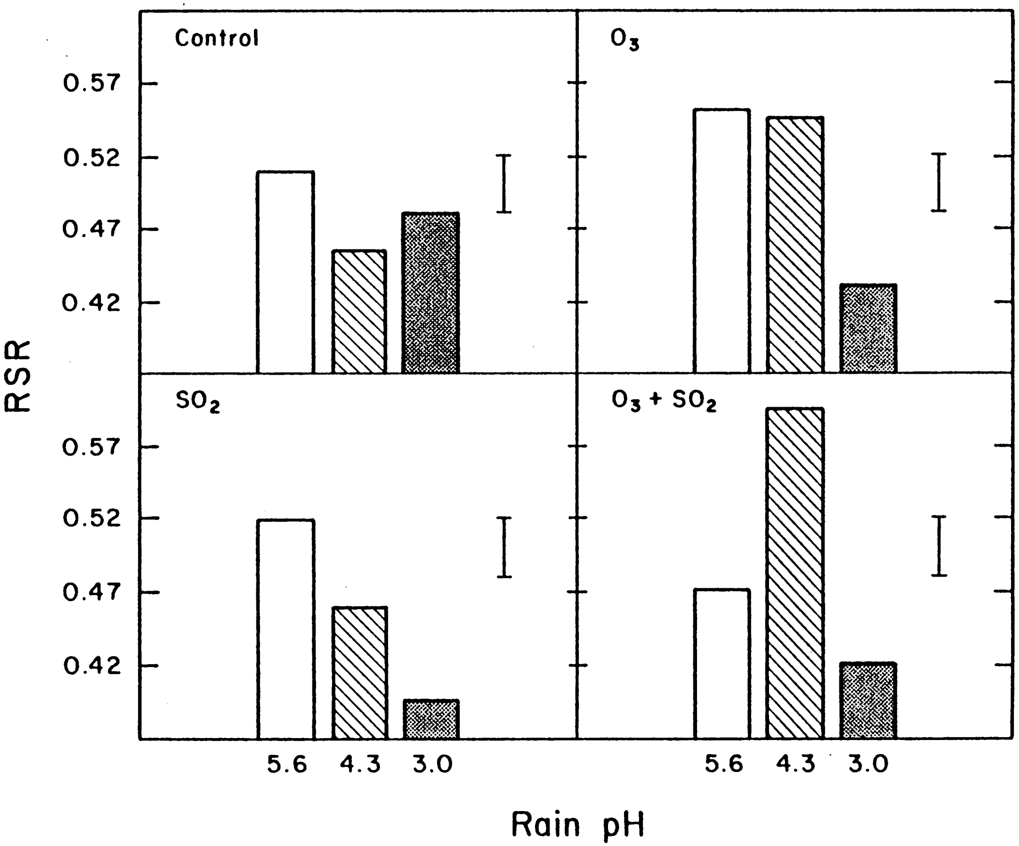

Figure 8. Mean RSR (root dry wt./above ground dry wt., g/g) of 15-wk-old white ash, exposed to simulated rain and O_3, SO_2 or O_3 + SO_2 for 6 weeks. Vertical bars represent least significant difference (LSD, $p = 0.05$)

RSR was 27 and 34% greater for seedlings treated with pH 4.3 rain solutions than pH 5.6 or 3.0 solutions, respectively. No other contrasts were significant for this variable.

DISCUSSION

Dry matter accumulation of green and white ash seedlings was not significantly affected by either O_3 or SO_2 fumigation over a six week period, except O_3-treated plants had significantly less leaf biomass than controls, for white ash (Tables 6 and 7). Kress and Skelly (15) exposed seedlings of several different tree species to increasing concentrations of O_3 for up to 45 days. They reported differences in biomass accumulation response between green and white ash only at 0.15 ppm O_3.

Jensen (11) and Kress and Skelly (15) independently reported green ash to be more sensitive to O_3 than white ash, regarding suppression in shoot elongation. Results from this present study support the findings of Jensen (11) and Kress and Skelly (15) regarding the sensitivity of these species, with respect to shoot elongation.

The combination of O_3 + SO_2, across all other treatments, did not affect the growth response and visible injury of green or white ash seedlings more than O_3 or SO_2 alone, except for HTI for green ash (Fig 3A) in which the response was additive. Jensen (12) reported similar results regarding growth inhibition, for white ash. He found no significant differences in white ash growth when fumigated with O_3, SO_2 or O_3 + SO_2. In several studies with other tree species (3,16,19) however, the combined effects of O_3 + SO_2 caused a significantly greater reduction in growth than single pollutant effects. Variation in species sensitivity, duration of exposure, pollutant concentration and tree age may all contribute to the different responses among species.

Green ash has been reported (13,15) as being more sensitive than white ash to O_3, and white ash more sensitive than green ash to SO_2 (13), regarding visible foliar injury. In this present study white ash was found to be more sensitive than green ash to O_3 or SO_2 alone, but similar in response to pollutant combinations.

Increases in simulated rain acidity resulted in no significant changes in biomass accumulation, but in quadratic growth responses in LAR and RSR, for green ash (Fig 4A and 5A). White ash exhibited significant linear decreases in these variables (Figs 4B and 5B); however these responses were influenced by significant pollutant X rain pH interactions (Table 7).

This statistically significant quadratic response to LAR, for green ash occurred with LAR being the greatest for pH 4.3 treated seedlings. Reasons for these alterations in response are unknown, but may be related to increased nutrient uptake and availability by certain plant species in moderately acidic environments (2). This allocation shift is further illustrated by a quadratic response in RSR, for green ash, with RSR being the least for seedlings treated with pH 4.3 rain solutions. Ferenbaugh (7) attributed a decrease in root dry weight and subsequent decrease in RSR in Phaseolus vulgaris L. treated with acid rain to a reduction in the amount of photoassimilates translocated. Neufeld et al. (22) reported similar results for several species of tree seedlings. Lee et al. (17) however, observed a stimulation in above ground biomass and decrease in RSR for several agronomic crop species at pHs 3.5 and 4.0, compared with plants treated with pHs 3.0 or 5.6. His results are similar to those observed for green ash in this present study.

The timing of rain application affected certain variables of plant growth for both species (Table 8). Generally, biomass decreases were observed for plants exposed to simulated rain after fumigation with gaseous pollutants. Chappelka et. al. (3) and Elkiey and Ormrod

(6) reported that growth was significantly inhibited when plants were fumigated with wet leaf surfaces rather than dry leaf surfaces. The present results suggest that this effect may be related to leaf wetability and leaf surface characteristics (8), or different environmental conditions during fumigation (light, temperature, RH, etc.).

For green ash, a significant pollutant X rain pH treatment interaction was evident for LWR (Fig. 6). In non-fumigated green ash controls, LWR decreased linearly, with the decrease being the greatest between pH 5.6 and 4.3, whereas in O_3- and O_3 + SO_2-treated plants, a quadratic response occurred with increasing rain acidity, with seedlings treated with pH 4.3 rain exhibiting the greatest increase in LWR. Leaf weight ratio (LWR) is the amount of dry mass retained in the foliage compared with the dry weight of the entire plant and a change in LWR is a reflection of photosynthate allocation shifts in dry weight (10).

The combined effects of gaseous pollutants and simulated acid rain resulted in a significant pollutant X rain pH treatment interaction in LAR and RSR for white ash. Ozone- and SO_2-treated plants exhibited statistically significant linear increases in LAR and linear decreases in RSR with increasing rain acidity (Figures 7 and 8). Although the trends were linear in nature, the increase in LAR was greatest between pH 5.6 and 4.3 in O_3-treated seedlings. The decrease in RSR was greatest between pH 4.3 and 3.0 for O_3-treated seedlings. Chappelka and Chevone (4) observed similar results with 10-wk-old white ash exposed to increasing concentrations of O_3 and simulated acid rain. They found a significant linear decrease in RSR for seedlings exposed to either 0.05 or 0.10 ppm O_3 as rain pH decreased from 5.6 to 3.0. These data reflect a change in the allocation pattern of photosynthates in the foliage at the expense of root growth.

When white ash were fumigated with O_3 + SO_2 in combination with simulated rain, LAR and RSR exhibited a quadratic response in this present study. Reasons for this are unknown, but indicate a possible antagonism between these pollutants.

This current work represents the first report of the effects of O_3, SO_2 and acid rain, alone or in combination on the growth of green and white ash, and one of the few studies investigating the combined effect of gaseous pollutants and acidic precipitation on tree growth (3,14,24). Results from this present study indicate that although green and white ash are morphologically similar (1) they respond differently to air-borne contaminants. This differential response was reflected in visible injury (gaseous pollutants and simulated rain), shoot elongation (gaseous pollutants), and shifts in biomass allocation (gaseous pollutants + simulated acid rain). These results support the findings of other researchers (3,7,-21,22,27) that one of the primary effects of air pollutants on plants is to alter the patterns of photoassimilate translocation.

Since it is known that gaseous pollutants, primarily O_3 and acid rain, can impact the same geographic areas (20) the linear decrease RSR and linear increase in LAR with O_3 or SO_2 in combination with simulated acid rain for white ash in this study, and linear decrease in root dry weight and RSR, for white ash, in another study (4), are cause for concern. The retention of photosynthate in the foliage at the expense of root growth may predispose these trees to other stresses (drought, root rotting microorganisms, etc.). Additional research is needed using different tree species and field testing to determine if such effects are occurring in the natural ecosystem.

LITERATURE CITED

1. Anonymous. 1965. Silvics of Forest Trees of the United States. USDA Agric. Handb. No. 271.

2. Blamey, F. P. C., D. G. Edwards, C. J. Asher and Moo-Key Kim. 1982. Response of sunflower to low pH. pp. 66-72. In Plant Nutrition 1982, Vol. 1., Avon Litho Ltd., Stratford-Upon-Avon, UK.

3. 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. Expt. Bot. 25:233-244.

4. Chappelka, III, A. H. and B. I. Chevone. 198_. White ash seedling growth response to ozone and simulated acid rain. Can. J. For. Res. (In Press).

5. Chevone, B. I., Y. S. Yang, W. E. Winner, I. Storks-Cotter and S. J. Long. 1984. A rainfall simulator for laboratory use in acidic precipitation studies. J. Air Pollut. Contr. Assoc. 34:355-359.

6. Elkiey, T. and D. P. Ormrod. 1981. Sulphur and nitrogen nutrition and misting effects on the response of bluegrass to ozone, sulphur dioxide, nitrogen dioxide or their mixture. Water, Air, and Soil Pollut. 16:177-186.

7. Ferenbaugh, R. W. 1976. Effects of simulated acid rain on Phaseolus vulgaris L. (Fabaceae). Amer. J. Bot. 63:283-288.

8. Haines, B. L., J. A. Jernstedt and H. S. Neufeld. 1985. Direct foliar effects of simulated acid rain II. Leaf surface characteristics. New Phytol. 99:407-416.

9. 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.

10. Hunt, R. 1982. Plant Growth Curves. Univ. Park Press, Baltimore.

11. Jensen, K. F. 1973. Response of nine forest trees

species to chronic ozone fumigation. Plant Dis. Rep. 57:914-917.

12. Jensen, K. F. 1981. Air pollutants affect the relative growth rate of hardwood seedlings USDA For. Ser. Res. Pap. NE-470.

13. Karnosky, D. F. and K. C. Steiner. 1981. Provenance and family variation in response of *Fraxinus americana* and *F. pennsylvanica* to ozone and sulfur dioxide. Phytopathology 71:804-807.

14. Krause, G. H. M., K. D. Jung and B. Prinz. 1984. Forest effects in West Germany. pp. 297-332. *In*: Symposium on Air Pollution and the Productivity of the Forest. Izzak Walton Leaque of America, Arlington, VA.

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

16. Kress, L. W., J. M. Skelly and K. H. Hinkelmann. 1982. Growth impact of O_3, NO_2 and/or SO_2 on *Plantanus occidentalis*. Agric. and Environ. 7: 265-274.

17. Lee, J. J., G. E. Neely, S. C. Perrigan and L. C. Grothaus. 1981. Effect of simulated sulphuric acid rain on yield, growth and foliar injury of several crops. Environ. Expt. Bot. 21:171-185.

18. Lefohn, A. S. and D. T. Tingey. 1984. The co-occurrence of potentially phytotoxic concentrations of various air pollutants. Atmos. Environ. 18:2521-2526.

19. 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 O_3, SO_2, and NO_2. Can. J. For. Res. 14:150-153.

20. McLaughlin, S. B. 1985. Effects of air pollution on forests: A critical review. J. Air Pollut. Contr. Assoc. 35:512-534.

21. McLaughlin, S. B., R. K. McConthy, 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.

22. Neufeld, H. S., 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.

23. Reinert, R. A. 1984. Plant response to air pollutant mixtures. Ann. Rev. Phytopathol. 22:421-442.

24. Skeffinton, R. A. and T. M. Roberts. 1985. The effects of ozone and acid mists on Scots pine saplings. Oecologia 65:201-206.

25. 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. pp. 69-73. In: Internat. Symp. in Hydromet. Amer. Water Res. Assoc.

26. Steele, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York.

27. Tingey, D. T., R. G. Wilhour and C. Standley. 1976. The effect of chronic ozone exposures on the metabolic content of ponderosa pine seedlings. For. Sci. 22:234-241.

CHAPTER III

WHITE ASH SEEDLING GROWTH RESPONSE TO OZONE AND SIMULATED ACID RAIN

INTRODUCTION

Ozone (O_3) is considered the major phytotoxic pollutant in eastern North America (30), and has been reported as causing significant adverse effects on plant growth and productivity, even in the absence of visible foliar injury (8,9). Increases in rainfall acidity, due to the wet deposition of sulfur and nitrogen compounds, has raised concern over potential detrimental effects on terrestrial ecosystems (13,19). However, at present only limited information is available on the effects of acid rain on plant growth at hydrogen ion concentrations which approximate those occurring naturally (19,26).

Although both of these air contaminants (O_3 and acid rain) are observed in the same geographic area, only a few studies have been conducted on the combined effects of these pollutants on tree growth (6,23,29). The purpose of this study, therefore, was to examine the effects of O_3 and simulated acid rain, alone and in combination, on the growth and productivity of white ash (Fraxinus americana L.) seedlings under laboratory exposure conditions.

White ash was selected since it is a major commercial hardwood species and is a commonly planted landscape tree in eastern North America, with its natural range extending from Nova Scotia, westward to eastern Minnesota and south to east Texas and north Florida (2). This species has been reported as being sensitive to intermediate in response to O_3 based on visible foliar injury (24) and intermediate (24) to tolerant (20) to O_3 with respect to height growth and biomass production. No investigation of the effect of acid rain on white ash growth has previously been reported.

MATERIALS AND METHODS

Plant Material

Stratified, open-pollinated, white ash seed (Herbst Brothers Seedmen, Inc., Brewster, NY 10509) were surface sterilized with 0.2% sodium hypochlorite, rinsed in tap water and sown in flats containing vermiculite. Seedlings at two weeks postemergence were transplanted to 10-cm diameter plastic pots containig a 2:2:1 (v/v/v) mixture of steam pasturized Weblite ^{R} (Weblite Corp., Inc., Roanoke, VA 24061), vermiculite and peat moss with 1 g of 14-14-14 (N-P-K) Osmocote (Sierra Chemical Co., Milpitas, CA 95035) added per pot.

Seedlings were grown in a greenhouse supplied with charcoal-filtered air to reduce ambient O_3 concentrations to approximately 0.025 ppm. Greenhouse temperatures ranged from 20-35^{o}C and lighting was supplemented (approx. 600 umol/m^2/s photon flux densitiy) to maintain a 14 hr photoperiod. Pollutant and rain exposures were initiated five weeks after seedling emergence.

Simulated Rain and Ozone Exposures

Rain was applied to the seedlings using a simulator developed on the principle of droplet formation from needle tips (7). Simulated rain solutions, with ionic concentrations similar to the average ambient rainfall in southwestern Virginia (30), were prepared as previously described (7). See Table A-2 for ion concentrations. The pH of the solutions was adjusted to either 5.6, 4.3 or 3.0 by the addition of 1M NaOH or a mixture of 1M H_2SO_4 and 0.5M HNO_3. The sulfate/nitrate ratio was maintained at 2.2:1 in all solutions. Rain was applied to the plants at a rate of 0.75 cm/hr for 1 hr, 2 times a week for 5 weeks, either just before or just after fumigation with O_3.

Seedlings were exposed to either 0.00, 0.05, 0.10 or 0.15 ppm O_3, 4 hr a day (0900-1300), 5 days a week for 5 weeks in 12 continuously stirred tank reactors (CSTRs) (16). Environmental conditions within the CSTRs were maintained at 24 ± 2^{o}C, 55 ± 10% RH and 600-700 umol/m^2/s photon flux density. The concentrations of O_3 used were realistic since O_3 episodes commonly occur in the eastern United States having durations of 1-3 days, with peak hourly means often exceeding 0.10 ppm (30).

Ozone was generated with a Model T-408 Welsbach Laboratory Ozonator (Welsbach Ozone Systems Corp., Philadelphia, PA 19129) and monitored with a Dasibi Model 1003-RS Ozone Analyzer (Dasibi Environ. Corp., Glendale, CA 91205). Periodically the monitor was calibrated with a Photocal 3000 Automated Ozone Calibration System (Columbia Scientific Industries, Austin, TX 78776), according to USEPA quality assurance methods.

Measurement of Plant Response

Every seven days, starting with the first day of fumigation, seedling height was measured from the cotyledonary node to the base of the terminal bud, and diameter measured at the cotyledonary node, using a calipers accurate to 0.10 mm. Cumulative increases (height and diameter), measured at weeks one through five were calculated for each treatment. Seedlings were periodically examined for visible foliar injury.

Twenty plants were harvested initially (5 weeks postemergence), dried at 70°C for 36 hr, and mean root, leaf and stem dry weights determined (0.02, 0.01 and 0.01

g, respectively). At the termination of the experiment, all seedlings were harvested and dry weights determined. Mean relative growth rates and root/shoot ratios were then calculated using classical growth analysis (18): mean relative growth rate (RGR) for each plant component= [ln(finial dry weight)- ln(mean initial dry weight)]/number of weeks; TRGR, RGSTEM, RGROOT and RGLEAF= mean total, stem, root and leaf relative growth rates; and root/shoot ratio (RSR)= root dry wt./(stem + leaf dry wt.). Chlorophyll content of the leaf (simple leaf) at the 3rd node from the cotyledonary node for each seedling, was determined at the end of the study using the ethanol extraction procedure of Knudson et al. (22).

Experimental Design and Data Analysis

The overall design for fumigation exposures was a split-plot, replicated three times. Three blocks of 4 CSTRs each were used to allow for variation among chambers due to differences in temperature, RH and light intensity. Whole plot treatments were O_3 concentrations, with one O_3 concentration/CSTR. Sub-plot treatments were a 3 X 2 factorial combination of three simulated rain pHs and two application times of rain. Each treatment combination was replicated 2 times/CSTR (12 trees/CSTR, 36 trees/O_3 treatment, 48 trees/rain pH, 72 trees/rain application time, 144 total trees). The experiment was repeated twice and, due to similar statistical results, data for each replicate were combined for further analysis.

Data were analyzed by analysis of variance (ANOVA). Analysis of covariance (COANOVA) was utilized to determine if any growth component was significantly influenced by a covariate (initial seedling height, 5 wks postemergence). Variance for O_3 treatments was partitioned into linear, quadratic and cubic components and variance for rain treatments was partitioned into linear and quadratic components using orthogonal polynomial contrasts (31). Six orthogonal contrasts using fixed levels of O_3 across either linear or quadratic components of rain pH were developed to determine if significant combined effects of rain pH and O_3 occurred. These contrasts included: 1) control (0.00 ppm) vs linear; 2) 0.05 ppm vs linear; 3) 0.10 ppm vs linear; 4) 0.15 ppm vs linear; 5) control vs quadratic; and 6) 0.10 ppm vs quadratic.

RESULTS

The mean squares for dry matter production, chlorophyll content and plant growth components are presented in Table A-3. Initial seedling height was a significant covariate for all variables, except RSR, and the data were adjusted appropriately for this covariate. Significant linear responses ($p < 0.05$) with increasing O_3 concentrations from 0.00 to 0.15 ppm were observed for root, leaf and total dry weight, HTI (total height increase), TRGR and RSR. No significant effects were observed for TCHL (total chlorophyll content) or DIA (total stem diameter increase) in response to O_3 fumigation.

The cumulative shoot height growth curve of white ash seedlings exposed to increasing O_3 concentrations is shown in Fig. 9. Height growth was less than controls when plants were fumigated with O_3 concentrations greater than 0.05 ppm. This inhibition was linear in response ($p < 0.05$) to increasing O_3 concentrations and after 5 weeks of exposure, cumulative shoot elongation was 13% less in seedlings exposed to 0.15 ppm O_3 than controls. Differences between treatments first became apparent after three weeks of exposure.

At O_3 exposures of 0.10 and 0.15 ppm, growth inhibition of leaf and total dry weight became evident (Fig. 10). Root dry weight was less than controls at any O_3 concentration greater than 0.00 ppm. These decreases fit a linear response ($p < 0.05$) for all biomass variables (root, leaf and total dry weight and RSR), except stem dry weight. A 26, 11, 20 and 16% loss in root, leaf and total dry weight and RSR, respectively, occurred when 0.15 ppm

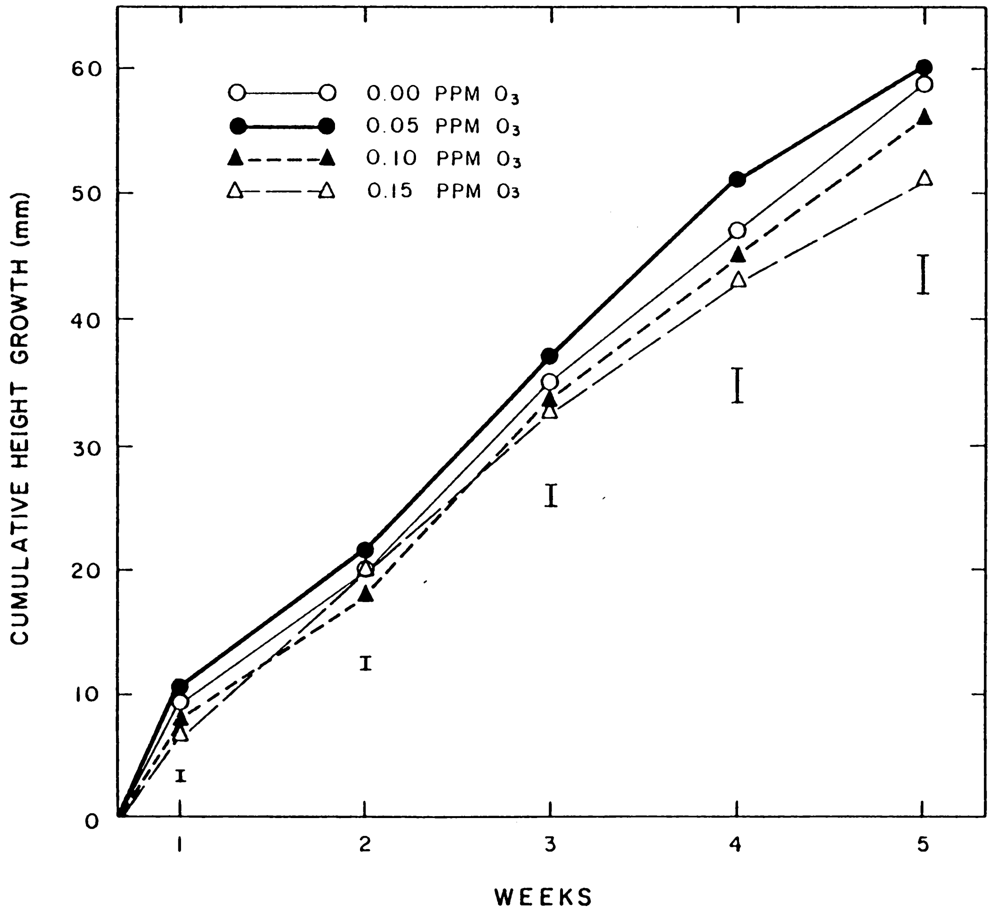

Figure 9. Cumulative shoot height increase (mm) of white ash seedlings during 5 weeks of fumigation with ozone. Vertical bars represent least significant difference (LSD, p = 0.05). Data are adjusted for initial seedling height.

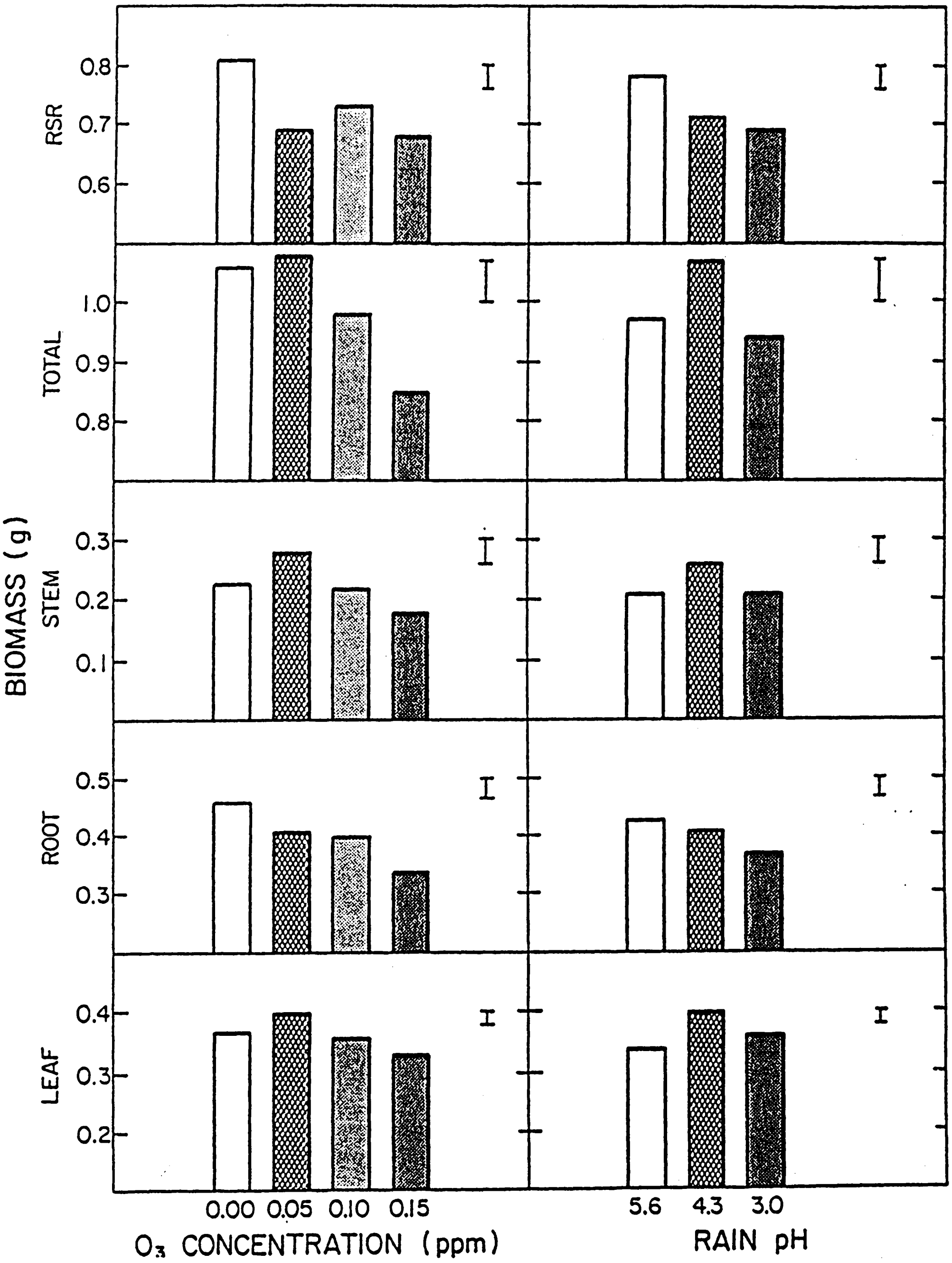

Figure 10. Mean dry weight of 10-wk-old white ash for rain pH treatments by O_3 treatment. RSR = root/shoot (above ground biomass) ratio (w/w). Vertical bars represent least significant difference (LSD, $p = 0.05$). Data are adjusted for initial seedling height.

O_3-treated plants were compared with controls.

Changes in mean relative growth rates for all plant components in response to increasing O_3 concentrations are shown in Fig. 11. Significant linear decreases ($p < 0.05$) were observed for all relative growth rates as O_3 concentration increased. An 8, 7, 4 and 10% inhibition in TRGR, RGSTEM, RGLEAF and RGROOT, respectively, occurred when 0.15 ppm O_3-treated plants were compared with controls.

Significant linear responses ($p < 0.05$) for rain pH occurred with root dry weight and RSR. These growth variables were 12% less in plants treated with pH 3.0 rain than in plants exposed to pH 5.6 rain solutions (Fig. 10). A significant linear decrease ($p < 0.05$) in RGROOT also occurred with increasing rain acidity (Fig. 11).

Significant quadratic responses ($p < 0.05$) were observed for stem, leaf and total dry weight (Fig. 10), TRGR, RGSTEM and RGLEAF (Fig. 11), HTI (Fig. 12), and DIA. Growth was the greatest for seedlings treated with pH 4.3 and the least for those exposed to either pH 5.6 or 3.0 rain solutions. Stem diameter increase (DIA), although not shown, followed the same pattern. No significant differences in TCHL were observed among any rain pH treatments.

The cumulative shoot height growth curve of white

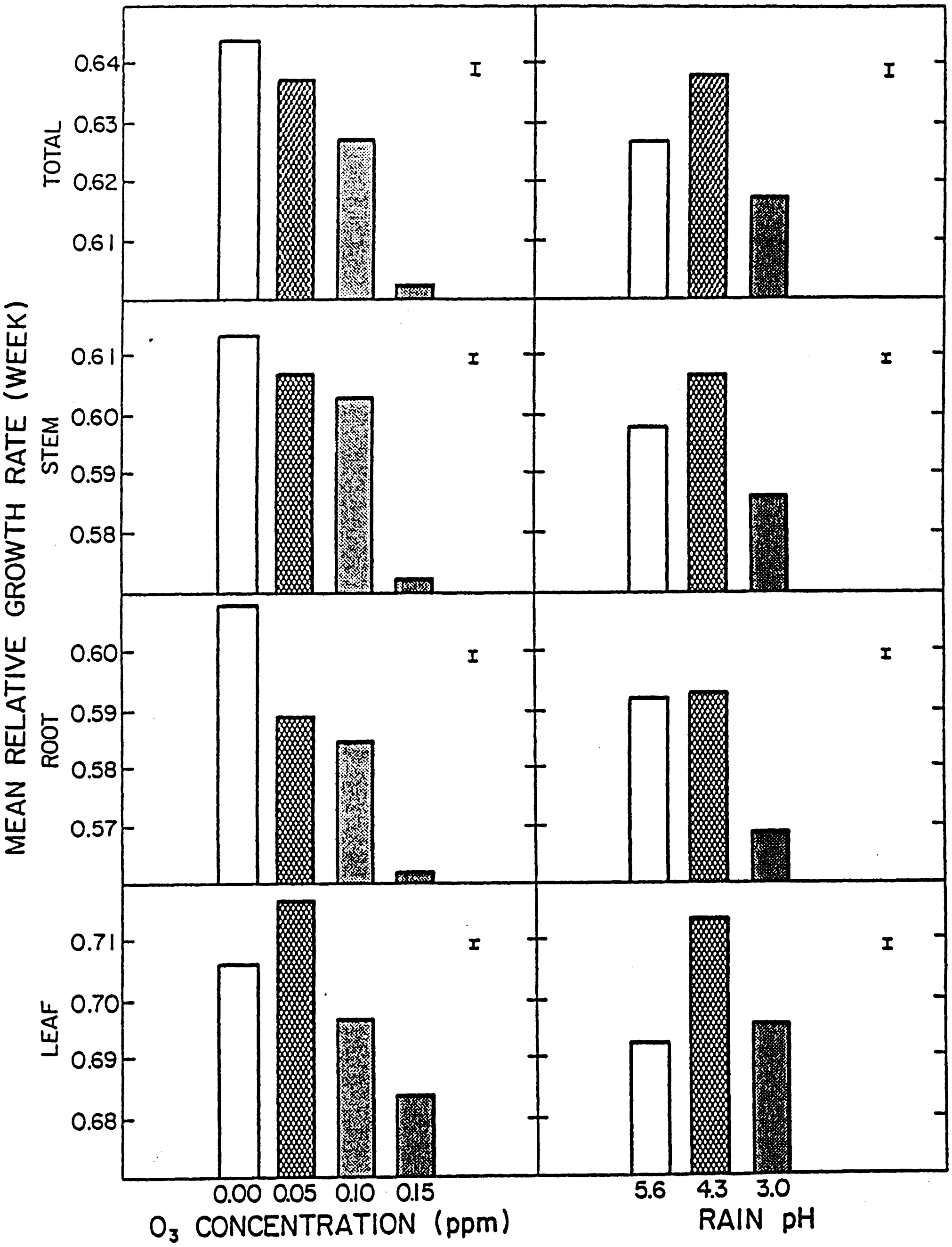

Figure 11. Mean relative growth rates (per week) of 10-wk-old white ash for rain pH treatments by O_3 treatment. Vertical bars represent least significant difference (LSD, $p = 0.05$). Data are adjusted for initial seedling height.

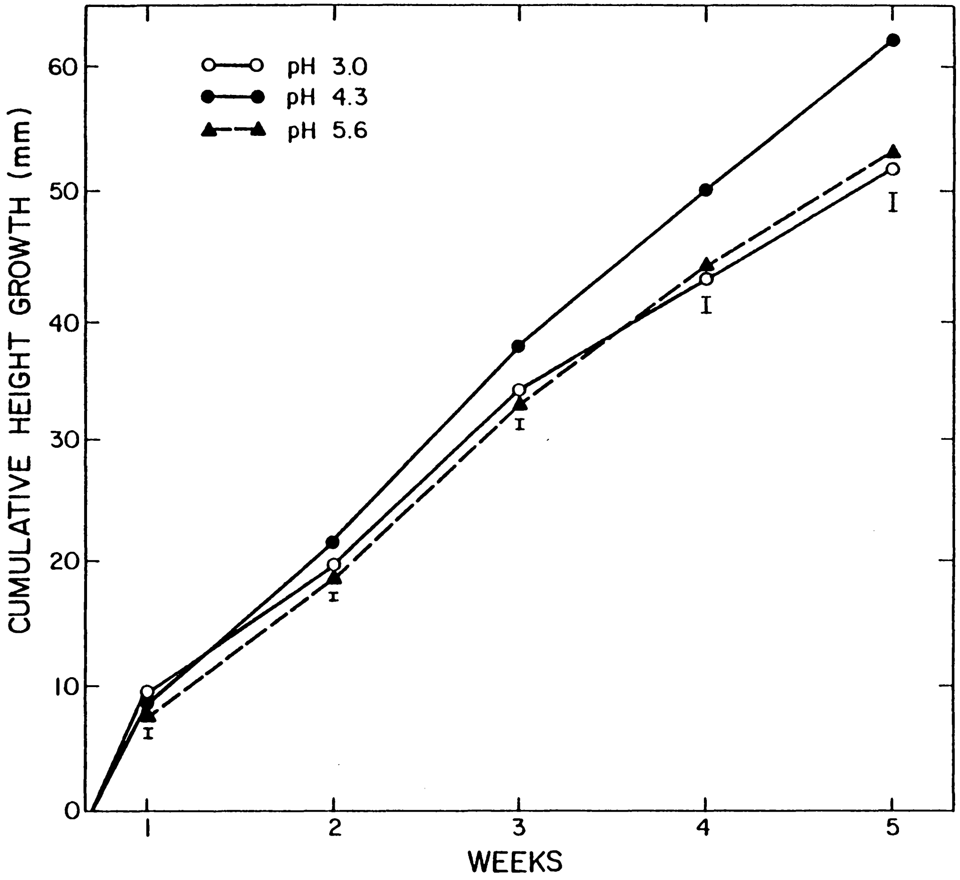

Figure 12. Cumulative shoot height increase (mm) of white ash seedlings during 5 weeks of simulated rain application. Vertical bars represent least significant difference (LSD, p = 0.05). Data are adjusted for initial seedling height.

ash seedlings exposed to decreasing rain pH is shown in Fig. 12. Seedlings exposed to pH 4.3 rain for 5 weeks had 13% greater HTI than plants treated with either pH 5.6 or 3.0 rain solutions. Differences in treatments became apparent after two weeks of rain exposure.

Visible foliar symptoms developed on plants exposed to 0.15 ppm O_3 and/or pH 3.0 solutions. Ozone symptoms first appeared on the older leaves beginning with the third week of fumigation, and were characterized initially by purple-white stipples on the adaxial leaf surface. Stipples generally coalesed and eventually resulted in tan-brown necrotic lesions. Symptoms occurred on 61% of the plants treated with 0.15 ppm O_3 (n= 72).

Acid rain injury was characterized by distinct, necrotic, circular lesions (0.5-1.0 mm dia) on the adaxial leaf surface, located at or near vascular tissue. New, fully-expanded leaves appeared the most sensitive. Lesions were widely scattered and approximately 0.5% of the leaf area exhibited macroscopic injury. Symptoms were observed on 61% of the plants exposed to pH 3.0 solutions (n= 96). Incidence of foliar injury resulting from combined pollutant treatments occurred on 54% of the plants treated with 0.15 ppm O_3 and pH 3.0 simulated rain (n= 24).

At an O_3 concentration of 0.05 ppm, root dry weight, RGROOT and RSR were 23, 8 and 20% less ($p < 0.05$) for seedlings treated with pH 3.0 solutions compared to seedlings exposed to seedlings exposed to pH 5.6 simulated rain (Table 9). At 0.10 ppm O_3, significant differences ($p < 0.05$) of 7 and 23% in RGROOT and RSR, respectively, occurred between seedlings exposed to pH 3.0 compared with those treated with pH 5.6 solutions.

The timing of rain application relative to O_3 exposure did not alter any growth variable measured. In addition, no statistically significant interactions occurred with any treatment combination.

DISCUSSION

Results from these experiments demonstrate that O_3 and simulated acid rain, alone or in combination, can alter the growth of white ash seedlings under controlled environmental exposure conditions. Increasing O_3 concentrations greater than 0.05 ppm O_3 resulted in less growth for the majority of variables measured. Root biomass, however, was less than controls when fumigated with O_3 at any concentration (0.05, 0.10 and 0.15 ppm). Kress and Skelly (24) reported that low concentrations (0.05 ppm) caused an initial stimulation in growth of white ash. A substantial inhibition of growth was observed, however, at

Table 9. Effects of ozone and rain pH treatments on root dry weight (g), mean relative root growth ratio (RGROOT, per week) and root/shoot ratio (RSR, g/g).[a]

	Root dry weight			$\overline{\text{RGR}}$OOT (week^{-1})			RSR		
	rain pH								
Ozone (ppm)	5.6	4.3	3.0	5.6	4.3	3.0	5.6	4.3	3.0
0.00	0.44	0.50	0.44**	0.60	0.63	0.61	0.79	0.79	0.85
0.05	0.47	0.40	0.36*	0.61	0.60	0.56*	0.79	0.64	0.63*
0.10	0.43	0.44	0.35	0.60	0.60	0.56*	0.82	0.75	0.63*
0.15	0.36	0.31	0.34	0.56	0.54	0.55	0.71	0.66	0.68

[a] * = sig. linear effects ($p < 0.05$), ** = sig. quadratic effects ($p = 0.06$).

higher concentrations (0.10-0.15 ppm), with root dry weight being the most sensitive variable measured. Our results support those of Kress and Skelly (24).

The inhibition in root biomass, RSR and RGRs is a reflection of the affect of increasing O_3 concentrations on physiological plant processes. Relative growth rate is a measure of plant productivity and expresses the efficiency of the plant as a producer of new material (18). When RGR was divided into its component parts, O_3 effects were most apparent on root growth, as reflected by losses in root dry weight, RGROOT and RSR.

Growth inhibition could result from a decrease in photosynthesis (5), an increase in respiration (3) or a decrease in the the rate of translocation of photosynthate from the leaves to the roots (21,27,32). Jensen (21) reported that in Fraxinus pennsylvanica Marsh. seedlings exposed to O_3 for six weeks, stem and leaf dry weight was less than in control plants. Roots of fumigated seedlings contained significantly less starch, sucrose and reducing sugars than nonfumigated plants. He concluded that this decrease in root carbohydrate content resulted from the inability of leaves to replenish depleted food reserves in the roots. Similar results have been reported for coniferous tree species (27,32), where assimilated substances (sugar and starch) remained in the foliage and were not translocated to the roots.

Increases in simulated rain acidity resulted in linear or quadratic growth responses, depending upon the variable measured. The linear decrease in RSR in seedlings exposed to pH 3.0 solutions compared to plants treated with pH 5.6 solutions resulted from a lower rate of root growth and supports the studies of Ferenbaugh (14), Neufeld et al. (28) and Lee et al. (25), which indicate that acid rain treatments cause significant reductions in root biomass. Ferenbaugh (14) attributed decreases in root dry weight in Phaseolus vulgaris L. plants to a reduction in photoassimilates in the leaves available for translocation. Neufeld et al. (28) reported similar results for several species of tree seedlings.

A significant quadratic response occurred with simulated rain exposure for all above-ground growth variables measured. Chappelka et. al. (6) reported similar results in Liriodendron tulipifera L. seedlings for leaf dry weight. Lee et al. (25) observed a stimulation in above-ground biomass for several agronomic crop species at pHs 3.5 and 4.0, compared with plants treated with pHs 3.0 or 5.6. Reasons for this stimulation in growth are unknown, but may be related to increased nutrient availability and uptake by certain plants in moderately acidic environments (4).

This is the first report of visible foliar injury resulting from simulated acid rain exposure for white ash, and is coincident with observations reported by other researchers (1,11,12) with other plant species.

The timing of the rain application did not affect any growth variable for white ash. Chappelka et al. (6) and Elkiey and Ormrod (10) found growth was significantly inhibited when plants were fumigated with wet leaf surfaces versus those with a dry leaf surface. Our results suggest that this effect may be species specific and could be related to leaf wetability and leaf surface characteristics (15), or different environmental conditions during fumigation (light, temp, RH, etc.).

The combination of O_3 and simulated acid rain resulted in linear reductions in root dry weight, RGROOT and RSR at O_3 concentrations (0.05 and 0.10 ppm) and rain pHs ($\leq$ 4.3) which are common in ambient field conditions (30). Chappelka et al. (6) observed similar results in *L. tulipifera* seedlings. Ozone exposures resulted in linear reductions in root dry weight, TRGR, leaf area increase and unit leaf rate (net assimilation rate), as rain acidity increased.

The variation in seedling growth resulting from O_3 as rain pH decreases may be related to the chemical activity of this gas at different solution pHs (17). In alkaline solutions O_3 is rapidly decomposed and is more soluble than O_2, but in acidic conditions O_3 is considerably more stable.

Changes in nutrient availability and mobilization response to ozone and acid solutions may affect translocation of photoassimilates and thereby alter tree growth. Laboratory experiments in West Germany (23) demonstrated that increased foliar leaching of certain nutrients from Norway spruce (Picea abies L., Karst.) needles occurred with the combination of O_3 and acidic mists. Skeffington and Roberts (29) found that increasing concentrations of O_3 resulted in increased concentrations of most ions in new needles of Pinus sylvertris L. as well as a decrease only in P content in older needles. Ozone also caused a reduction of the foliar uptake of NO_3 from the acid mist pollution.

This current work represents the first report of the effects of O_3 and acid rain on white ash seedling growth. Although 0.15 ppm O_3 depressed plant growth more than any other treatment, the combination of 0.05 or 0.10 ppm O_3 and acid rain caused significant reductions in certain growth variables under controlled environmental conditions. Chappelka et al. (6) have similarly reported that the combination of these pollutants in the laboratory can inhibit yellow-poplar seedling growth. Additional research is needed, however, using different tree species, soil types and field tests to determine if such effects are occurring in natural forest ecosystems.

LITERATURE CITED

1. Adams, C. M., N. G. Dengler and T. C. Hutchinson. 1984. Acid rain effects on foliar histology of Artemisia litesii. Can. J. Bot. 62:463-474.

2. Anonymous. 1965. Silvics of Forest Trees of the United States. USDA Agric. Handb. No. 271.

3. Barnes, R. L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut. 3:123-138.

4. Blamey, F. P. C., D. G. Edwards, C. J. Asher and Moo-Key Kim. 1982. Response of sunflower to low pH. pp. 66-72. In Plant Nutrition 1982, Vol. 1., Avon Litho Ltd., Stratford-upon-Avon, UK.

5. Carlson, R. W. 1979. Reduction in the photosynthetic rate of Acer, Quercus and Fraxinus species caused by sulphur dioxide and ozone. Environ. Pollut. 18:159-170.

6. 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. Expt. Bot. 25:233-244.

7. Chevone, B. I., Y. S. Yang, W. E. Winner, I. Storks-Cotter and S. J. Long. 1984. A rainfall simulator for laboratory use in acidic precipitation studies. J. Air Pollut. Contr. Assoc. 34:355-359.

8. Duchelle, S. F., J. M. Skelly and B. I. Chevone. 1982. Oxidant effects on forest tree seedlings in the Appalachian Mountains. Water, Air and Soil Pollut. 16:363-373.

9. Duchelle, S. F., J. M. Skelly, T. L. Sharick, 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 National Park of Virginia. J. Environ. Manag. 17:294-308.

10. Elkiey, T. and D. P. Ormrod. 1981. Sulphur and nitrogen nutrition and misting effects on response of bluegrass to ozone, sulpher dioxide, nitrogen dioxide

or their mixture. Water, Air, and Soil Pollut. 16: 177-186.

11. Evans, L. S., N. F. Gmur and F. DaCosta. 1977. Leaf surface and histological perturbations of leaves of Phaseolus vulgaris and Helianthus anus after exposure to simulated acid rain. Am. J. Bot. 64: 903-913.

12. Evans, L. S., N. F. Gmur and F. DeCosta. 1978. Foliar response of six clones of hybrid poplar to simulated acid rain. Phytopathology 68:847-856.

13. Evans, L. S., G. R. Hendrey, G. L. Stensland, D. W. Johnson and A. J. Francis. 1981. Acidic precipitation: Considerations for an air quality standard. Water, Air, and Pollut. 16:469-509.

14. Ferenbaugh, R. W. 1976. Effects of simulated acid rain on Phaseolus vulgaris L. (Fabaceae). Am. J. Bot. 63:283-288.

15. Haines, B. L., J. A. Jernstedt and H. S. Neufeld. 1985. Direct foliar effects of simulated acid rain II. Leaf surface characteristics. New Phytol. 99: 407-416.

16. 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.

17. Horvath, M., L. Bilitzky and J. Huttner. 1985. Ozone. Elsevier Sci. Publ. Co., New York.

18. Hunt, R. 1982. Plant Growth Curves. Univ. Park Press, Baltimore.

19. Jacobson, J. S. 1984. Effects of acid aerosol, fog, mist and rain on crops and trees. Phil. Trans. Roy. Soc. London (B) 305:327-328.

20. Jensen, K. F. 1973. Response of nine forest tree species to chronic ozone fumigation. Plant Dis. Rep. 57:914-917.

21. Jensen, K. F. 1981. Ozone fumigation decreased the root carbohydrate content and dry weight of green ash seedlings. Environ. Pollut. (A) 26:147-152.

22. Knudson, L. L., T. W. Tibbitts and G. E. Edwards. 1977. Measurement of leaf chlorophyll concentration. Plant Physiol. 60:606-608.

23. Krause, G. H. M., K. D. Jung and B. Prinz. 1984. Forest effects in West Germany. pp. 297-332. In: Symposium on Air Pollution and the Productivity of the Forest. Izaak Walton League of America, Arlington, VA.

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

25. Lee, J. J., G. E. Neely, S. C. Perrigan and L. C. Grothaus. 1981. Effect of simulated sulphuric acid rain on yield, growth and foliar injury of several crops. Environ. Expt. Bot. 21:171-185.

26. McLaughlin, S. B. 1985. Effects of air pollution on forests: A critical view. J. Air Pollut. Contr. Assoc. 35:512-534.

27. 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.

28. Neufeld, H. S., 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.

29. Skeffington, R. A. and T. M. Roberts. 1985. The effects of ozone and acid mists on Scots pine saplings. Oecologia 65:201-206.

30. 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. pp. 69-73. In: Inernt. Symp. Hydromet. Amer. Water Res. Assoc.

31. Steele, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York.

32. Tingey, D. T., R. G. Wilhour and C. Standley. 1976. The effect of chronic ozone exposures on the metabolitic content of ponderosa pine seedlings. For. Sci. 22:234-241.

CHAPTER IV

ALTERATION OF GROWTH AND GAS EXCHANGE OF YELLOW-POPLAR SEEDLINGS BY OZONE AND SIMULATED ACID RAIN

INTRODUCTION

Recently, much concern has developed over increases in rainfall acidity, due to wet deposition of sulfur and nitrogen compounds, and its potential impact on terrestrial ecosystems (24). At the present time, there is limited information demonstrating effects of acid rain on plant growth at hydrogen ion concentrations similar to those occurring naturally (20).

In contrast, ozone (O_3) is considered the major phytotoxic pollutant in eastern North America (24) and has been reported as causing significant reductions in plant growth. These effects can occur even in the absence of macroscopic, foliar symptoms (4,6,16).

Exposure of trees to air pollutants can cause various adverse effects such as, foliar discoloration, premature defoliation, and reductions in height growth and biomass productivity (3,4,6). Growth inhibition may result from a decrease in photosynthesis (2,28), an increase in respiration (1), a decrease in the rate of translocation of photosynthate from leaves to other plant parts (22,26), or a combination of these factors (1,26).

Previous studies (3,4) have shown that gaseous air pollutants in combination with simulated acid rain can cause an alteration in growth of white ash (Fraxinus americana L.) and yellow-poplar (Lirodendron tulipifera L.) seedlings, under controlled environmental conditions. The mechanism(s) underlying such effects, however, are not well understood. The purpose of this study, therefore, was to examine the alterations in gas exchange characteristics and growth of yellow-poplar seedlings in response to low concentrations of O_3 and simulated acid rain. The specific objectives were 1) to examine the effects of four different O_3 concentrations (0.00, 0.05, 0.10 and 0.15 ppm) and three levels of simulated rain (pHs 5.6, 4.3 or 3.0) on the growth of yellow-poplar seedlings, under laboratory exposure conditions and 2) to determine the effects of O_3 (0.00 and 0.10 ppm) and simulated acid rain (pHs 5.6, 4.3 or 3.0) on yellow-poplar seedling gas exchange characteristics, under laboratory exposure conditions.

METHODS AND MATERIALS

Plant Material

Yellow-poplar seed were collected from a single tree on the campus of Virginia Polytechnic Institute and State University in order to minimize variability in any genotypically controlled response to air pollutant stress. Seedlings collected from this source are reported as being intermediate to tolerant in sensitivity to O_3 (3,19). Seeds for the two experiments reported in this study, were collected in different years. Seeds were surface sterilized with 0.2% sodium hypochlorite, rinsed in tap water, stratified at 4°C for 12 weeks, then sown in flats containing vermiculite. Five-week-old seedlings were transferred to 10-cm diameter plastic pots containing a 2:2:1 (v/v/v) mixture of steam pasteurized Weblite ^{R} (Weblite Corp., Inc., Roanoke, VA 24061), vermiculite and peat moss with 1 g of 14-14-14 (N-P-K) Osmocote (Sierra Chemical Co., Milpitas, CA 95035) added per pot. Peters', water soluble, general purpose, 20-20-20 (N-P-K) fertilizer (W. R. Grace & Co., Foelsville, PA 18051) was added to the planting medium at a rate of 12 g/1000 ml deionized H_2O (approximately 100 ml solution/pot) during week eight postemergence to ensure adequate plant nutrition. Seedlings were grown in a greenhouse supplied with air filtered by charcoal to reduce ambient O_3 concentrations to approximately 0.025 ppm. Greenhouse temperatures ranged from 20-35°C and ambient light was supplemented with high pressure sodium vapor lamps (approx. 500-600 umol/m^2/s photosynthetic photon flux density, PPFD) to maintain a 14 hr photoperiod. Pollutant and rain exposures were initiated nine weeks after seedling emergence.

Simulated Rain and Ozone Pollutant Exposures: Experiment 1

Rain was applied to the seedlings using a simulator developed on the principle of droplet formation from needle tips (5). Artificial rain solutions, with ionic concentrations similar to the average ambient rainfall in southwestern Virginia (24), were prepared as previously described (5). See Table A-2 for ion concentrations. The pH of the solutions was adjusted to either 5.6, 4.3 or 3.0 by the addition of 1M NaOH or a mixture of 1M H_2SO_4 and 0.5M HNO_3. The sulfate/nitrate ratio was maintained at 2.2:1 in all solutions. Rain was applied to the plants at a rate of 0.75 cm/hr for 1 hr, 2 times a week for 5 weeks, just prior to fumigation with O_3.

Seedlings were exposed to either 0.00, 0.05, 0.10 or 0.15 ppm O_3, 4 hr a day (0900-1300), 5 days a week for 5 weeks in 12 continuously stirred tank reactors (CSTRs) (7). Environmental conditions within the CSTRs were maintained at 30 ± 2^0C, $70 \pm 5\%$ RH and 350-400 umol/m^2/s photon flux density. The concentrations of O_3 used were realistic since O_3 episodes commonly occur in the eastern United States having durations of 1-3 days, with peak hourly means often exceeding 0.10 ppm (24).

Ozone was generated with a Model T-408 Welsbach Laboratory Ozonator (Welsbach Ozone Systems Corp., Philadelphia, PA 19129) and monitored with a Bendix Model 8002 Ozone Analyzer (Bendix Corp., Lewisburg, WV 24901). Periodically, the monitor was calibrated with a Photocal 3000 Automated Ozone Calibration System (Columbia Scientific Industries, Austin, TX 78776), according to USEPA quality assurance methods.

Simulated Rain and Ozone Pollutant Exposures:Experiment 2

Simulated rain and O_3 exposures were similar to experiment 1, except that only 2 concentrations of O_3 were applied to the seedlings (0.00 and 0.10 ppm). Environmental conditions within the CSTRs were different, due to installation of new lights and a temperature control system, and were 25 ± 2^0C, 45 ± 15% RH and 600-700 umol/m^2/s PPFD.

Measurement of Leaf Gas Exchange:Experiment 2

Net photosynthesis (mg $CO^2/m^2/s$) and leaf conductance (cm/s) were measured with a Li-Cor Model LI-6000 Portable Photosynthesis System (Li-Cor Ltd., Lincoln, NE 68504). A cuvette with a volume of 1100 cm^3, and a gas flow rate of 12 ml/s was used. A constant leaf area of 8.45 cm^2 was placed in the cuvette for all gas exchange measurements. Boundary layer resistance within the cuvette was reduced by circulating fans to approximately 0.45 s/cm. Four plants/treatment combination were utilized for gas exchange measurements. Observations were taken 2 times a week for 3 weeks, beginning with the 3rd week of fumigation, immediately after fumigation with O_3, on the leaf at the 6th node from the cotyledonary node (leaf fully expanded at wk 3). Average environmental conditions in the leaf chamber were leaf temperature= 32.1 $\pm$ 1.48^{0}C, chamber temperature=32.3 $\pm$ 1.34^{0}C, RH=27.1 $\pm$ 4.6%, photon flux density=635.9 $\pm$ 19.6 umol/m^2/s, CO_2 concentration=373.2 $\pm$ 20.6 ppm, and vapor pressure deficit (VPD)=3.53 $\pm$ 0.31 kPa. The infrared gas analyzer was calibrated twice during the study using a Li-Cor model 6000-01 gas calibration cylinder, with CO_2 concentrations of 350 and 400 ppm.

Measurement of Plant Response

Measurements to determine plant response to O_3 and acid rain exposures were similar for both experiments. Every seven days, beginning with the first day of fumigation, seedling height was measured from the cotyledonary node to the base of the terminal bud. Cumulative increases, measured at weeks one through six were calculated for each treatment. Seedlings were periodically examined for visible foliar injury.

Ten plants were harvested at 9 weeks postemergence (initiation of pollutant exposures), dried at 60^{0}C for 36 hr and mean root, leaf and stem dry weights determined (0.08, 0.14 and 0.08 g, respectively). At the termination of the experiment(s), all seedlings were harvested, dried and biomass determined as previously described.

Total leaf area (ALA) was determined by measuring the length and width (LW) of each leaf/plant at 2-week intervals, beginning with the first day of fumigation for experiment 1 and at the initiation and termination of experiment 2. One hundred twenty five leaves in all ontogenilogical stages were selected, LW determined, and ALA measured using a Li-Cor Model 3000 Portable Area Meter. A regression equation relating ALA to LW was then developed: $Y= -0.66 + 0.80X$, $R^2=0.98$, where y=ALA and X=LW. This equation was then used to estimate leaf area.

Relative growth responses were calculated using growth analysis (9,17). The following growth parameters were calculated: Mean relative growth rate for total (TRGR), stem (RGSTEM), root (RGROOT) and leaf (RGLEAF) relative growth rates; leaf area ratio (LAR)=leaf area/ plant dry wt; leaf wt ratio (LWR)=leaf dry wt/total plant dry wt; specific leaf area (SLA)=leaf area/leaf dry wt;

root/shoot ratio (RSR)=root wt/(stem+leaf dry wt); and mean unit leaf rate (ULR), (net assimilation rate)= [(-final plant dry wt-initial dry wt)/wk] X [(ln final leaf area)-ln initial leaf area)]/(final leaf area-initial leaf area).

Experimental Design and Data Analysis: Experiment 1

A completely randomized design, replicated 3 times (3 chambers per pollutant treatment) was used. Data were analyzed by analysis of variance (ANOVA) as a 4 X 3 factorial combination of O_3 and simulated rain pH treatments (6 trees/treatment combination, 72 total trees). This experiment was repeated 2 times (144 total trees).

Analysis of covariance (COANOVA) was utilized to determine if any growth component was significantly influenced by a covariate, and the data were adjusted where appropriate. Variance for O_3 treatments was partitioned into linear, quadratic and cubic components and variance for rain pH treatments was partitioned into linear and quadratic components using orthogonal polynomial contrasts (25).

Six orthogonal contrasts using fixed levels of O_3 across either linear or quadratic components of rain pH treatments were developed to determine if a combined effect of O_3 and simulated rain occurred. These contrasts included: 1) control (0.00 ppm) vs linear; 2) 0.05 ppm vs linear; 3) 0.10 ppm vs linear; 4) 0.15 ppm vs linear; 5) control vs quadratic; and 6) 0.10 ppm vs quadratic.

Experimental Design and Data Analysis: Experiment 2

A completely randomized design replicated 3 times was used. Data were analyzed by ANOVA as a 2 X 3 factorial combination of O_3 treatments and simulated rain pH treatments (6 trees per treatment combination, 36 total trees). The experimental design for leaf gas exchange was similar, except time (measurements taken 6 times over a 3 wk period) was included as a block variable.

As previously mentioned for experiment 1 , COANOVA was used to determine if a significant covariate was present. Variance for rain pH treatments was partitioned as described for experiment 1.

Two orthogonal contrasts, using fixed levels of O_3 across linear components of rain pH treatment were developed to determine if a combined effect of O_3 and simulated rain occurred. These contrasts were 1) control vs linear; and 2) 0.10 ppm O_3 vs linear.

RESULTS

Mean dry weights, TRGR and total height increase (HTI) for experiments 1 and 2, across all pollutant treatments are shown in Table 10. Yellow-poplar seedlings

Table 10. Mean dry weights, total mean relative growth rate per week ($\overline{\text{TRGR}}$) and cumulative height increase (HTI, mm) for 15-week-old yellow-poplar, by experiment, across O_3 and rain pH treatments.

Experiment	Biomass (g) stem	root	leaf	total	TRGR	HTI
1	0.33	1.20	1.28	2.83	0.36	60.9
2	0.97	1.13	1.35	3.45	0.40	152.2

from experiment 1 produced substantially less stem biomass (66%), total dry weight (18%) and shoot height growth (60%) than plants from experiment 2. Because of these differences in growth and different environmental conditions during fumigation, the two experiments were not directly compared.

Treatment Effects: Experiment 1:

The mean squares for dry matter production and plant growth components are presented in Table 11. Initial seedling height was a significant covariate for the majority of the variables and the data were adjusted appropriately for this covariate.

Increasing concentrations of O_3 from 0.00-0.15 ppm resulted in significant linear increases ($p=0.05$) in SLA and RSR (Table 11), however, RSR was influenced by a significant O_3 X rain pH interaction. Specific leaf area (Fig. 13) was 9% greater for seedlings fumigated with 0.15 ppm O_3 compared with non-fumigated plants. No other significant main effects occurred for O_3 or rain pH treatments. No symptoms of visible injury due to any O_3 or rain pH treatment were observed.

The combined effects of O_3 X rain pH treatment resulted in significant interactions for mean stem and leaf dry weight, RGSTEM, RGLEAF, RSR and cumulative leaf

Table 11. Mean squares and levels of significance of analysis of variance for dry matter and selected plant growth responses of 15-week-old yellow-poplar exposed to gaseous air pollutants and simulated acid rain under laboratory exposure conditions.

Source of variation	df	Mean squares[a]											
		Biomass											
		stem	leaf	root	total	RSR	HTI	LAI	LAR	LWR	SLA	$\overline{\text{TRGR}}$	$\overline{\text{ULR}}$
Ozone	3												
Linear	1	0.003	0.102	0.083	0.017	0.281*	21.6	301.2	259.1	0.010	8935.6*	0.000	0.135
Quadratic	1	0.002	0.021	0.217	0.185	0.102	61.6	4024.3	106.5	0.000	19.7	0.000	0.351
Cubic	1	0.004	0.047	0.000	0.036	0.000	22.4	7998.7	640.4	0.000	1585.8	0.000	0.661
Rain pH	2												
Linear	1	0.013	0.222	0.132	0.617	0.071	928.7	4414.3	24.1	0.000	983.1	0.002	0.638
Quadratic	1	0.013	0.085	0.028	0.069	0.026	752.3	7046.0	57.0	0.001	619.5	0.000	0.018
O_3 x pH	6	0.020*	0.207*	0.078	0.062	0.128*[c]	424.8	10745.6*[d]	426.7	0.003	1566.7	0.002	2.231
Covariate[b]	1	1.438**	5.763**	1.309**	19.462**	0.967**	7094.3**	221257.5**	162.8	0.006	640.0	0.076**	2.556*
Error	135	0.007	0.102	0.114	0.392	0.067	277.6	5405.7	478.8	0.004	1413.8	0.002	0.597

[a] Calculated F - values significant at the 0.05 or 0.01 levels are denoted by a * or **, respectively; RSR = root/shoot (leaf + stem) ratio (g/g), HTI = total height increase (mm); LAI = leaf area increase (cm^2), LAR = leaf area ratio (cm^2/g), LWR = leaf wt/total wt (g/g), SLA = specific leaf area (cm^2/g), TRGR = tota mean relative growth rate (week) and ULR = mean unit leaf rate ($mg/cm^2/wk$).

[b] Covariate = initial seedling height.

[c] = p = 0.08.

[d] = p = 0.06.

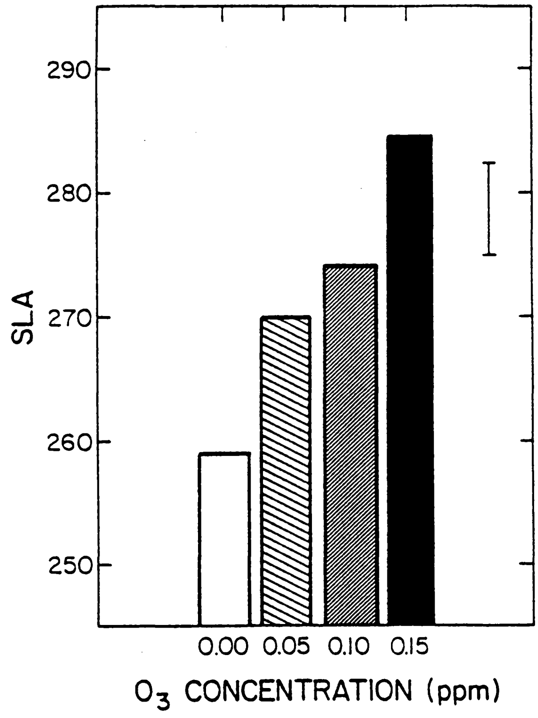

Figure 13. Mean specific leaf area (total leaf area/leaf dry wt. cm^2/g) of 15-wk-old yellow-poplar exposed to four different O_3 concentrations for 6 wks. Vertical bars represent least significant difference (LSD, $p = 0.05$).

area increase (Tables 11 and 12). The mean cumulative leaf area increase for each rain pH treatment within O_3 treatments is graphically represented in Fig. 14. A significant linear decrease and linear increase (p=0.05) occurred for the 0.05 and 0.15 ppm O_3 treatments, respectively, with decreasing rain pH from 5.6 to 3.0, after 6 weeks of exposure. The total cumulative leaf area increase was 19% less in the 0.05 ppm O_3 treatment at pH 3.0 compared with pH 5.6, and increased 18% in the 0.15 ppm O_3 treatments, between these pHs.

Mean stem dry weight exhibited a significant linear decrease (p=0.05) for plants fumigated with 0.05 or 0.10 ppm O_3 as rain pH decreased from 5.6 to 3.0 (Fig. 15). Dry weights were 18 and 24% less for 0.05 or 0.10 ppm O_3 fumigated seedlings, respectively, which were treated with pH 3.0 rain solutions compared with those treated with pH 5.6 solutions. No other contrasts were significant for this variable.

At 0.05 ppm O_3, leaf dry weight exhibited a significant (p=0.01) linear decrease as rain acidity increased (Fig. 16). Mean leaf dry weight was 24% less for 0.05 ppm O_3-treated seedlings exposed to pH 3.0 rain solutions than pH 5.6 solutions. No other linear or orthogonal contrasts were significant for this variable.

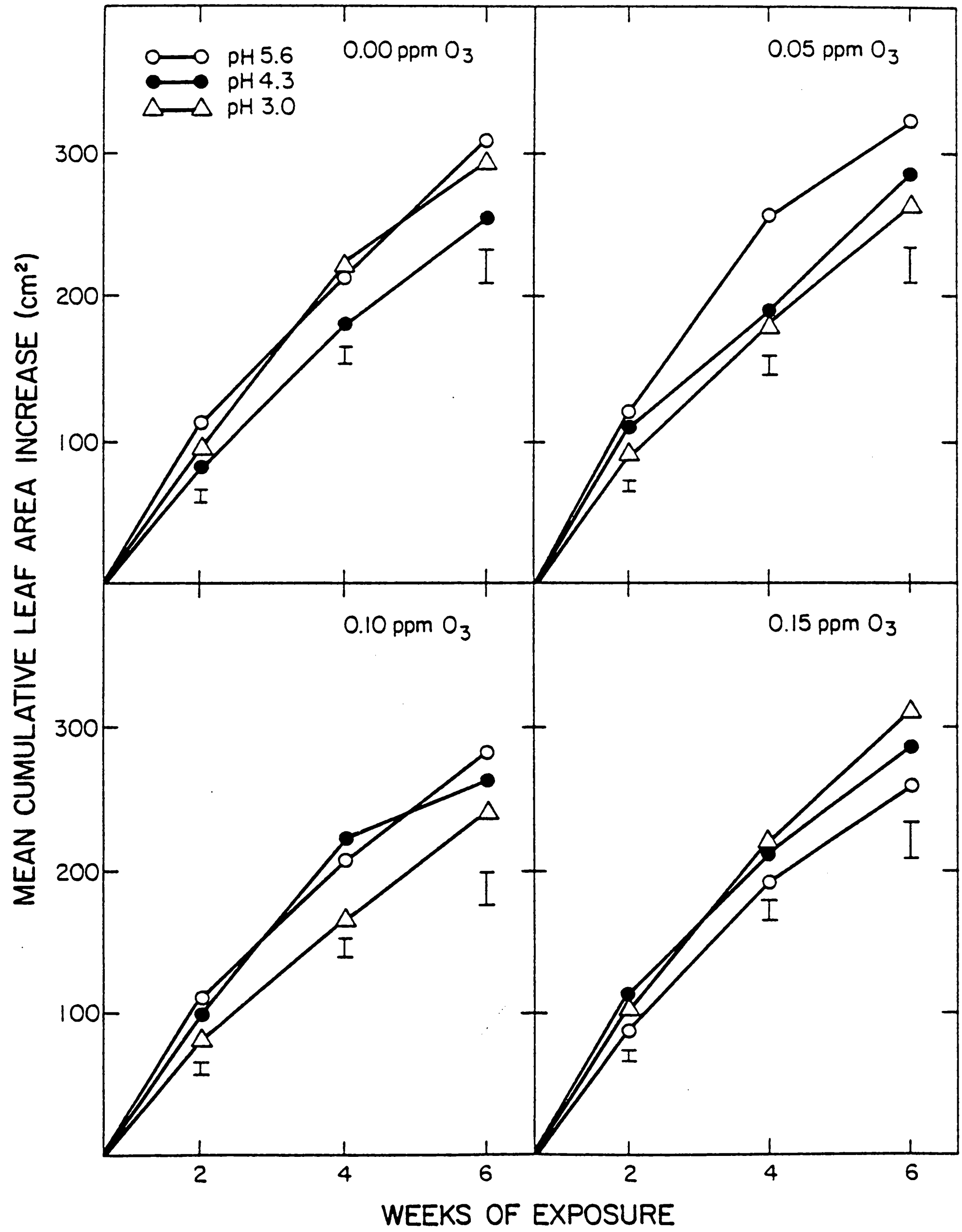

Figure 14. Mean cumulative leaf area increase (cm^2) of yellow-poplar seedlings during 6 wks fumigation for rain pH treatments by O_3 treatments. Vertical bars represent least significant difference (LSD, $p = 0.05$).

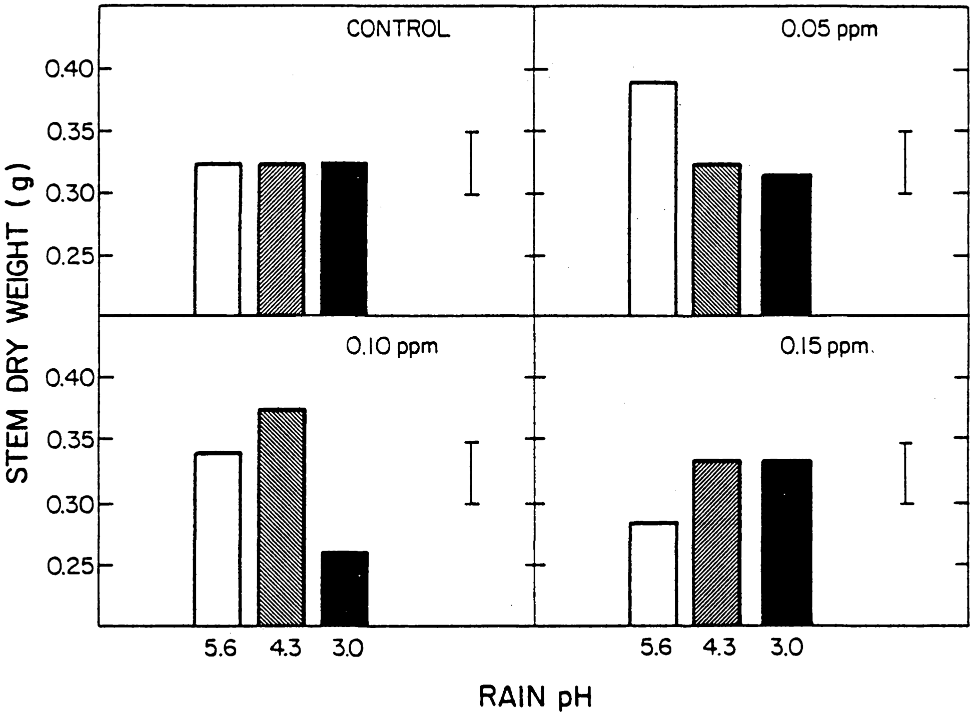

Figure 15. Mean stem dry wt. (g) of 15-wk-old yellow poplar, for rain pH treatment, by O_3 treatment. Vertical bars represent least significant difference (LSD, p = 0.05).

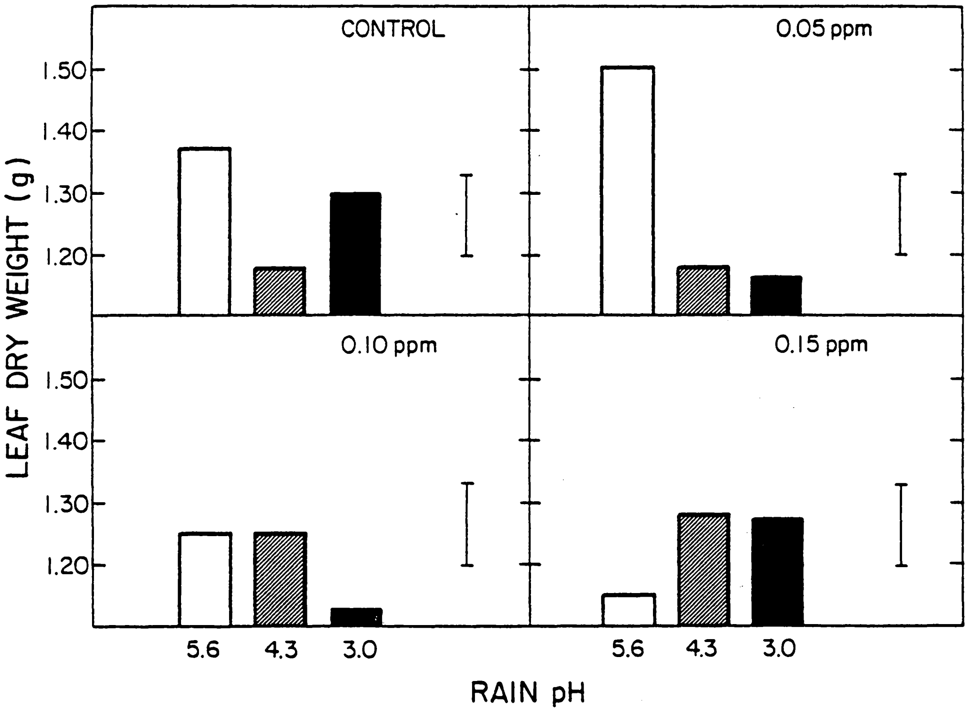

Figure 16. Mean leaf dry wt. (g) of 15-wk-old yellow-poplar, for rain pH treatment, by O_3 treatment. Vertical bars represent least significant difference (LSD, $p = 0.05$).

Table 12. Mean selected growth rates by O_3 concentration and rain pH treatment, for 15-week-old yellow-poplar.[a]

O_3 Conc. (ppm)	RGSTEM			RGLEAF			RSR		
	5.6	4.3	3.0	5.6	4.3	3.0	5.6	4.2	3.0
0.00	0.22	0.23	0.21	0.36	0.35	0.36	0.79	0.78	0.78
0.05	0.25	0.22	0.22	0.39	0.34	0.33*	0.68	0.74	0.80
0.10	0.23	0.24	0.19	0.35	0.35	0.35	0.80	0.69	0.86
0.15	0.18	0.22	0.24*	0.33	0.36	0.36	1.04	0.86	0.73*

[a] Adjusted for initial seedling height; means followed by a * indicates a sig. linear response at the 0.05 level of significance.

Changes in RGSTEM, RGLEAF and RSR for O_3 concentrations within rain pH treatments are shown in Table 12. Significant linear responses (p=0.05) occurred for RGSTEM, RGLEAF and RSR. At 0.05 ppm O_3 RGLEAF was 15% less for plants treated with pH 3.0 rain solutions compared to those treated with pH 5.6. Root/shoot ratio and RGSTEM were 30% less and 25% greater, respectively, for 0.15 ppm fumigated seedlings treated with pH 3.0 than pH 5.6 rain solutions. In addition, RSR was 24% greater for 0.15 ppm O_3-treated plants than controls treated with pH 5.6, but 6% less than controls, for seedlings treated with pH 3.0 rain.

Treatment Effects: Experiment 2:

The mean squares for dry weight production and plant growth components are presented in Table 13. Initial seedling height was a significant covariate for several growth variables and vapor pressure deficit (VPD) was a significate covariate for leaf conductance and net photosynthesis and the data were adjusted appropriately for these covariates.

A significant O_3 treatment effect was observed in LAR and ULR (Table 13). Leaf area ratios were 97 and 84 cm^2/g for controls and fumigated seedlings, respectively. Unit leaf rates were 4.25 and 4.98 $mg/cm^2/wk$ for controls

Table 13. Mean squares and levels of significance of analysis of variance for dry matter, selected plant growth responses and leaf gas exchange characteristics of 15-week-old yellow-poplar exposed to ozone and simulated acid rain, under laboratory exposure conditions.

Source of Variation	df	Biomass (a)				RSR	HTI	LAI	LAR	LWR	SLA	$\overline{\text{TRGR}}$	$\overline{\text{ULR}}$	Leaf gas exchange characteristics[c]	
		stem	leaf	root	total									PHOTO	Cond.
Ozone	2	0.006	0.000	0.048	0.030	0.010	399.1	4831.4	874.9*	0.002	1461.6	0.000	2.971*	0.021	0.196**
Rain pH	2														
Linear	1	0.158	0.054	0.193	1.280	0.002	838.0	17207.4	139.2	0.004	4283.2	0.003	0.003	0.018	0.048*
Quadratic	1	0.101	0.290	0.000	1.004	0.047	1.9	9180.8	171.3	0.006	556.8	0.002	0.398	0.015	0.007
O_3*pH	2	0.160*	0.054	0.107	0.596	0.017	570.1	8963.0	360.7	0.002	1239.2	0.002	2.917	0.027	0.002
Covariate	1	1.113**	0.542*	0.837*	7.514**	0.002	2924.9*	18.8	1779.2**	0.008	2904.0	0.016**	11.682**	0.205**	1.341**
Error	19	0.042	0.080	0.119	0.496	0.011	903.1	7037.2	156.9	0.001	1218.6	0.001	0.629	0.012	0.011

[a] Calculated F-values significant at the 0.05 or 0.01 levels are denoted by a * or **, respectively; RSR = root/shoot (leaf + stem) ratio-(g/g), HTI = total height increase (mm), LAI = leaf area increase (cm^2), LAR = leaf area ratio (cm^2/g), LWR = leaf wt/total wt (g/g), SLA = specific leaf area (cm^2/g), TRGR = total mean relative growth rate (week) and ULR = mean unit leaf rate (mg/cm^2/wk), PHOTO = mean photosynthesis (mg CO_2/m^2/s) and cond. = mean leaf conductance (cm/s).

[b] Covariate = initial seedling height

[c] Covariate - Vapor pressure deficit; Error = 120, a significant block effect was also observed; block = time of measurement.

and fumigated seedlings,respectively. Leaf conductance was 15% less when O_3 treated plants were compared with controls (Table 14). A significant linear response (p=0.05) occurred for leaf conductance for rain pH treatments, across O_3 treatments. Conductances were 9% less for plants treated with pH 3.0 rain solutions than those treated with pH 5.6 solutions. No other significant main effects occurred for O_3 or rain pH treatments. No symptoms of visible injury due to any O_3 or rain pH treatment were observed.

The combined effects of O_3 X rain pH treatment resulted in significant interactions for mean stem dry weight (Table 13) and RGSTEM (p=0.05). For plants treated with 0.10 ppm O_3, mean stem dry weight and RGSTEM exhibited significant linear decreases with decreasing rain pH; there were 33 and 23% decreases in these variables, respectively, when fumigated plants treated with pH 3.0 rain solutions were compared with plants treated with pH 5.6 solutions (Fig. 17).

In addition to these significant interactions ($p < 0.05$) the combined effects of O_3 X rain pH treatment (interactions significant p=0.10) resulted in significant linear responses (p=0.05) for plants treated with 0.10 ppm O_3 with decreasing rain pH, for cumulative leaf area

Table 14. Mean foliar gas exchange characteristics by O_3 concentration and rain pH treatments for 15-week-old yellow-poplar.[a]

	O_3 conc. (ppm)		Rain pH[b]		
	0.00	0.15	5.6	4.3	3.0
leaf conductance (cm/s)	0.536	0.456**	0.524	0.484	0.479*
photosynthesis (mg $CO_2/m^2/s$)	0.266	0.240	0.275	0.237	0.247

[a] Adjusted for vapor pressure deficit; ** = sig. dif. at 0.01 level.

[b] Means followed by a * indicate a sig. linear response at the 0.05 level of significance.

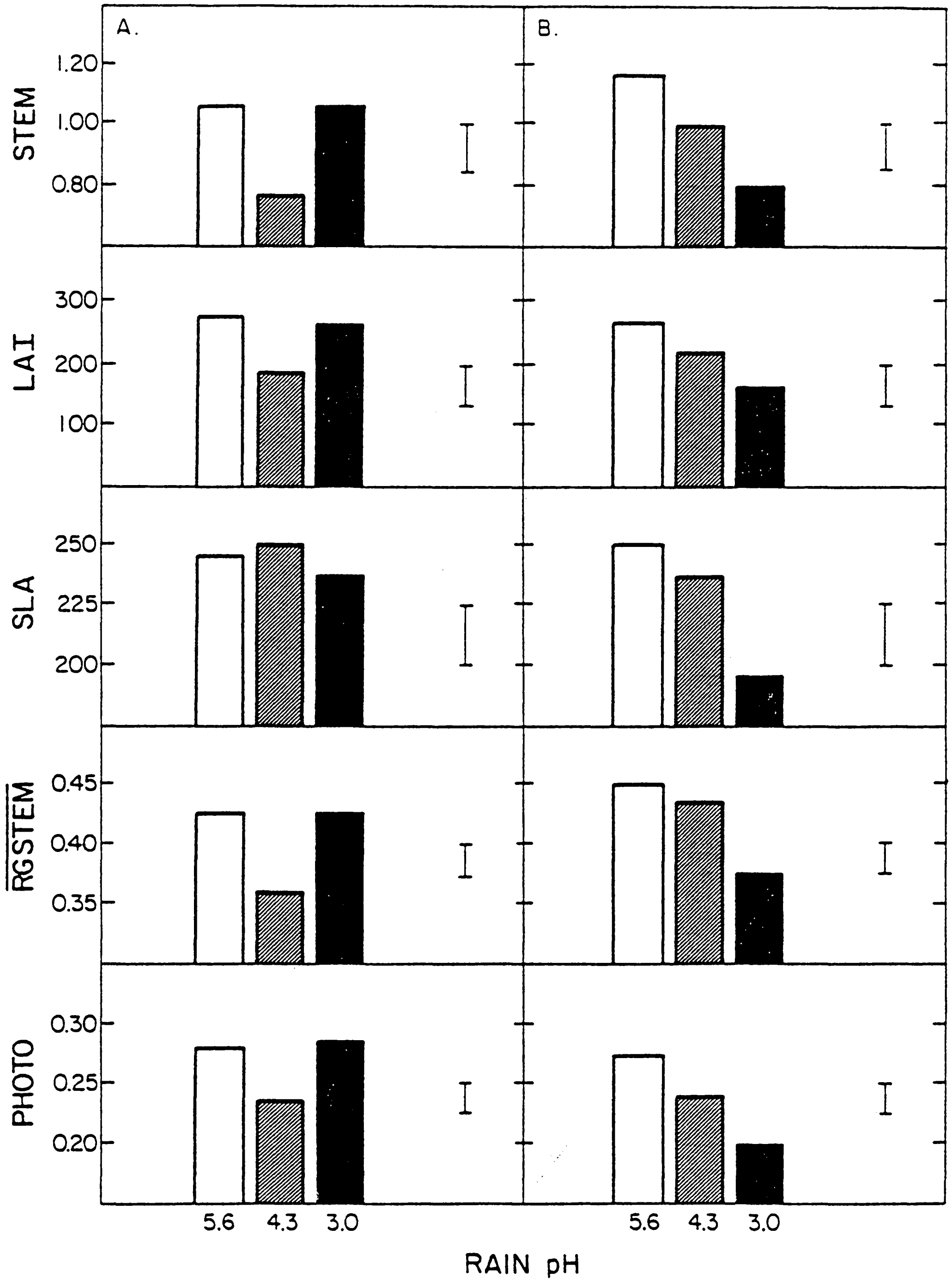

Figure 17. Mean stem dry wt. (g), LAI (cumulative leaf area increase, cm^2), SLA (specific leaf area, cm^2/g), RGSTEM (relative stem growth rate per wk.), and PHOTO (photosynthesis, mg $CO_2/m^2/s$) of 15-wk-old yellow-poplar for rain pH treatment by (a) 0.00 ppm O_3 and (b) 0.10 ppm O_3. Vertical bars represent least significant difference (LSD, p = 0.05).

increase, SLA and net photosynthesis (Fig. 17). There were 40, 22 and 24% decreases in cumulative leaf area increase, SLA and net photosynthesis, respectively, for 0.10 ppm O_3 fumigated plants treated with pH 3.0 rain solutions than those treated with pH 5.6 solutions. There were no significant linear responses observed for any variable examined in seedlings exposed to charcoal-filtered air as rain pH decreased (Fig. 17).

In order to determine if the stomatal response to VPD was altered by O_3 and/or simulated rain, stepwise multiple linear regression was used to generate the best fit model. Model parameters included O_3, rain pH, O_3 X rain pH, VPD, leaf temp., air temp., CO_2 concen., PPFD, O_3 X VPD, rain pH X VPD and the natural logs. of these values. The best fit for leaf conductance was $Y_S = -0.043 - 0.582(\ln VPD) + 0.002(PPFD) - 0.224(O_3 \text{ X } VPD)$, where Y_S=leaf conductance. When the slopes of the regression lines for control; $Y_S = -0.828 - 0.429(\ln VPD)$ and 0.10 ppm O_3-treated plants; $Y_S = 0.320 - 0.731(\ln VPD)$ were compared statistically using indicator variables, they were significantly different ($p=0.05$) indicating that stomatal response to VPD was altered in the presence of O_3. Conductance for O_3-treated plants decreased more rapidly in response to VPD than controls at similar PPFDs. Neither rain pH nor the combination of O_3 X rain pH was found to influence the response of leaf conductance to VPD.

DISCUSSION

Results from these two experiments demonstrate that O_3 and simulated acid rain can alter growth and foliar gas exchange characteristics of yellow-poplar seedlings under controlled environmental conditions. The combination of O_3 and simulated acid rain, in general, caused more deleterious effects than single pollutant exposures (Tables 11 and 13). In this present study, O_3 exposure did not cause alterations in seedling height growth or biomass accumulation which supports the findings of others (3,10,16,19). Exposure to O_3, however, did cause alterations in SLA in experiment 1 and ULR, LAR and stomatal conductance in experiment 2.

Increasing O_3 concentrations from 0.00 to 0.15 ppm caused linear increases in SLA , across rain pH treatments in experiment 1 (Fig. 13). Although not significantly different SLA was slightly lower (5%), for seedlings treated with 0.10 ppm O_3 compared to controls, in experiment 2.

The response of SLA to O_3 has been reported as being variable in other studies (12,13). Jensen (12) with silver maple (Acer sarcharinum L.) reported SLA to be higher for O_3-treated plants compared with controls. Similar to results in this present study, Jensen (13) also reported SLA to be higher for yellow-poplar fumigated with 0.10 ppm O_3 once a week , for 20 weeks, compared with controls; however, SLA was lower than controls when O_3 was applied twice weekly. Reasons for these discrepancies in results are unknown, but SLA is known to be very sensitive to changes in environmental conditions (9). For example, leaves which are grown under lower PPFD have higher SLAs than leaves exposed to higher PPFD (shade vs sun leaves). Different exposure temperatures, PPFD, VPDs, etc., may account for these differences in response, between and among studies.

When yellow-poplar seedlings exposed to 0.10 ppm O_3 were compared with controls (experiment 2), across rain pH treatments, LAR was 13% less and ULR 14% greater for the O_3-treated plants. Jensen (13) reported similar results for yellow-poplar fumigated with 0.10 ppm O_3, 2 times a week, for 20 weeks. He found LAR to be 27% less and ULR to be 16% greater for the fumigated seedlings, after 16 weeks, and concluded that the production of these leaves required large amounts of carbohydrate, which would have been provided by the high ULR. However, when yellow-poplar were fumigated only once a week, he found a 34%

decrease in ULR and no decrease in LAR after 16 weeks. Differences in these results are attributed to changes in environmental conditions during fumigation, however these effects were not quantified. There was no change in ULR and LAR between O_3-treated seedlings and controls in experiment 1, and in another study with yellow-poplar (3).

Stomatal conductance decreased in response to O_3 when plants fumigated with 0.10 ppm O_3 were compared with controls (Table 14). A reduction in stomatal conductance to O_3 has been observed for many plant species (24,28). The mechanism behind this response is still unclear at the present time (27). Although not significantly different, net photosynthesis was also 10% less for O_3-treated plants in this present study, and this decrease may be due in part to the decreases in stomatal conductances observed in these seedlings (23,27).

Ozone was found to modify stomatal sensitivity to VPD in the present study. The alteration of stomatal response to VPD has previously been reported for other environmental stresses (14), but this is the first known report of a change in stomatal response to VPD by O_3. Johnson and Ferrell (14) reported that drought stress was found to modify stomatal response to VPD in <u>Pseudotsuga menziesii</u> (Mirb.) Franco. The stomata of Douglas fir were sensitive to VPDs, ranging from 0.5 to 3.5 KPa, between -0.5 MPa and -2.0 MPa xylem water potential, but below -2.0 MPa, sensitivity decreased. As VPD increased in the present study, stomatal conductance decreased more rapidly for O_3-treated seedlings than controls. The mechanism behind this response is unknown, but may be related to an increase in peristomatal transpiration (18). Since the range of VPDs used was limited (mean VPD= 3.53 ± 0.31 KPa) further research is needed in this area, over a broader range of VPDs.

The only significant effect of simulated acid rain in yellow-poplar seedlings was a linear decrease in stomatal conductance with decreasing rain pH. Neufeld et al. (21) reported that stomatal conductance of yellow-poplar was unchanged by simulated acid rain treatments, however they observed that stomatal conductance of sweetgum (Liquidambar styraciflua L.) leaves was reduced after 16 exposures to pH 2.6 rain compared with exposures to pH 5.6 rain. They postulated that these decreases were the result of one of three factors, alone or in combination: 1) loss of turgor by guard cells as a result of acid rain induced plasmolysis, thus causing partial stomatal closure, 2) entry of H^+ ions into the guard cells, thus causing a disruption in the ion balance in these cells, and 3) guard cells may be sites of cation leaching from the leaves, causing stomatal dysfunction. However, photosynthesis was not reduced significantly in either the current study or the study of Neufeld et al. (22) by simulated acid rain treatments.

The combination of O_3 + simulated acid rain resulted in significant linear response for several growth variables (Fig. 14-17, Table 12). Chappelka et al. (3) previously reported for yellow-poplar, that O_3 exposures of 0.10 ppm resulted in linear decreases in RGR for all plant parts, root dry weight and ULR with decreasing rain pH. The results from these two studies are similar, except that TRGR, RGROOT, root dry weight and ULR, did not decrease significantly with increasing rain acidity for O_3-treated plants in the present study. Reasons for these discrepancies are unknown, but are probably related to differences in environmental conditions in the greenhouse and fumigation chambers, between the two studies. Jensen (13), using yellow-poplar exposed to 0.10 ppm O_3, 12 hours a day, once or twice a week, for 20 weeks, reported that there were significant differences in growth between control seedlings which were moved from the greenhouse to the fumigation chambers once or twice a week; seedlings moved only once a week exhibited the greatest growth. No reasons were given for these differences in growth, but they are probably related to differences in lighting and temperature, etc., between the two groups of seedlings.

Inhibition in growth is a result of a decrease in photosynthesis (2,23,28), an increase in respiration (1), an alteration in the pattern of photosynthate allocation (22,26), or a combination of these factors (1). This is the first report in the literature on the direct effect of O_3 + simulated acid rain in photosynthesis (Fig. 17). Since a similar linear decrease in stomatal conductance did not occur, photosynthesis must have been directly affected (23,27).

The variation in seedling growth resulting from O_3 in combination with simulated acid rain may be related to the chemical activity of this gas at different solution pHs (8). In alkaline solutions O_3 is rapidly decomposed and is more soluble than O_2, but in acidic conditions O_3 is considerably more stable. Although it is not known if this response is similar in both plant cytoplasm and aqueous solutions, this may be a possible explanation for the different effects observed with the three pH treatments.

Root/shoot ratio decreased linearly in 0.15 ppm O_3-treated seedlings with decreasing rain pH. This decrease occurred due to an increase in above-ground biomass at the expense of root growth as reflected by a significant increase in RGSTEM and cumulative leaf area. A decrease in RSR indicates a disproportionate decrease in root dry matter compared with above-ground biomass, and is a reflection of a shift in the allocation pattern of photosynthate or energy utilization. These seedlings may be more susceptible to other environmental stresses, such as drought, due to the large increase in above-ground biomass relative to root growth.

In summary, the combination of O_3 + simulated acid rain resulted in significant linear responses for several growth variables in this study. At concentrations (0.05-0.10 ppm) which normally occur in the ambient atmosphere (24), stem dry weight, leaf dry weight, RGSTEM, RGLEAF, and cumulative leaf area increase decreased with an increase in the acidity of rain. Root/shoot ratio decreased linearly in 0.15 ppm O_3-treated seedlings with decreasing rain pH. These decreases in growth may be partly explained by a linear decrease in mean net photosynthesis for O_3-treated seedlings with decreasing rain pH.

Since both O_3 and acidic precipitation in the ambient atmosphere can occur in the same geographic area

(20), the alteration in tree seedling growth response resulting from the combination of these two atmospheric contaminants demonstrated in this study and others (3,4), is cause for concern. Additional research is needed, using different tree species, soil types and field tests to determine if such effects are occurring in the natural forest ecosystem.

LITERATURE CITED

1. Barnes, R. L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut. 3:123-138.

2. Carlson, R. W. 1979. Reduction in the photosynthetic rate of _Acer_, _Quercus_, and _Fraxinus_ species caused by sulphur dioxide and ozone. Environ. Pollut. 18:159-170.

3. 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 combina tion. Environ. Expt. Bot. 25:233-244.

4. Chappelka, III, A. H. and B. I. Chevone. 1986. White ash seedling growth response to ozone and simulated acid rain. Can. J. For. Res. (In Press).

5. Chevone, B. I., Y. S. Yang, W. E. Winner, I. Storks-Cotter and S. J. Long. 1984. A rainfall simulator for laboratory use in acidic precipitation studies. J. Air Pollut. Contr. Assoc. 34:355-359.

6. Duchelle, S. F., J. M. Skelly, T. L. Sharick, 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 National Park of Virginia. J. Environ. Manag. 17:294-308.

7. 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.

8. Horvath, M., L. Bilitzky and J. Huttner. 1985. _Ozone_. Elsevier Sci. Publ. Co., New York.

9. Hunt, R. 1982. _Plant Growth Curves_. Univ. Park Press, Baltimore.

10. Jensen, K. F. 1973. Response of nine forest tree species to chronic ozone fumigation. Plant Dis. Rep. 57:914-917.

11. Jensen, K. F. 1981. Air pollutants affect the

relative growth rate of hardwood seedlings. USDA For. Ser. Res. Pap. NE-470.

12. Jensen, K. F. 1982. Growth relationships in silver maple seedlings fumigated with O_3 and SO_2. Can. J. For. Res. 13:298-302.

13. Jensen, K. F. 1985. Response of yellow-poplar seedlings to intermittent fumigation. Environ. Pollut. (A) 38:183-191.

14. Johnson, J. J. and W. K. Ferrell. 1983. Stomatal response to vapour pressure deficit and the effect of plant water stress. Plant, Cell and Environ. 6:451-456.

15. Krause, G. H. M., K. D. Jung and B. Prinz. 1984. Forest effects in West Germany. pp. 297-332. In: Symposium on Air Pollution and the Productivity of the Forest. Izaak Walton League of America, Arlington, VA.

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

17. Ledig, T. T. 1974. Concepts of growth analysis. pp. 166-182. In: Proceedings of the 3rd North American Forest Biology Workshop. Colorado State Univ., Fort Collins.

18. Losch, R. and J. D. Tenchunen. 1981. Stomatal response to humidity-phenomenon and mechanism. pp. 137-161. In: Stomatal Physiology, P. G. Jarvis and T. A. Mansfield eds., Cambridge, Univ. Press, New York.

19. 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 O_3, SO_2, and NO_2. Can. J. For. Res. 14:150-153.

20. McLaughlin, S. B. 1985. Effects of air pollution on forests: A critical review. J. Air Pollut. Contr. Assoc. 35:512-534.

21. Nuefeld, H. S., 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.

22. Okano, K., O. Ito, G. Takeba, A. Shimizu and T. Totsuka. 1984. Alteration of ^{13}C-assimilate partitioning in plants of Phaseolus vulgaris exposed to ozone. New Phytol. 97:155-163.

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

24. Skelly, J. M., B. I. Chevone and Y. S. Yang. 1982. Effects of ambient concentrations of air pollutants indigenous to the Blue Ridge Mountains of Virginia. pp. 69-73. In: Internat. Symp. in Hydrometer. Amer. Water Res. Assoc.

25. Steele, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York.

26. Tingey, D. T., R. G. Wilhour and C. Standley. 1976. The effect of chronic ozone exposures on the metabolic content of ponderosa pine seedlings. For. Sci. 22: 234-241.

27. Unsworth, M. H. and V. J. Black. 1981. Stomatal response to pollutants. pp. 187-203. In: Stomatal Physiology., P. G. Jarvis and T. A. Mansfield. eds., Cambridge, Univ. Press, New York.

28. Yang, Y. S., J. M. Skelly, B. I. Chevone and J. B. Birch. 1983. Effects of long-term ozone exposure on photosynthesis and dark respiration of eastern white pine. Environ. Sci. Technol. 17:371-373.

SUMMARY

Sulfur dioxide (SO_2) and oxides of nitrogen (NO_x) are the most phytotoxic primary pollutants that are emitted into the atmosphere (21). In addition, these air contaminants are precursors to secondary pollutants, such as ozone (O_3) and acidic precipitation (acid rain). These latter pollutants can be transported long distances from urban sources to rural areas and remote forested regions (17,21). Ozone is considered the most phytotoxic air contaminant in the eastern United States (20). Acid rain is also potentially detrimental to terrestrial vegetation, although its phytotoxicity has not yet been documented at hydrogen ion concentrations normally found in the ambient environment (8). Since both O_3 and acid rain can impact the same area (16), the overall objective in this dissertation was to determine if the combination of these pollutants had an effect on seedling growth of three forest tree species which are native to the southern Appalachian Mountains. In order to minimize variations environmental factors which influence plant response to air pollutants, these studies were conducted under controlled laboratory conditions.

The specific objectives were: 1) to determine the effects of ozone, sulfur dioxide and simulated acid rain, alone or in combination on the growth of yellow-poplar seedlings, 2) to determine the effects of these three pollutants on the growth of green and white ash seedlings, 3) to determine the effects of ozone and simulated acid rain on the growth of white ash seedlings, and 4) to determine any alterations in foliar gas exchange characteristics and growth reductions of yellow-poplar seedlings in response to ozone and simulated acid rain.

Potted yellow-poplar and green and white ash were grown from seed, in a soilless mixture of Weblite, peatmoss and vermiculite in a greenhouse receiving charcoal-filtered air, and fumigated 4 hr/d, 5 d/wk for 6 wk, in order to accomplish the first two objectives. Pollutant treatments included controls (no pollutants), 0.10 ppm O_3, 0.08 ppm SO_2 and 0.10 ppm O_3 + 0.08 ppm SO_2. In addition, seedlings were exposed to artifical rain solutions of pHs 5.6, 4.3 or 3.0 from a laboratory rain simulator, just before or after fumigation with gaseous pollutants.

The concentrations of O_3 and SO_2 used were realistic, since O_3 episodes commonly occur in the eastern United States having durations of 1-3 days, with peak hourly means often exceeding 0.10 ppm (20). Concentrations of SO_2 often exceed 0.10 ppm for several hours in the vicinity of coal-fired power plants, during atmospheric inversions (17). While elevated levels of O_3 + SO_2 do not commonly co-occur in the ambient atmosphere, they represent the effect of pollutant mixtures on plant growth and, in several cases, have been shown to have a more harmful effect on tree growth than O_3 or SO_2 alone (13,15). The rain pHs used are also relevent to natural conditions since they represent a range from theoretically pure rain (pH 5.6) (14), a pH (4.3) which is representative of the average annual pH in southwestern Virginia (20), to an extremely low pH (3.0) which represents a worst case which may occur. The lowest rain pH in Virginia has been reported as pH 3.30 for a single event (20). Rain solutions were prepared with ionic concentrations similar to the average ambient rainfall in southwestern Virginia.

Yellow-poplar exhibited the most deleterious response in height growth and biomass production when exposed to O_3 + SO_2, and this effect was generally additive in nature. Growth, however, was not significantly inhibited by these two pollutants, when applied alone. Mahoney et al. (15) reported that O_3 + SO_2 significantly decreased shoot elongation and dry matter production of yellow-poplar seedlings, compared with the single pollutants or non-fumigated controls. Using the same half-sib family, I conclude these results confirm those of Mahoney et al. (15). Jensen (10), however, in the only other known study examining the response of yellow-poplar seedling growth to O_3 and SO_2, found the effect of O_3 + SO_2 to be less than additive, and reported this species as being more sensitive to O_3 and SO_2, singly than in combination. In a later study Jensen (11) reported that O_3 + SO_2 had a greater effect on yellow-poplar growth than O_3 alone when plants were fumigated twice weekly for 20 weeks, however, this effect on growth did not occur when the pollutants were applied only once a week. He offers no explanation why these differences occurred between and among his studies with yellow-poplar, and also provides no information regarding environmental variations (temperature, lighting, humidity, etc.) during fumigations, which may have influenced yellow-poplar seedling response.

Although O_3 + SO_2 suppressed yellow-poplar growth more than any other treatment, the response to O_3 and SO_2 alone was decreased or increased as the acidity of rain increased, respectively. Ozone exposures resulted in a linear decrease in root dry weight, leaf area increase, relative growth rate and unit leaf rate (net assimilation rate), as the acidity of rain increased, whereas fumigation with SO_2 resulted in an increase in these growth variables as rain acidity increased. Chlorophyll content, however, was increased in both O_3- and SO_2-treated plants as the acidity of rain increased.

The reasons for these results are unknown, but could be related in part, to the chemical activity of O_3 and SO_2 in aqueous solutions. In alkaline solutions O_3 is rapidly decomposed and is more soluble than O_2, but in acidic conditions O_3 is generally more stable (6). Sulfur dioxide, however, reacts differently than O_3 in aqueous solution, depending upon the solution pH (5). The formation of HSO_3^- and SO_3^{2-} from SO_2 in aqueous solution has been reported as being 1000 times greater at pH 6.0 than pH 3.0, with $SO_2 \cdot H_2O$ being more prevalent at the lower pH. The increase in HSO_3^- and SO_3^{2-} as pH increases may account for the reductions in growth observed with the high pH. Although it is not known if these chemical reactions are similar in both plant cytoplasm and aqueous solution, this phenomenon may be a possible explanation for the different effects observed with gaseous pollutants in combination with the three pH treatments. The linear increase in chlorophyll with O_3 X acid rain has not been reported previously in the literature. It appears that chlorophyll biosynthesis is unaffected by this treatment.

The timing of rain application had a significant effect on yellow-poplar growth. Generally, growth was significantly less for plants exposed to gaseous pollutants with wet leaf surfaces, compared to seedlings to which rain was applied after fumigation. Elkiey and Ormrod (3) reported that the severity of pollutant injury (O_3, SO_2, NO_2) was increased dramatically in Poa pratensis L., by misting the plants twice daily with deionized H_2O for 5 minutes during the fumigation period. They determined that stomatal conductance was greater in misted plants compared to non-misted controls. These results suggest that water itself can enhance gaseous pollutants effects, probably through increased humidity at the surface and subsequently, increased stomatal conductance.

The effects of air pollutants on green and white ash were compared directly, since these species are morphologically similar (1), but respond differently to air pollutant stress (9,13). Biomass accumulation of green and white ash was not significantly affected by O_3 or SO_2 fumigation, over a six week period, except that white ash leaf dry weight was suppressed by the application of O_3. Cumulative shoot elongation of green ash exposed to O_3,

SO_2 and O_3 + SO_2 was less than controls, after the six week exposure period. Fumigation inhibited height growth approximately 15%, compared with controls, for all gaseous pollutant treatments. There was no significant effect on white ash height growth by any gaseous pollutant treatment. Kress and Skelly (13) and Jensen (9), independently reported that green ash was more sensitive to O_3 than white ash, regarding decreases in shoot elongation. Results from this study support their findings.

The combination of O_3 + SO_2 did not affect the growth response of all variables measured for green and white ash seedlings greater than either pollutant alone. Jensen (10) reported similar results regarding inhibition of growth rates for white ash in response to O_3, SO_2 or O_3 + SO_2 exposure. Green and white ash seedlings did not respond to O_3 + SO_2 exposure as did yellow-poplar seedlings, where growth was suppressed the greatest by O_3 + SO_2 stress. Reasons for these discrepancies between species are unknown, but are likely to be related to variations in tolerance to pollutant combinations.

Simulated rain acidity caused no significant changes in biomass accumulation, but did cause a quadratic response in leaf area ratio (LAR, leaf area/plant wt) and root/shoot ratio (RSR, root wt/above ground biomass), for green ash. These results reflect shifts in the allocation of photosynthate, rather than the direct inhibition of biomass accumulation. The increase in LAR with green ash at pH 4.3, relative to other rain pH treatments, may be related to increased nutrient uptake by this species in a moderately acidic environment (2). As the acidity increased (pH 3.0), plant response to H^+ may override a nutrient effect, thus causing a decrease in LAR at pH 3.0.

The combined effects of gaseous pollutants and simulated acid rain resulted in several significant pollutant X rain pH treatment interactions for green and white ash. For green ash, non-fumigated controls exhibited a linear decrease in leaf weight ratio (LWR, leaf wt/plant wt), whereas in O_3- and O_3 + SO_2-treated plants, a quadratic response occurred with increasing rain acidity; LWR was the greatest for these plants treated with pH 4.3 rain, indicating a shift in photosynthate allocation for these seedlings in favor of the photosynthetic portions of the plant.

A significant pollutant X rain pH treatment interaction occurred with white ash. Ozone- and SO_2-treated plants exhibited significant linear increases in LAR and linear decreases with RSR as the acidity of rain increased. These data reflect a change in the allocation pattern of photosynthate in the foliage at the expense of root growth. By decreasing the below ground portion of the plant in favor of the above ground portion, these plants may be predisposed to other stresses, such as, drought or root rotting micro-organisms. As a species, white ash appears to be more sensitive to the combination of gaseous pollutants and acid rain than green ash, as reflected by the linear increase in LAR and decrease in RSR. This current work represents the first report of the effects of O_3, SO_2 and acid rain, alone or in combination, on green and white ash growth.

The timing of rain application affected growth for both green and white ash. Generally, biomass decreases were observed for plants exposed to simulated rain after fumigation with gaseous pollutants. These results are dissimilar to those previously reported for yellow-poplar where growth was inhibited in plants exposed to artifical rain solutions before fumigation. These results suggest that this effect may be the result of differences in leaf wetability. Also, the variation in environmental conditions during fumigation may have affected plant response. Average temperatures in the CSTR chambers, were slightly higher ($\geq$ $2^{o}C$) for the study with green and white ash than yellow-poplar. These increased temperatures could cause a more rapid drying of the leaf surfaces, thus modifying plant response.

The alteration in growth response of yellow-poplar and white ash to O_3 + acid rain observed in the first two studies led to a more detailed investigation of the combined effects of these two pollutants. Also, since O_3 is the most phytotoxic pollutant in the eastern United States (20) and impacts plants in the the same area as acid precipitation (16), it is important to understand at what O_3 concentrations and rain pHs growth alterations can occur.

To accomplish the first O_3 + acid rain study, 5-wk-old white ash were exposed to four O_3 concentrations (0.00, 0.05, 0.10 or 0.15 ppm) for 4 hr/d, 5 d/wk, for 5 wk., in combination with simulated rain applied at three pHs (5.6, 4.3, 3.0) 1hr/d, 2d/wk at 0.75 cm/hr. Rain was applied either just before or after O_3 fumigation. Younger white ash (5-wk-old) were used in this study because in the earlier study the seedlings grew so tall (approx. 20-25 cm height increase) and accumulated so much biomass that they became unmanageable. This was also the reason that these seedlings were only fumigated for 5 wk, instead of 6 wk.

Significant linear decreases occurred with increasing O_3 concentrations for root, leaf and total dry weights, height increase, RSR and mean relative growth rate of all plant parts. Kress and Skelly (13) reported a substantial inhibition in white ash seedling growth at concentrations higher than 0.05 ppm O_3. In the earlier study with white ash, leaf dry weight was the only growth variable in objective 3 to be significantly suppressed by 0.10 ppm O_3, however, O_3 exposure did result in slight non-significant reductions in other growth variables in this study. Discrepancies in these results may be related to differences in the relative growth rates between the two studies. The rate of growth was approximately twice as great for the younger white ash seedlings compared to the older ones. This increase in growth rate may exaserbate the pollutant response.

Increases in simulated rain acidity resulted in linear or quadratic growth responses, depending upon the variable measured. Significant linear decreases in root growth and RSR occurred with increasing rain acidity. Results from this study support the findings of other researchers (4,18) who reported that the primary effect of acid rain treatments was to alter root growth. These results also reinforce previous findings where RSR decreased linearly with increasing rain acidity in the older white ash seedlings. Comparisons between LARs were not made, since leaf area was not measured for the younger white ash.

A significant quadratic response occurred with simulated rain exposures for all above ground growth variables measured. Growth was the greatest for seedlings treated with pH 4.3 and the least for those treated with pHs 5.6 or 3.0. No quadratic response was found for any variable measured in the older white ash. Reasons for these differences are unknown, but could be related to the growth rate and ability of the younger seedlings to absorb and utilize nutrients provided in rain at moderately acidic pHs.

The combination of O_3 + acid rain resulted in linear reductions in root growth and RSR at O_3 concentrations (0.05 and 0.10 ppm) and rain pHs ($\leq$ 4.3) which are common in ambient field conditions. Although there was no significant reduction in root growth, RSR did significantly decrease linearly for the older O_3-treated white ash seedlings as rain pH decreased. Results from these two studies indicate that the primary effect of O_3 + acid rain in white ash is to decrease root growth in favor of above ground biomass. This effect may predispose these plants to other environmental stresses.

The timing of rain application did not affect any growth variable measured. However, with the older white ash seedlings, growth was increased for plants fumigated with wet leaves, compared to those which rain was applied after fumigation. Reasons for these discrepancies are unknown, but as mentioned previously are probably related to differences in environmental parameters between studies.

The three previous studies in this disertation were designed to demonstrate that gaseous pollutants in combination with acid rain can cause alterations in growth of tree seedlings, under controlled environmental conditions. The mechanism(s) underlying such effects, however, are poorly understood. Gas exchange characteristics and yellow-poplar seedling growth therefore were investigated to determine the relationship of changes in net photosynthesis on seedling biomass allocation in response to low concentrations of O_3 and simulated acid rain. Two seperate experiments were designed as follows: 1) to examine the effects of four different O_3 concentrations (0.00, 0.05, 0.10 or 0.15 ppm) and three pHs of simulated rain (5.6, 4.3, 3.0) on the growth of yellow-poplar seedlings, and 2) determine the effects of O_3 (0.00

and 0.10 ppm) and simulated acid rain (pHs 5.6, 4.3, 3.0) on yellow-poplar seedling gas exchange characteristics and relate these effects to growth response of these seedlings. In both studies seedlings were exposed to O_3, 4 hr/d, 5 d/wk and simulated rain 1 hr/d, 2 d/wk, before fumigation with O_3.

Increasing O_3 concentrations from 0.00 to 0.15 ppm caused no effects on height growth or biomass production of yellow-poplar. However, specific leaf area (SLA, leaf area/leaf wt) increased with increasing O_3 concentrations. Jensen (11) reported variable results with SLA for yellow-poplar. Seedlings exposed to 0.10 ppm O_3 applied once a week, for 20 weeks, exhibited higher SLA, compared with controls, however when O_3 was applied two times a week, SLA was lower than controls. Since SLA is reported to be very sensitive to environmental changes (7), different conditions during fumigation may be responsible for these discrepancies in response.

When 0.10 ppm O_3-treated yellow-poplar seedlings were compared with controls LAR decreased and unit leaf rate (ULR) increased. There was no change in these variables between treatments in earlier studies. Jensen (11) found an increase in ULR and decrease in LAR with yellow-poplar fumigated with O_3 twice a week, but he observed a decrease in ULR when seedlings were fumigated only once a week. These differences were attributed to environmental factors, which however, were not quantified.

Stomatal conductance of yellow-poplar decreased in response to O_3 when 0.10 ppm O_3-treated seedlings were compared with controls. A reduction in stomatal conductance to O_3 has been observed for many plant species (19). Although not significantly different, net photosynthesis was also slightly reduced for these seedlings. These responses, however, were not reflected by growth decreases.

In addition, O_3 was found to modify stomatal sensitivity of yellow-poplar to vapor pressure deficit (VPD). The alteration of stomatal response to VPD has previously been reported for plants in response to other environmental stresses (12), but this is the first known report of a change in stomatal response to VPD by O_3; as VPD increased, stomatal conductance decreased more rapidly for O_3-treated seedlings than controls. The mechanism behind this response is unknown. Since the range of VPDs used in this study was limited (mean VPD= 3.53 ± 0.31 KPa) and rather high, further research is needed in this area, using a broader range of VPDs.

The only effect of simulated acid rain on yellow-poplar was a linear decrease in stomatal conductance with increasing rain acidity. No subsequent significant reductions in net photosynthesis or growth were observed.

At O_3 concentrations (0.05 or 0.10 ppm) which normally occur in the ambient environment, significant linear decreases in stem dry weight, leaf dry weight, relative stem and leaf growth rates and cumulative leaf area increase occurred with increasing rain acidity. These decreases in growth may be partly explained by a linear decrease in net photosynthesis with decreasing rain pH for these seedlings. Root/shoot ratio exhibited a significant linear decrease with decreasing rain pH for 0.15 ppm O_3-treated seedlings. This was the result of a disproportionate allocation of photosynthate from the above-ground plant parts to the roots, as reflected in a subsequent increase in RGSTEM and cumulative leaf area increase as rain pH decreased, for 0.15 ppm O_3-treated plants.

LITERATURE CITED

1. Anonymous. 1965. Silvics of Forest Trees of the United States. USDA Argric. Handb. No. 271.

2. Blamey, F. P. C., D. G. Edwards, C. J. Asher and Moo-Key Kim. 1982. Response of sunflower to low pH. pp. 66-72. In Plant Nutrition 1982, Vol. 1., Avon Litho Ltd., Statford-upon-Avon, UK.

3. Elkiey, T. and D. P. Ormrod. 1981. Sulfur and nitrogen nutrition and misting effects on the response of bluegrass to ozone, sulphur dioxide, nitrogen dioxide or their mixture. Water, Air and Soil Pollut. 16:177-186.

4. Ferenbaugh, R. W. 1976. Effects of simulated acid rain on *Phaseolus vulgaris* L. (Fabaceae). Amer. J. Bot. 63:283-288.

5. Gravenhorst, G., S. Beilke, M. Betz and H. W. Georgii. 1981. Sulfur dioxide absorbed in rain water. pp. 41-55. In Effects of Acid Precipitation on Terestrial Ecosystems, T. Hutchinson and M. Havas eds. Plenum Press, New York.

6. Horvath, M., L. Bilitzky and J. Huttner. 1985. Ozone. Elsvier Sci. Publ. Co., New York.

7. Hunt, R. 1982. Plant Growth Curves. Univ. Park Press, Baltimore.

8. Jacobson, J. S. 1984. Effects of acid aerosol, fog, mist and rain on crops and trees. Phil. Trans. Roy. Soc. London (B). 305:327-328.

9. Jensen, K. F. 1973. Response of nine forest tree species to chronic ozone fumigation. Plant Dis. Rep. 57:914-917.

10. Jensen, K. F. 1981. Air pollutants affect the relative growth rate of hardwood seedlings. USDA For. Ser. Res. Pap. NE-470.

11. Jensen, K. F. 1985. Response of yellow-poplar seedlings to intermittent fumigation. Environ. Pollut. (A) 38:183-191.

12. Johnson, J. J. and W. K. Ferrell. 1983. Stomatal response to water vapour pressure deficit and the effect of plant water stress. Plant, Cell and Environ. 6:451-456.

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

14. Likens, G. E. and T. J. Butler. 1982. Recent acidification of precipitation in North America. Atmos. Environ. 15:1103-1109.

dioxide. Phytopathology 75:679-682.

15. Mahoney, M. J., J. M. Skelly, B. I. Chevone and L. D. Moore. 1984. Response of yellow poplar (<u>Liriodendron tulifera</u> L.) seedling shoot growth to low concentrations of O_3, SO_2 and NO_2. Can. J. For. Res. 14:150-153.

16. McLaughlin, S. B. 1985. Effects of air pollution on forests: A critical review. J. Air Pollut. Contr. Assoc. 35:512-534.

17. Mudd, J. B. and T. T. Kozlowski. 1975. <u>Response of Plants to Air Pollution</u>, pp. 9-51. Academic Press, New York.

18. Nuefeld, H. S., 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.

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

20. Skelly, J. M., B. I. Chevone and Y. S. Yang. 1982. Effects of ambient concentrations of air pollutants indigenous to the Blue Ridge Mountains of Virginia. pp. 69-73. <u>In</u> Internt. Symp. in Hydrometer. Amer. Water Res. Assoc.

21. Smith, W. H. 1981. <u>Air Pollution and Forests</u>. Springer-Verlag, New York, 379 pp.

CONCLUSIONS

The purpose of this section is to discuss some of the major conclusions pertaining to the effects of gaseous pollutants and simulated acid rain on the growth of young seedlings of three forest tree species native to the southern Appalachian Mountains, under laboratory exposure conditions. Results demonstrated that yellow-poplar was intermediate to tolerant in response to O_3, SO_2 or simulated acid rain when applied singly. Green and white ash, however, were intermediate to sensitive in response to these pollutants.

With yellow-poplar, the only variable affected by O_3 or SO_2 was stomatal conductance, where the application of O_3 resulted in a significant decrease. Ozone exposure caused significant decreases in total biomass and RSR in white ash, wheras sulfur dioxide did not cause any adverse effects on growth. Both O_3 and SO_2 significantly decreased green ash height growth. This was the only variable altered by these pollutants for green ash. These results demonstrate that as a species white ash was the most sensitive to O_3 and green ash the most sensitive to SO_2, regarding growth.

The only effect of simulated acid rain on yellow-poplar was a linear decrease in stomatal conductance with increasing rain acidity. Increases in rain acidity resulted in no significant change in green ash biomass. Components of growth analysis, such as LAR and RSR, however, exhibited a quadratic response with simulated acid rain, with the greatest effect occurring in plants treated with pH 4.3 rain solutions. White ash, in contrast, exhibited a significant linear increase in the amount of photosynthetic leaf area (LAR) and linear decreases in root biomass and root growth rate and a subsequent decrease in RSR, as the acidity of rain increased. These results demonstrated that the effect of simulated acid rain varied depending upon the plant species observed.

The combined effects of all these pollutants also caused different responses in growth among the three tree species examined. The combined effect of O_3 + SO_2 resulted in substantial decreases (greater than additive) for the majority of growth variables measured for yellow-poplar. In contrast, the combination of these pollutants did not cause a greater effect on green and white ash growth than individual pollutants. In fact, in many cases the combined effect was less than additive. Reasons for these differences between species are unknown, but may be related to differences in pollutant effects on plant metabolism.

When comparing the effects of gaseous pollutants in combination with acid rain on growth of these three tree species, it was apparent that yellow-poplar and white ash were the species most severely affected. Their response to these pollutants, however, was different. The most consistant effects of O_3 + acid rain on yellow-poplar was to decrease photosynthetic area (leaf area) and above-ground biomass. In addition, foliar gas exchange measurements indicated that decreases in growth resulting from the combined effects of O_3 + simulated acid rain, may have resulted from a linear decrease in mean net photosynthesis with decreasing rain pH, for O_3-treated seedlings. Since a similar decrease in stomatal conductance did not occur, photosynthesis was directly affected. Results with white ash, in contrast, indicated a decrease in below-ground biomass in favor of above-ground biomass, i. e., root biomass and RSR decreased and LAR increased for O_3-treated seedlings as the acidity of rain increased.

Sulfur dioxide + simulated acid rain resulted in an increase in the majority of growth variables measured for yellow-poplar. With white ash, however, SO_2-treated seedlings exhibited the same response in combination with simulated acid rain as O_3-treated seedlings. The growth response of green ash was not altered by SO_2 in combination with simulated acid rain.

Rain acidity did not alter the growth response of yellow-poplar to O_3 + SO_2, although growth was the least for this gaseous pollutant combination at any rain pH. With green and white ash, the combination of these pollutants caused no adverse effects on height growth or biomass, and growth was similar to single pollutant treatments at any rain pH.

Again, reasons for these different responses between species to the combination of gaseous pollutants + acid rain are not known. They may be related to differences in pollutant effects on plant metabolism or carbon utilization patterns.

The results from this dissertation indicate that the combined effect of gaseous pollutants and simulated acid rain can alter the growth of three forest tree species, native to the southern Appalachian Mountains, under laboratory conditions. Additional research is needed using different tree species, tree ages, soil types and field tests to determine if such effects are occurring in natural forest ecosystems.

APPENDIX

Table A-1. Major ion chemistry of simulated rain before and after passing through rain simulator for experiments presented in chapters I and II.

Ion conc.	Stock solution	60-min sample
pH	4.29	4.42
H^+ μeq L^{-1}	51.2 ± 0.00	38.0 ± 0.00
Conductivity (μ mohs/cm)	39 ± 0.00	37 ± 0.00
Calcium[a]	1.60 ± 0.00	1.50 ± 0.00
Magnesium	0.05 ± 0.00	0.06 ± 0.00
Ammonium	0.45 ± 0.014	0.54 ± 0.007
Potassium	0.235 ± 0.007	0.25 ± 0.00
Chloride	0.44 ± 0.035	0.47 ± 0.007
Sodium	0.46 ± 0.00	0.445 ± 0.021
Nitrate (pH 5.6, 4.3)	2.70 ± 0.00	2.45 ± 0.07
Nitrate (pH 3.0)	3.5 ± 0.00	3.25 ± 0.07
Sulfate (pH 5.6, 4.3)[b]	5.40 ± 0.00	4.80 ± 0.00
Sulfate (pH 3.0)	57 ± 0.00	52 ± 0.00
Sodium	0.46 ± 0.00	0.445 ± 0.021

[a] Ion concentrations expressed in mg/L; 2 replicates per sample.

[b] Sulfate concentrations were determined on solutions of ph 5.6, 4.3 (no addition of H_2SO_4) and pH 3.00 (H_2SO_4 added to adjust pH).

Table A-2. Major ion chemistry of simulated rain before and after passing through rain simulator for experiments presented in chapters III and IV.

Ion conc.	Stock solution	60-min sample
pH	4.26	4.30
H^+ μ eq L^{-1}	55 ± 0.00	50 ± 0.00
Conductivity (μ mohs/cm)[a]		
Calcium[b]	1.19 ± 0.07	1.23 ± 0.007
Magnesium	0.10 ± 0.04	0.21 ± 0.002
Ammonium	0.47 ± 0.004	0.50 ± 0.016
Potassium	0.212 ± 0.00	0.277 ± 0.010
Chloride	0.48 ± 0.037	0.43 ± 0.015
Sodium	0.558 ± 0.00	0.606 ± 0.013
Nitrate (pH 5.6, 4.3)	2.98 ± 0.106	3.11 ± 0.070
Nitrate (pH 3.0)	19.0 ± 0.54	19.2 ± 0.18
Sulfate (pH 4.3, 5.6)[c]	5.44 ± 0.25	5.52 ± 0.17
Sulfate (pH 3.0)	41.7 ± 0.49	42.5 ± 0.49

[a] Not measured.

[b] Ion concentrations expressed in mg/L; 2 replicates per sample.

[c] Sulfate and nitrate concentrations were determined on solutions of pH 5.6, 4.3, (no addition of H_2SO_4) and pH 3.00 (H_2SO_4 added to adjust pH).

Table A-3. Mean squares and levels of significance of analysis of variance for biomass production, selected growth responses and total chlorophyll content of 10-week-old white ash exposed to ozone and simulated acidic rain.

Source of[a] variation	df	Mean Squares								
		Biomass production								
		Stem	Root	Leaf	Total	DIA	HTI	$\overline{RGR}$	RSR	TCHL
Block	5	0.064	0.099**	0.064*	0.225	0.145	424.768	0.009	0.633**	13.748
Ozone	3									
Linear	1	0.118	0.493**	0.096*	1.836**	0.216	2645.054*	0.069	0.388**	110.962
Quadratic	1	0.137	0.004	0.075	0.506	0.829	393.345	0.006	0.078	92.623
Cubic	1	0.063	0.034	0.019	0.042	0.006	76.235	0.000	0.262*	43.781
Block x ozone	15	0.054	0.019	0.019	0.132	0.219	496.669	0.003	0.049	29.837
Rain pH	2									
Linear	1	0.000	0.113*	0.009	0.063	0.078	11.210	0.005	0.032*	8.329
Quadratic	1	0.188*	0.017	0.153**	0.910**	0.406*	3469.365*	0.015*	0.044	18.076
Timing of rain	1	0.008	0.005	0.001	0.019	0.013	0.974	0.000	0.122	3.759
Ozone x pH	6	0.055	0.036	0.016	0.138	0.051	511.913	0.003	0.100	13.329
Ozone x time	3	0.024	0.005	0.019	0.010	0.165	398.811	0.003	0.008	5.783
Time x pH	2	0.015	0.011	0.002	0.027	0.000	83.458	0.000	0.014	4.283
Ozone x time x pH	6	0.049	0.017	0.016	0.138	0.024	288.392	0.002	0.635	12.546
Covariate[b]	1	0.580**	0.830**	0.620**	6.053**	2.542**	6813.265*	0.249**	0.002	39.014*
Error	242	0.044	0.020	0.017	0.135	0.096	635.769	0.004	0.053	10.721

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[a] Calculated F-values significant at the 0.05 or 0.01 levels are denoted by * or **, respectively; pollutant x block interaction used as error to test for block and pollutant effects; DIA = total diameter increase (mm), HTI = total height increase (mm), $\overline{RGR}$ = mean relative growth rate (week), RSR = root/shoot (above-ground biomass) ratio (w/w), TCHL = total chlorophyll content (μg/mg dry weight).

[b] Covariate = initial tree height.