

# Growth Response of Pinus Ponderosa Seedlings and Mature Tree Branches to Acid Rain and Ozone Exposure

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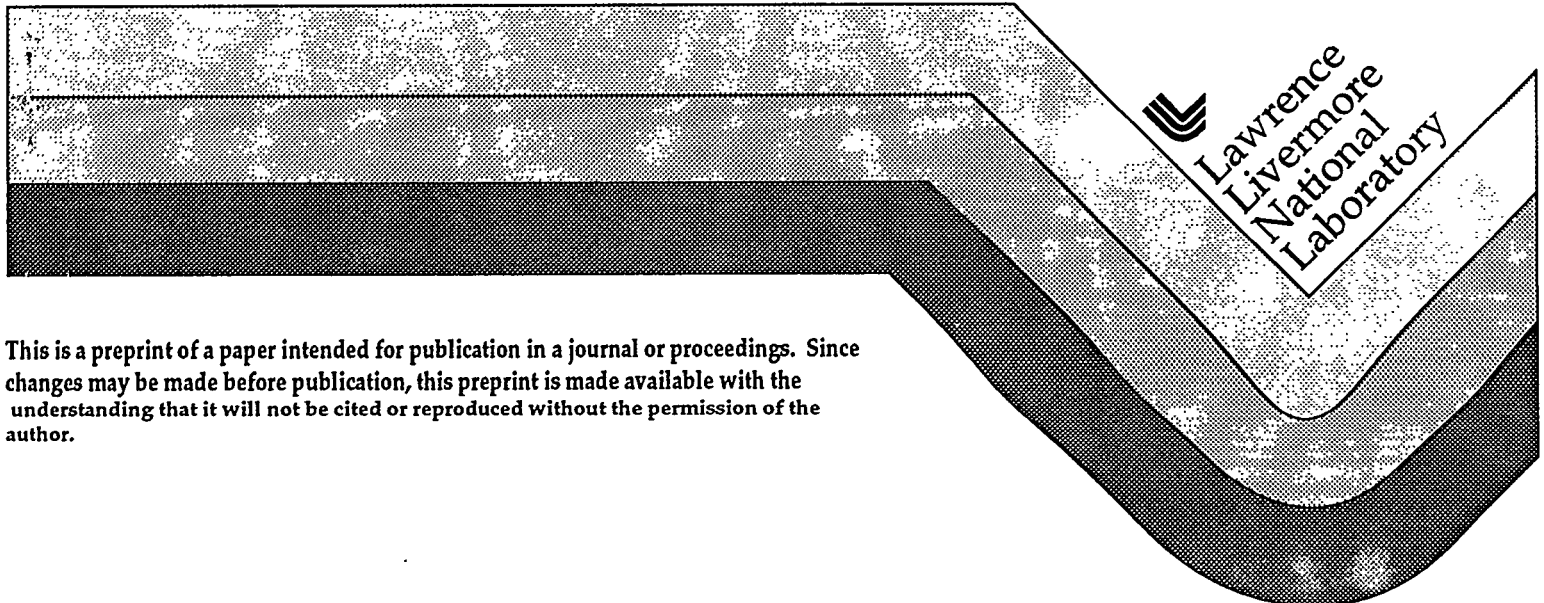
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This was prepared for submittal to the  
*Proceedings of the Air Pollution and Multiple Stresses*  
*16th International Meeting for Specialists in Air Pollution*  
*Effects on Forest Ecosystems*  
*Fredericton, New Brunswick, Canada*  
*September 6-7, 1994*

October 1994



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GROWTH RESPONSE OF PINUS PONDEROSA SEEDLINGS AND  
MATURE TREE BRANCHES TO ACID RAIN AND OZONE EXPOSURE

by

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## Abstract

Stem or branch diameter growth and elongation were measured on seedlings and mature tree branches of Pinus ponderosa exposed to all combinations of three acid rain treatments (no rain, pH 5.1 rain or pH 3.0 rain) and three ozone exposure regimes (charcoal-filtered air, ambient ozone or twice-ambient ozone) over a 13-month period. Three genotypes (3087, 3088 and 3399) were evaluated using replicate trees of graft origin and corresponding half-sib seedlings.

Mature tree branch elongation and diameter growth were decreased 10 to 12 percent under twice-ambient ozone for all three genotypes. Twice-ambient ozone did not significantly influence seedling diameter growth and was associated with a decrease in shoot elongation for only the half-sib genotype 3088. Seedling diameter was increased approximately 15 percent with exposure to pH 3.0 rain. Stimulatory effects of pH 3.0 rain on branch and stem elongation were limited to seedling diameter growth and to elongation of branches exposed to twice-ambient ozone.

These data indicate that the effects of ozone and acid rain on growth by Pinus ponderosa: 1) may vary significantly between seedling and mature branch life-stages; 2) may differ among genotypes; and 3) may be interactive.

## Introduction:

Forests of the central and southern Sierra Nevada in California have been subjected to chronic damage by ozone and other atmospheric pollutants for the past several decades (Peterson *et al.* 1989). Until recently, pollutant exposure of northern Sierra Nevada forests has been mild but increasing population and changes in land use throughout the Sacramento Valley and Sierra Nevada foothills may lead to increased pollutant damage in these forests. Although, better documented in other regions of the United States, little is known regarding the potential for acidic precipitation damage to Sierra Nevada forests. Only recently have studies directed towards understanding the potential interactive effects of ozone and acidic precipitation been undertaken (Temple *et al.* 1992).

A key issue in resolving potential regional impacts of pollutants on forests is the extent to which research results can be scaled across genotypes and life-stages. Most of the pollution research to date has been performed using seedlings with varying degrees of genetic control. It is important to determine if the results obtained in such studies can be extrapolated to mature trees and to different genetic sources.

In this paper, we present results from a one-year study examining the interactive effects of foliar exposure to acidic rain and ozone on the growth of ponderosa pine (*Pinus ponderosa*), a conifer known to be sensitive to ozone (Miller *et al.* 1989). The response to pollutants is characterized for both seedlings and mature tree branches of three genotypes grown in a common environment.

## Materials and Methods:

Branches of mature clonal trees and half-sib seedlings of three *Pinus ponderosa* genotypes (3087, 3088, 3399) were exposed to three levels of ozone and three levels of acidic rain in a factorial treatment structure. The clonal trees were generated from buds of three 70-to-80 year-old forest grown superior trees grafted onto 3 year-old root stocks and outplanted at the US Forest Service Tree Improvement Center in Chico, California (CTIC). At the time of the study, the grafted trees were 12 to 15 years of age and had mature tree branch morphology and cone production characteristics. The seedlings used were produced from seed collected from the same superior trees that provided scion for grafting. The seedlings were one-year-old when planted in 1990. The three parent trees for the study plants were located within one-mile of each other at 1190 m to 1220 m elevation on the Eldorado National Forest in the central Sierra Nevada (USFS seed zone 526). Relative to other genotypes in the production orchard at CTIC originating from the same elevation and breeding zone, these families represented the full range of phenotypic vigor as defined by overall size, branch development, foliage density, foliage retention, and foliage color. Trees of clone 3088 had the least vigor and trees of clone 3399 had the greatest vigor.

Ozone exposures were conducted from November, 1991 through November 1992. Branches of mature trees and seedlings were enclosed in Branch Exposure Chambers (BECs) (Houpis *et al.* 1991) and exposed to charcoal filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB). Exposures were continuously monitored and controlled by means of a computerized data acquisition and control program interfaced with ozone monitors and an ozone generator. Ambient ozone concentration (12-h average, 0900 to 2100 h) ranged from  $0.01 \mu\text{L L}^{-1}$  in January to  $0.07 \mu\text{L L}^{-1}$  in July and August. Relative to the ambient values, ozone concentrations for the CF and 2xAMB treatments were 55 and 190 percent, respectively, when averaged over the study.

Simulated acidic rain applications of 5 cm deposition per event, were conducted weekly from mid-January through April, 1992. Acidic rain was prepared from deionized water by the addition of trace salts in concentrations similar to that of naturally occurring rainfall in the central Sierra Nevada, and a mixture of  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$  (1:3 v/v) to achieve rain solutions of pH 5.1 or pH 3.0. Rain solutions were applied through calibrated nozzles mounted internally at the top of the BECs. The rain with a mean droplet diameter of 0.3 mm was applied at a deposition rate of  $5.8 \text{ cm h}^{-1}$ . Throughfall and runoff was

collected at the base of the BECs to prevent the solution from reaching the soil. Thus the simulated rain treatments were used to investigate direct foliar effects, rather than soil mediated effects, of acid rain deposition.

Repeated measurements of diameter and stem length were made for both mature branches and seedlings over the study period. Diameter was measured at the cotyledon scar for seedlings and at the base of the 1991 stem segment for the mature branches. Diameter measurements were made at approximate monthly intervals beginning in August 1991. Measurements of seedling terminal shoot length and mature branch length were made from February through November, 1992. For both seedlings and branches, stem length was measured from the base of the 1992 growth to the tip of the terminal bud on the terminal shoot (or a dominant lateral shoot if the terminal shoot was missing or damaged).

Absolute measures of diameter and stem length were normalized relative to values for the first measurement event. This was done to account for the effect of variation in initial size on subsequent growth. The dependent variables for diameter and height, therefore, were cumulative percent increase in dimension with respect to 1) basal diameter of seedlings; 2) basal diameter of the 1991 branch stem segment; 3) seedling total height prior to 1992 bud-break; or 4) the length of the 1991 branch segment. As defined, the normalized variables for seedlings represent percent growth relative to total size prior to the study while for mature branches, normalized variables represent percent growth relative to dimensions of tissue produced during 1991.

Diameter growth and elongation of stems were analyzed using Repeated Measures Analysis of Variance (RMANOVA) procedures. Separate analyses were performed for the mature branch and seedling lifestages due to constraints on the model imposed by differences in the nesting of treatments between lifestage experimental units. For seedlings, genotype was nested within acidic rain and ozone. For mature branches, ozone was nested within acidic rain and genotype.

## Results:

### Branch and Stem Elongation

Mature branch elongation was significantly less under the 2xAMB treatment relative to either the CF or AMB treatment. For the study period, mean branch growth was 39.4, 43.6 and 28.8 percent for the CF, AMB and 2xAMB treatments, respectively. Differences in cumulative branch elongation were not consistent throughout the study period. From February through March, there was no significant difference in branch elongation among ozone treatments. Between the end of March and the third week of April, branch growth in the 2xAMB treatment was substantially less than that for the CF and AMB treatments resulting in a difference of 6 to 7 percent cumulative elongation by early May.

Mature branch elongation response to ozone differed significantly with acid rain treatment (Figure 1). For the NAP treatment, branch elongation for the CF and AMB treatments were nearly identical and 10 to 12 percent greater than that for 2xAMB. When exposed to pH 5.1 rain, mean elongation was greatest for the AMB treatment and least for the 2xAMB treatment (55 and 23 percent, respectively). Under the most-acidic rain, there was less than 2 percent difference in total branch elongation among the three ozone treatments.

Cumulative height growth by half-sib seedlings of genotypes 3088 and 3399 was significantly greater than that for genotype 3087. By the end of the study, percent height growth for the genotypes 3088, 3399 and 3087 was 79.6, 75.6 and 64.2 percent, respectively.

Seedling height growth response to ozone differed among genotypes (Figure 2). For genotype 3087, the greatest cumulative growth was observed for the CF treatment and least for the AMB treatment, but differences among treatment means were not statistically significant. Height growth by seedlings of genotype 3088 was substantially reduced for the 2xAMB treatment, relative to the CF and AMB treatments. Cumulative percent height growth was 85.9, 83.9 and 69.1 percent for the AMB, CF and 2xAMB treatments, respectively. Although there was a slight tendency for genotype 3399 seedlings exposed to the AMB treatment to grow the least, differences in cumulative percent height growth



among the ozone treatments were less than 6 percent for any measurement period. These data suggest a differential sensitivity to ozone among seedlings of three genotypes.

### Diameter Growth

Diameter growth for seedlings exposed to pH 3.0 rain was greater than that for seedlings exposed to the pH 5.1 and NAP treatments (Figure 3). While, treatment mean values for the pH 3.0 treatment were consistently greater than those for the pH 5.1 and NAP treatments, the greatest difference among treatment mean values occurred late in the study. At the final measurement, percent diameter growth was 117.8, 97.8 and 97.0 percent for the pH 3.0, pH 5.1 and NAP treatments, respectively. Some caution must be used in assessing the magnitude of diameter growth enhancement by the pH 3.0 treatment. As illustrated in Figure 3, the mean diameter growth for the pH 3.0 treatment exceeded the values for the pH 5.1 and NAP treatments by 5 to 6 percent as early as November, 1991, prior to the application of acid rain exposures in 1992. If it is assumed that there was an inherent 5 to 6 percent difference in potential growth performance in seedling in the pH 3.0 treatment, then the magnitude of diameter growth enhancement of pH 3.0 exposure was probably 14 to 15 percent rather than the 20 percent implied by measurements made at the end of the study.

There was a tendency for decreased diameter growth for mature branches exposed to 2xAMB ozone relative to the CF and AMB treatments. Through May, ozone treatment level means did not differ by more than 4.1 percent and values for the CF and 2xAMB treatments were very similar. Subsequently, differences between diameter growth in the AMB and 2xAMB treatments increased and the values for the CF treatment were either similar to the values for AMB or distinctly intermediate to the values for the AMB and 2xAMB treatments. Mean differences for the AMB and 2xAMB treatments increased to 11.4 percent by the end of the study.

There was little difference in mature branch diameter growth among genotypes as, by seasons end, mean cumulative growth ranged from 45.1 percent for genotype 3087 to 49.7 percent for genotype 3088. Acid rain treatment also had no significant impact on branch diameter growth.

Throughout most of the study period there was little difference in cumulative percent diameter increase among the three half-sib genotypes. But, at the final measurement in November, 1992, percent diameter growth for genotype 3088 (112 percent) was greater than that for genotype 3087 (97 percent). The final percent growth for genotype 3399 (103 percent) was intermediate and did not differ significantly from that for the other two genotypes.

There was no significant effect of ozone on diameter growth by the half-sib seedlings.

### Discussion:

In response to 2xAMB ozone, significant decrease in seedling growth was limited to genotype 3088. These seedlings demonstrated decreased height growth but not diameter growth. In contrast, Temple *et al.* (1992) found no ozone-related growth responses following four months exposure but did observe increased current-year needle and stem biomass and decreased diameter growth by seedlings of ponderosa pine in response to three seasons of elevated ozone exposure. Following a single growing season exposure, Edwards *et al.* (1990) found that loblolly pine seedling biomass was decreased by 7 to 16 percent when exposed to twice ambient ozone, relative to sub-ambient ozone, but seedling height and diameter growth were not significantly different among treatments. These results suggest that seedling growth response to ozone may be family specific and dependent upon cumulative exposure. They also indicate that seedling height and diameter growth may not be as sensitive as indicators of carbon allocation as is shoot and needle biomass.

Acidic rain main effects were less pronounced than were ozone main effects and were limited to an enhancement of seedling diameter growth. Fertilization effects of acidic precipitation have been observed for seedlings of other species with the implication that the nitrogen from  $\text{HNO}_3$  may be utilized by the plants. Our data for mature branch elongation suggests that the acidic rain fertilization effect may mitigate potential decreases due to 2xAMB ozone exposure. Roberts and Canon (1992) demonstrated

that terminal height growth and new shoot biomass of well-watered red spruce seedlings tended to be greater when exposed to both acidic rain and ozone than for ozone alone.

Although ozone is recognized as being the principal source of pollutant damage to California ponderosa pine, our data demonstrate that interactive effects with acidic rain, genotype, and season can modify growth response to the oxidant. We have also demonstrated that simple and interactive effects of the pollutants differ between seedlings and mature trees. These results indicate the complexity involved in scaling growth responses to multiple pollutants across genotypes and life stages.

#### Acknowledgements and Disclaimer:

This research was funded through the Atmospheric Acidity Protection Program of the California Air Resources Board (contract A132-101) and McIntire-Stennis Project 4635-MS. The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board.

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\*This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

#### List of Figures:

Figure 1. Mean percent branch elongation by clonal ponderosa pine trees exposed by simulated acid rain and ozone treatment. Acid rain treatments include no-rain (NAP), pH 5.1 rain and pH 3.0 rain. Ozone treatments include charcoal-filtered (CF), ambient (AMB) and twice ambient (2xAMB). Values are means and vertical bars represent one standard error of the mean.

Figure 2. Mean percent height growth of ponderosa pine seedlings of three half-sib genotypes by ozone treatment. Ozone treatments include charcoal-filtered (CF), ambient (AMB) and twice ambient (2xAMB). Values are means and vertical bars represent one standard error of the mean.

Figure 3. Seasonal pattern of ponderosa pine seedling diameter growth by simulated acid rain treatment. Acid rain treatments include no-rain (NAP), pH 5.1 rain and pH 3.0 rain. Values are means and vertical bars represent one standard error of the mean.

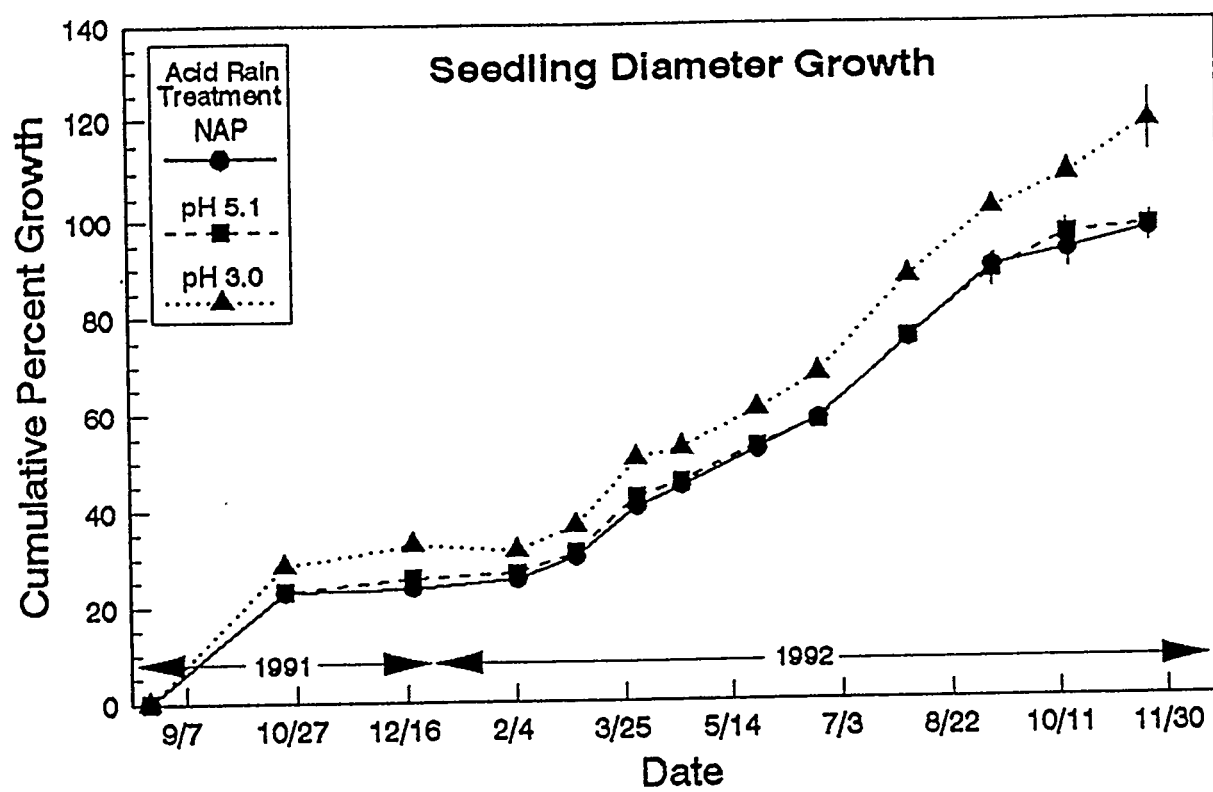


Figure 3.

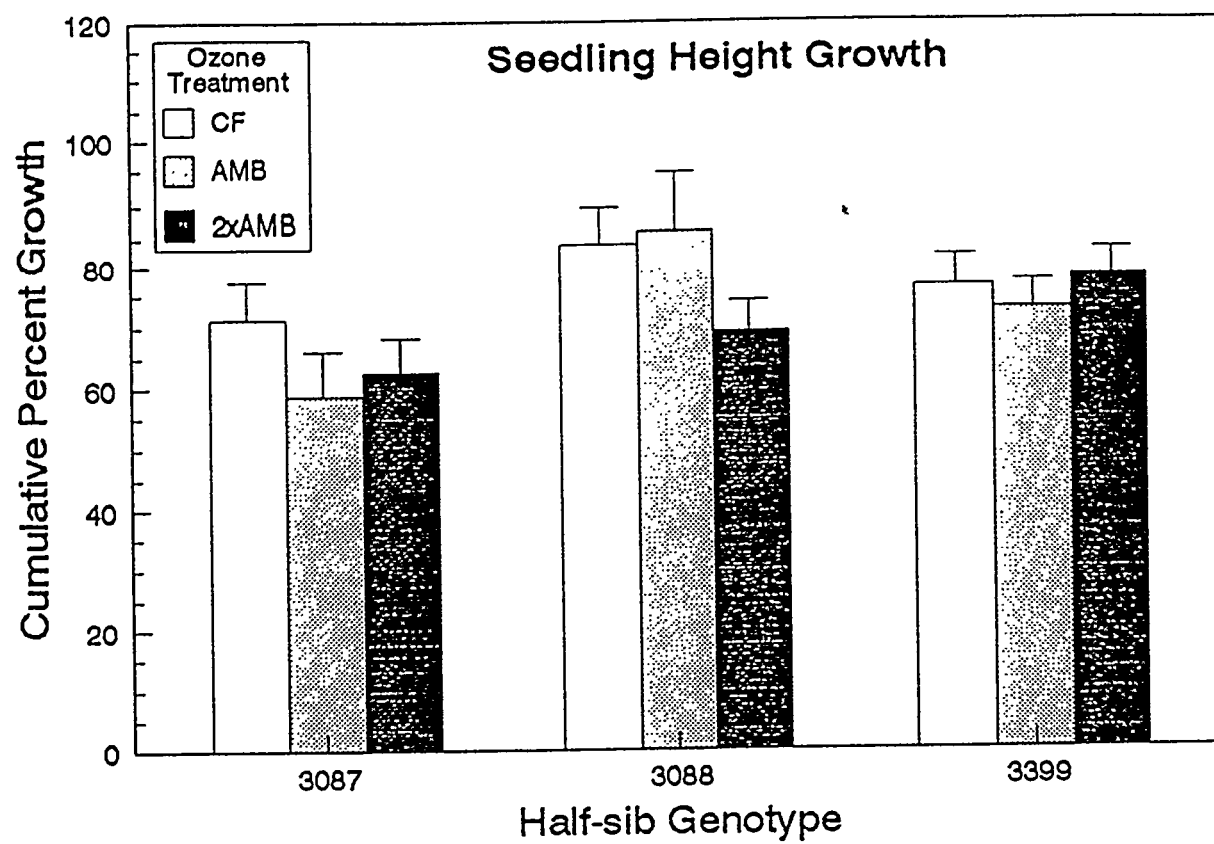


Figure 2.

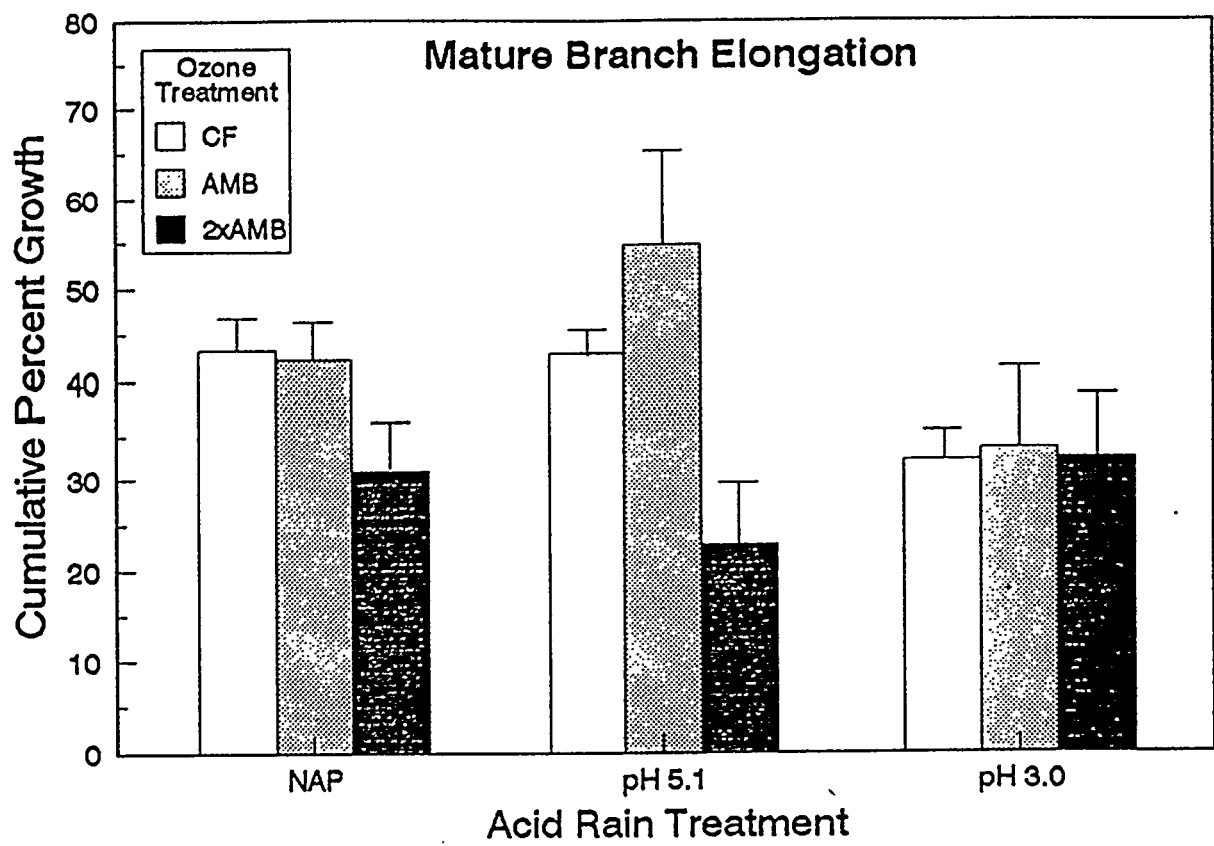


Figure 1.