The Impact of Ozone, Water Stress, and Acid Rain on the Growth and Physiology of Fraser Fir Seedlings

by

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Abstract

Three studies were conducted to determine the effects of ozone, water stress, and simulated acidic rain on the growth and physiology of Fraser fir seedlings. In Experiment I, seedlings were first exposed to 2 levels of moisture stress conditioning (MSC) for four weeks, and 3 levels of ozone (< 0.02, 0.05, and 0.10 ppm) and three levels of water stress (control, moderate, and severe) for 10 wks. The 2 MSC levels were a control (well-watered at all times) and a stress treatment in which seedlings were not watered until pre-dawn needle water potential measurements (Ψ) fell below -1.0 MPa. The 3 levels of water stress were a control (well-watered at all times); a moderately stressed treatment (seedlings watered when Ψ levels fell between -0.8 and -1.0 MPa); and a severely stressed treatment (seedlings watered when Ψ fell below -1.2 MPa). Fraser fir seedlings were exposed to 3 levels of simulated rain (pH 3.0, 4.3, and 5.6) and 2 levels of ozone (< 0.02 and 0.10 ppm) in Experiment II for 10 wks. Fraser fir seedlings in Experiment III were exposed to ozone levels of < 0.02, 0.05, and 0.10 ppm ozone and control, moderate, and severe water stress (as specified above) for 10 wks during the first year. Seedlings were then exposed to 2 levels of ozone (< 0.02 and 0.10 ppm) for 10 wks in the following year. Analysis of data indicate that ozone of 0.10 ppm significantly decreased net photosynthesis (Pn) in one study but was not significant in reducing Pn

in the other two studies. Ozone also failed to reduce growth significantly; however, decreasing trends were often apparent. Fraser fir biomass, transpiration (Ts), and needle conductance (Cs) were significantly decreased by water stress. Water-use efficiency was also improved as a result of prior moisture stress. Simulated acidic rain did not result in any significant changes in biomass accumulation, height, or diameter increment over the ten week period of the study. A solution of pH 3.0 did result in significantly higher root surface area and significantly reduced Ts after 5 wks of exposure; these responses are possibly due to a fertilization effect. No treatment interactions were significant. However, the combined, cumulative effect of all of these stresses may contribute to an overall decline in forest ecosystem productivity.

Abstract

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Dedication

This thesis is dedicated in memory of my father, Paul Kuang-Jun Tseng, and to my mother, Margaret Evelyn Tseng.

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Introduction

The concern over the concomitant increase in air pollution and an apparent decline in forest productivity has created the need for assessing the potential deleterious effects of these pollutants, especially ozone, on forest tree health. Although considerable research has been conducted on the effects of ozone on major crop species, such investigations on tree species have been minimal. One major issue concerning ozone is the possible effect of this pollutant on the yield of tree species. Mature red spruce and Fraser fir in the Southeastern United States have undergone a visible decline at higher elevations. It is possible that this decline may be due, at least in part, to increasing frequencies and concentrations of ozone and other air pollutants. These pollutants may also be affecting seedling regeneration in stands on higher elevation sites. Relatively little research has been conducted to discern the possible effects of ozone on Fraser fir seedlings.

Another confounding variable that may interact with ozone is moisture stress. The combined effect could result in more extensive damage. It is generally accepted that water stress is the major environmental factor limiting plant productivity. Areas within the Southeast have had periodic occur-

rences of drought. This drought and a simultaneous increase in atmospheric ozone may have severe implications on the growth of Fraser fir seedlings.

Acid deposition has also been thought to be a contributing factor to the apparent decine of high elevation tree species. Increasing use of fossil fuel may add greater amounts of nitrates and sulfates in the atmosphere, leading to a decrease in precipitation pH. Many researchers have suggested that "acid rain" may act in conjunction with other gaseous pollutants. Crops and tree productivity could be affected significantly by these pollutants.

The research conducted herein was an investigation of the effects of ozone, moisture stress, and simulated acidic precipitation on 4-year-old Fraser fir seedlings. Specifically, the objectives of this study were to:

1. Investigate the effects of ozone on Fraser fir seedlings under different moisture stress regimes by:
    a. measuring root and shoot growth;
    b. determining net photosynthetic rates, transpiration, and needle conductance; and
    c. determining needle water potential parameters
2. Determine the effect of moisture stress conditioning by:
    a. measuring the alteration of needle conductivity, transpiration, and net photosynthesis; and
    b. evaluating any altered ozone and water stress sensitivity as a result of moisture stress conditioning.
3. Investigate the effects of ozone and simulated acid rain on Fraser fir seedlings by:
    a. measuring root, total shoot and new shoot growth; and
    b. determining net photosynthetic rates, transpiration and needle conductance.
4. Investigate the effects of ozone and Fraser fir seedlings under different moisture stress regimes on a subsequent ozone fumigation by:
    a. measuring root, total shoot, and new shoot growth; and
    b. determining net photosynthetic rates, transpiration, and needle conductance.

Altered drought and air pollutant susceptibility may be playing an important role in the spruce/fir ecosystem. It is important in the evaluation of the apparent decline of spruce/fir ecosystems to understand the potential roles that each stress factor may be playing on the health of the forest ecosystem.

Literature Review

Ozone

The deleterious effects of the photochemical oxidant ozone on a variety of plant species are well-documented (Kress and Skelly 1982, Kress and Miller 1983, Tingey and Hogsett 1985). Concern has arisen as studies have reported that ozone reduces growth of commercially important crops species as well as tree species. Due to ozone's regional occurrence and strong oxidizing properties, it is probably the most important phytotoxic air pollutant affecting terrestrial vegetation in the United States (Chevone 1985). High level episodes of ozone have been reported throughout areas of the United States, especially in the San Bernardino, California, area and throughout areas within the eastern United States. Periodic atmospheric ozone concentrations have reportedly reached 0.1 ppm or greater in all rural areas surveyed (Pinkerton and Lefohn 1986).

Ozone has been cited as a possible causal agent for the apparent decline (reduced growth, dieback, mortality) of red spruce (*Picea rubens* Sarg.) and Fraser fir (*Abies fraseri* (Pursh) Poir.) in the higher elevations of the Appalachian Mountains (Wargo 1985). Red spruce and Fraser fir which grow at elevations exceeding 1900 m in the southeastern U.S. are in a state of decline. Seventy-five to ninety percent defoliation of trees of ages 45 to 85 years is being experienced on some trees (Bruck 1985).

Although it is difficult to separate the effects of air pollutants from other environmental and biotic factors, it is believed that photochemical oxidants play a significant role in the decline of trees within the U.S. Ozone is soluble once it enters plant tissue and interferes with internal oxidation, affecting biochemical pathways within the cell. In a severe episode of ozone, foliar damage can occur, but growth can be inhibited without characteristic foliar symptoms (Smith 1981). There is considerable evidence, including field observations, that oxidants cause increased necrosis, reduced photosynthesis, reduced tree growth, increased arthropod activity and microbial pathogen activity, alteration of ecosystem and species composition, and a change in biomass accumulation (Smith 1985).

Some species are particularly sensitive to ambient ozone. A persistent problem in the northeastern United States has been oxidant stipple injury to grapevine foliage (Musselman and Melious 1984). Urban tree species have also exhibited various sensitivities to ozone (Karnosky 1981). The foliar symptom most commonly found was a randomly distributed leaf surface fleck, black or purple in color, on the adaxial surface of the leaf (Karnosky 1981). These stipples eventually coalesce and form tannish brown necrotic lesions (Chappelka and Chevone 1986). Coyne and Bingham (1982) evaluated ponderosa pine (*Pinus ponderosa* Laws.) in the San Bernardino National Forest for response to ozone. They reported visible foliar symptoms in addition to reduced photosynthesis. Needles exhibiting symptoms tended to be shorter, small in girth, and less dense. As ozone sensi-

tivity increased, specific leaf weight decreased as did photosynthetic rates and leaf conductance values. They found higher stomatal resistances directly related to the development of chronic oxidant-injury symptoms. Although visible symptoms are associated with ozone injury, Kress and Skelly (1982) state that more relevant information for determining ozone injury can be found by evaluating growth rather than assessing leaf injury.

In a study on several eastern forest tree seedlings, Kress and Skelly (1982) examined the effects of ozone at concentrations of 0.05, 0.10, and 0.15 ppm ozone on willow oak (*Quercus phellos* L.), green ash (*Fraxinus pennsylvanica* Marsh.), white ash (*F. americana* L.), loblolly pine (*Pinus taeda* L.), pitch pine (*P. rigida* Mill.), Virginia pine (*P. virginiana* Mill.), sweetgum (*Liquidambar styraciflua* L.), sycamore (*Platanus occidentalis* L.), sugar maple (*Acer saccharum* L.), and yellow-poplar (*Liriodendron tulipifera* L.). Ozone at 0.05 ppm resulted in a signficiant height growth suppression with loblolly pine. At 0.10 ppm ozone, significant height growth decreases were observed for pitch pine, sweetgum, sycamore, and green ash. With the exception of white ash and Virginia pine, all other species showed significant height growth decreases with 0.15 ppm ozone. Although significant growth effects were seen at 0.05 ppm, foliar injury was not observed.

Decreased growth of yellow-poplar has also been observed in response to ozone exposure. Chappelka et al. (1985) reported a decrease in root/shoot ratio, height growth, and dry matter production. In another investigation, Jensen (1985) intermittently fumigated yellow-poplar seedlings with an ozone concentration of 0.1 ppm once or twice a week for 20 weeks. Exposure to ozone resulted in a 35% reduction of relative growth rate and significant reductions in relative leaf area growth rate, net assimilation rates, leaf area ratio, and specific leaf area.

Reich and Lassoie (1985) fumigated hybrid poplar (*Populus deltoides X trichocarpa*) with 0.025, 0.05, 0.085, or 0.125 ppm ozone concentrations. Treatment effects did not appear until after six weeks. After 10 weeks, ozone decreased plant height, diameter, number of leaves per plant, and caused a decrease in leaf, stem, root, and total dry weight per plant.

White ash seedlings exposed to ozone concentrations of 0.00, 0.05, 0.10, or 0.15 ppm exhibited a significant linear decrease in root and leaf dry weight, total height increase, root/shoot ratio, and mean relative growth rate of the total plant, stem, root and leaves (Chappelka and Chevone 1986). In preliminary results by Chappelka, Chevone, and Seiler (1985), red spruce seedlings were shown to have significantly less shoot dry mass resulting in an increase in root/shoot ratio when exposed to ozone.

In addition to reductions observed in growth, ozone has also been shown to reduce many physiological functions (Hill and Littlefield 1969, Barnes 1972, Jensen 1981, Chevone and Yang 1985). Many of the growth reductions associated with the effects of ozone are due to a decrease in metabolic or physiological functions photosynthesis and transpiration. Hill and Littlefield (1969) evaluated the response of a number of species to ozone, including barley (*Hordeum vulgare* Gem), oat (*Avena sativa* Park), wheat (*Triticum aestivum* var. Lemhi), corn (*Zea mays* var. Bel B), and tomato (*Lycopersicon esculentum* var. Moscow). The ozone treatments resulted in a rapid reduction in stomatal aperture. This induced a reduction in photosynthesis and transpiration. Transpiration rates were reduced 48% and CO_2 assimilation reduced from 40 to 70% compared to the controls. Although fumigation rates were considered high for these treatments (0.4 to 0.9 ppm), most physiological processes eventually resumed their pre-fumigation rates after the stress was removed. However, the researchers noted that there may be a continued reduction of physiological function in species that are exposed to ozone stress for extended periods of time.

Bytnerowicz and Taylor (1983) noted a similar effect in bean plants (*Phaseolus vulgaris* L. var. 'Bush Blue Lake no. 274'). During fumigation, stomatal resistance on the abaxial leaf surface of primary leaves increased sharply but returned to prefumigation levels when the stress was removed.

Olszyk and Tingey (1984) evaluated the response of garden pea (*Pisum sativum* L. cv. Alsweet) and tomato to ozone exposure in the light and dark. Fusiococcin was applied to the pea plants to ensure open stomata in the dark. Both species exhibited 30% greater foliar necrosis in the light than in the dark. Ozone levels are reported to be highest from 1200 to 1600 hours, the point at which plants are transpiring at their highest rates (Hussar 1985).

In another greenhouse study, ozone reduced stem and leaf dry weight and reduced the starch, sucrose, and reducing sugar content in the roots of green ash seedlings (Jensen 1981). Ozone interfered with photosynthate production or utilization resulting in a slower rate of dry weight accumulation in the new stems and leaves.

In a study on the effects of low level ozone in hybrid poplar, Reich and Lassoie (1984) reported that exposure to ozone decreased both leaf diffusive conductance and water-use efficiency (WUE). Photosynthesis was also shown to decrease, but there were no consistent correlations between ozone induced reductions in WUE and in photosynthesis. Reich and Lassoie concluded that ozone exposure probably reduced WUE via a direct impact on the stomatal control system. They further concluded that the decrease in WUE may affect the ability of poplar to withstand dessication. This could potentially impact poplar plants exposed to both ozone and water stress.

Barnes (1972) evaluated the effects of 0.05 or 0.15 ppm ozone on the photosynthetic and respiration rates of slash pine (*Pinus elliottii* Engelm.), pond pine (*P. serotina* Michx.), eastern white pine (*P. strobus* L.), and loblolly pine. He noted a pronounced stimulation of respiration by ozone at 0.15

ppm on the older needles. Stimulation of respiration due to ozone would impose an additional drain on the carboydrate supply. No visible injury symptoms were observed yet photosynthetic rates decreased.

Three clones of eastern white pine were evaluated for their response to short-term ozone exposure effects on net photosynthesis, dark respiration, and transpiration (Yang, Skelly, and Chevone 1983). Increasing ozone concentrations of 0.10, 0.20, and 0.30 ppm O_3 caused a corresponding decrease in net photosynthesis of 7, 14, and 19% in the ozone-sensitive clone. Light transpiration rates were decreased significantly by 0.2 and 0.3 ppm ozone, but recovery occurred one hour after fumigation was terminated. An increase in dark respiration rates, similar to those found by Barnes (1972), was also observed.

It is clear that ozone has a tremendous potential to impact the growth as well as the physiological function of many plant species. The pronounced effect of ozone in the absence of foliar symptoms suggests that ozone could be causing a decrease in yield of many crops in addition to a decline among tree species long before the associated visible symptoms of such a decline are evident.

Water Stress

In contrast to the limited research on the effects of ozone, there has been a great deal of research on the effects of moisture stress on trees (Kramer 1983). Water availability is regarded as the most limiting environmental factor to the growth of plants. Water stress causes decreased growth and impacts nearly all physiological functions. Currently, interest exists in determining how plant spe-

cies may modify their morphology or physiology in order to cope better with water stress. Because periodic drought is common in the eastern United States, plant response to this factor in combination with other pollutant stresses is of high interest.

Although severe moisture stress causes death in many plants, mild moisture stress may actually increase the plant's ability to survive periods of drought. Phenotypic modifications to environmental factors can increase a plant's ability to survive periods of drought through reduced leaf area, thicker leaves, less responsive stomata, more negative osmotic potential, and increases in root/shoot ratio (Kramer 1983).

One of the most important acclimations to water stress is that of osmotic adjustment which has been exhibited by a number of species (Davies and Lakso 1978, Ackerson and Herbert 1981, Kramer 1983 Seiler and Johnson 1984b, Seiler 1985). Osmotic adjustment can occur either passively or actively. Passive adjustment results from an accumulation of solutes due to dehydration. This type of adjustment provides little advantage to the plant. Active osmotic adjustment occurs in a plant which actively accumulates solutes within its cells. The osmotic potential, a measure of solute concentration, becomes more negative at a given relative water content, enabling the plant to maintain turgor and growth at lower internal water potentials (Kramer 1983).

Ackerson and Herbert (1981) reported that cotton plants subjected to moisture stress exhibited greater turgor pressure and approached zero turgor potential at much lower water potentials than controls. Ike and Thurtell (1981) found osmotic adjustment in cassava (*Manihot* species) exposed to moisture stress. At any leaf water potential, the water content was higher in stressed plants, and the value of the leaf water potential was more negative in the stressed than in the unstressed plants at zero turgor pressure. They noted that stressed cassava had a larger decrease in water potential per unit decrease in relative water content than unstressed plants. In experiments conducted on 1-0

loblolly pine seedlings, Hennessey and Dougherty (1984) noted osmotic adjustment in moderately water-stressed seedlings four months after initiation of outplanting and irrigation treatments. Osmotic adjustment allows a plant to maintain cell turgor over a lower range of water potentials, enables a plant to continue cell expansion, and to maintain many biochemical, physiological, and morphological processes (Hsiao 1973).

Another drought tolerance mechanism found in tree species is the ability to store water. Davies and Lakso (1979) noted this ability in apples which released stored water from leaf and trunk tissue into the transpirational stream. Other modifications that apples exhibit include an increase in leaf elasticity, the ability of the stomates to remain open under lower water and turgor potentials, and, thus, the ability to grow in conditions of water stress.

Water stress has been shown to decrease the dry weight of roots and shoots in pine seedlings (Kaufmann 1968, Seiler and Johnson 1984b). A greater increase in root weight to shoot weight may improve seedlings' survival under conditions of moisture stress. A larger rooting volume enables the plant to exploit a greater amount of water at a given soil water potential. Cannell et al. (1978) noted that families of loblolly pine that exhibited the fastest growth when regularly watered were not necessarily those that exhibited fastest growth during periods of water stress. Seedlings that were small and grew slower when well-watered avoided water stress by producing greater root masses and lengths in proportion to their shoots.

Seiler and Johnson (1984a) noted a 35% reduction in the shoot dry weight of black alder (*Alnus glutinosa* (L.) Gaertn) after exposure to water stress. Black alder is generally considered sensitive to water stress. After a moisture stress period of 5 weeks, seedlings exhibited an increase in root/shoot ratio from 0.28 to 0.33. Loblolly pine seedlings grown in containers did not exhibit a decrease in shoot biomass due to moisture stress (Seiler and Johnson 1984). Root biomass de-

creased 39% in the pre-stressed seedlings in comparison to the controls. They concluded that the roots may have gone into a state of quiescense in response to water stress.

In another experiment with black alder, Seiler (1985) noted that water stress caused a significant reduction in leaf surface area (67%) and reduced shoot biomass. Osmotic potential was reduced over 0.4 MPa which resulted in a significant increase in turgor in pre-stressed plants at full hydration.

In addition to modifications in root or shoot biomass, plants also exhibit alterations in their physiology that enable them to withstand periodic moisture stress. Moisture-stress-conditioned loblolly pine seedlings exhibited a 30% reduction in transpiration and no significant decrease in initial photosynthesis; this resulting in a 67% increase in water-use efficiency compared to control seedlings (Seiler and Johnson 1985).

Water stress decreases the photosynthetic rates of many plants by inducing increased stomatal resistance to carbon dioxide. Photosynthesis may also be limited by the diffusional resistance to carbon dioxide in the mesophyll (Brix 1962). This decrease in photosynthetic rate and initial decrease in respiratory substrates caused a decrease in the respiration rates. Kaufmann (1968) noted that water stress reduced photosynthesis as well as translocation. Leaf conductance and transpirational rates were reduced in avocado trees (*Persea americana* Mill. cv. Bacon) when they were exposed to moisture stress (Sterne et al. 1977).

Although water stress may cause a decrease in photosynthesis, water stress conditioning can actually improve a plant's ability to continue photosynthesizing at lower water potentials. Ackerson and Herbert (1981) reported that, despite lower photosynthetic rates at high water potentials, preconditioned cotton plants continued photosynthesis at water potentials much lower than those of

the controls. Avocado plants pre-stressed to moisture had intermediate levels of photosynthesis when compared to unstressed trees and those undergoing stress that had not been preconditioned. In loblolly seedlings, Seiler and Johnson (1985) reported that seedlings which were moisture stress conditioned exhibited a more gradual decline in photosynthesis with decreasing needle water potentials.

The stomata of seedlings preconditioned to water stress often do not fully open immediately following rewatering. Leaf conductance following rewatering of black alder did not fully recover to prestress levels (Seiler 1985). This enables the seedling to conserve water and function longer during periods of drought. A similar response was noted by Hinckley (1973) in the response of black locust (*Robinia pseudoacacia* L.). When rehydrated following a period of water stress, stomata of black locust seedlings did not completely return to prestress levels after 72 hours. This adaptation may be important in the relative drought tolerance of this species. By inhibiting full stomatal opening following stress, plants may be better adapted to a subsequent period of stress.

Although water is an important environmental factor affecting the health and vigor of tree species, the plant's ability to acclimate to cycles of moisture stress may actually improve its overall survival. Through modifications such as osmotic adjustment, increased root/shoot biomass ratios, and increased water-use efficiency, naturally preconditioned seedlings in the forest may have an increased ability to withstand periodic cycles of drought.

Acidic Precipitation

In contrast to the relatively consistent reports on the short-term effects of ozone and water stress on plant growth and physiology, studies involving the effects of acid rain on crop species and trees have failed to present conclusive results. The forecasted increase in the consumption of coal has led many researchers to try to quantify the effects that the concomitant increase in precipitation acidity will have on crop and tree species. Increasing public concern on the effects of "acid rain" on natural ecosystems has also fueled research efforts.

Acidic rainfall can be defined as precipitation with a hydrogen ion concentration exceeding 2.5 μeq/l (pH < 5.6) (Evans 1982). Increasing use of fossil fuels has increased the amount of nitrates and sulfates in the atmosphere, leading to a decrease in the pH of precipitation. However, the amount and degree to which acid rain may affect terrestrial vegetation is unknown. In an excellent review of acidic deposition research, Irving noted that of the 14 crop cultivars (nine species) and 34 crops varieties (28 species) studied in field and controlled environments, respectively, only one field and six greenhouse studies resulted in negative responses. The majority of all reports could not attribute any reductions in growth or yield to an increase in rain acidity treatments in the short-term. Limitations to the comparison of research on acidic deposition studies are partly due to differing methodologies, inconsistent results, and incomplete explanations of experimental methods (Irving 1983).

In a study conducted to characterize the effects of "acid rain" on Aleppo pine, Matziris and Nakos (1977) irrigated one-year-old half-sib family seedlings with simulated rain pH of 3.1, 3.5, and 5.1. They reported that "acid rain" reduced the total height and sulfur content of needles. Both 3.1 and 3.5 pH rain suppressed the formation of terminal buds and increase seedling mortality.

The foliar response of six clones of hybrid poplar was investigated in response to simulated acid rain by Evans et al. (1978). Two clones exhibited galls resulting from hyperplasia and hypertrophy of the parenchyma cells. In two other clones, the upper epidermis, palisade parenhyma, and sponge parenchyma were injured. Rain pH of 2.7 resulted in leaf injury on 10% of the leaf area. They conclude that the adaxial surface is the most affected by simulated acidic rain exposure. Evans and Curry (1979) also noted hyperplastic and hypertrophic reactions in *G. max* after exposure to simulated acid rain.

In a study on the effects of acidic precipitation (pH 5.6, 4.0, 3.0 and 2.0) on deciduous tree species, Neufeld et al. (1985) investigated foliar damage, growth, and gas exchange variables. Foliar damage occurred only at pH 2.0 for yellow poplar, sycamore (*Platanus occidentalis* L.), sweetgum (*Liquidambar styraciflua* L.), and black locust. Height growth was reduced at pH 2.0 in *Platanus* and *Robinia*. They noted that photosynthetic rates in *Platanus* were reduced after rain exposure of pH 2.0 resulting from changes in mesophyll conductance to CO_2. *Liquidambar* exhibited a reduction in stomatal conductance after exposure to rain pH of 2.0. They concluded that reductions observed in biomass were linked to a decreased photosynthetic capacity caused by acid rain. However, it should be noted that pH levels of 2.0 are not commonly, if ever, found in actual field conditions.

MacDonald et al. (1986) found that acid rain pH levels of 2.0 inhibited germination of jack pine (*Pinus banksiana* Lamb.) and mortality of germinants was over 95%. Rain pH levels that approach typical precipitation in northern lower Michigan (> 3.0) did not significantly affect jack pine regeneration.

In contrast to studies noting reductions in productivity of tree species, Wood and Bormann (1977) reported that seedling productivity of *Pinus strobus* actually increased with acidity during a 20-wk

experimental period (pH range 5.6 to 2.3). They attribute an increase in productivity to the fertilization effect of excess NO_3^{-1}.

Lee and Weber (1979) evaluated the effect of simulated sulfuric acid rain at pH values of 3.0, 3.5, or 4.0 versus 5.6 on the emergence of eleven woody species. Eight species were affected by simulated acid rain, although the magnitude and direction of effects varied with species and treatment.

Amthor (1984) discussed the influence of acid rain on plant growth and concluded that, due to the inconclusive results concerning the effects of acid rain on metabolite leaching, acid-rain induced lesions, growth and productivity, and interaction with gaseous pollutants, no clear evidence suggests that acid rain alone will kill the crops and forests of Europe and North America. Effects of continual acidic precipitation may, over the course of many generations, alter the chemical equilibria of the soil system. This may result in significant site degredations and, hence, significant productivity declines. The impact of acid rain in conjunction with other pollutants may also cause detrimental long-term effects.

Ozone and Moisture Stress

Despite research on the effects of ozone and moisture stress separately, investigations on the combined effects of ozone and moisture stress are lacking. Recent studies by Temple et al. (1985) and Tingey and Hogsett (1985) indicate that water stress may actually reduce ozone injury in plants. It is important to understand the effects that these combined stresses have on plants, especially on trees, because both environmental stresses act concomitantly.

Periodic drought is common in much of the eastern United States during summer months (Heggestad et al. 1985). Ozone levels are also known to be highest during these summer months. To investigate the combined effect of soil moisture stress and ozone on soybean (*Glycine max* L.), Heggestad et al. (1985) conducted field experiments with five levels of ozone and two soil moisture stress regimes over a period of two years. They found that soybean yields were reduced significantly by ozone and moisture stress alone as well as in combination. Numbers of seeds were reduced 30% and numbers of pods were reduced 40% when compared to the no-stress treatments. In the treatment without soil moisture stress, ozone exposure caused a reduction in yields of 5%, whereas combined exposure to soil moisture stress and ambient ozone concentrations reduced yields by 25%. These data provide the first experimental evidence that plants may lose tolerance to soil moisture stress when exposed to ozone.

Contrary to the results found by Heggestad et al. (1985), Tingey and Hogsett (1985) found that water stress actually reduced ozone injury in bean plants. Bean plants were sprayed with either fusiococcin to induce stomatal opening or with abscisic acid (ABA) to close the stomates. In order to monitor the effects of ozone on the plants, Tingey and Hogsett (1985) monitored stress ethylene production and foliar chlorophyll concentrations. Plants treated with ABA or water stress (conditions that increased stomatal resistance) displayed a reduced response to ozone. The authors suggest that the primary means by which water stress protects plants against ozone injury is through stomatal closure.

In a 2-year field study on cotton (*Gossypium hirustum* L.), Temple et al. (1985) found that plant water status had a significant effect on the response of cotton to ozone. Data show that yields were relatively unaffected by ozone when plants were under water-stress, although yields were severely reduced by water-stress alone. In 1982, under more humid growing conditions, both normally irrigated and water-stressed plants were significantly more susceptible to ozone. The authors there-

fore concluded that exposure to water stress decreases the susceptibility of cotton plants to ozone injury.

Results from Heggestad et al. (1985) seem to conflict with those of Tingey and Hogsett (1985) and Temple et al. (1985). Clearly, further investigations on the effects of water stress in combination with ozone are necessary. It is important to determine how the effects of internal water status of the plant mediate the response to ozone exposure.

Ozone and Acid Rain

The reports on the effects of acid rain alone on vegetation have, for the most part, failed to be conclusive. However, many researchers have suggested that acid rain may act in conjunction with other gaseous pollutants, causing a significant effect on crop and tree productivity. Troiano et al. (1983) investigated the combined effects of ambient ozone and simulated acid rain (pH 4.0, 3.4, 2.8) on soybean. Whereas filtered air caused only a slight decrease in the vegetative mass with increasing acidity, ambient ozone concentrations with increasing levels of acidity caused a significant decrease in vegetative mass. They reported a significant interaction between the effects of acidity and ozone.

In another experiment on soybeans, simulated acid rain (pH 3.4, 4.2, 5.0) and a combination of 0.1 µl/l SO_2 and 0.05 µl/l O_3 resulted in no significant interactions (Norby et al. 1985). Acid rain failed to affect physiology; however, the air pollutants reduced photosynthesis, nitrogen fixation, and vegetative growth. Norby and Luxmoore (1983) also reported no significant interactions between pH and air pollutants on the effect of soybean growth.

The combined effects of simulated acid rain (pH 3.0, 4.3, 5.6) and varying ozone concentrations (0, 0.05, 0.10, and 0.15 ppm) on white ash (*Fraxinus americana* L.) were investigated by Chappelka et al. (1986). Increasing ozone concentrations resulted in significant linear reductions in root and leaf weight, total dry weight, total height increment, root/shoot ratio, and mean relative growth rate of the seedling, stem, root, and leaves. Decreasing pH levels also caused linear decreases in root dry weight, mean relative root growth, and root/shoot ratio. Ozone and pH interactions were significant for root dry weight, mean relative root growth, and root/shoot ratio at 0.05 ppm O_3 and less significant for 0.10 ppm O_3 for mean relative root growth and root/shoot ratio as pH decreased. Chappelka et al. (1985) also reported significant rain x air pollutant interactions for root dry weight and mean relative growth rate for yellow-poplar. In contrast, reports by Dochinger and Jensen (1985) on the effects of acid mist (pH 2.5, 3.5, 4.5, and 5.5) and air pollutants indicated no consistent trend for the effects of ozone in combination with acid mist.

In a study conducted on sugar maple (*Acer saccharum* L.) and northern red oak (*Quercus rubra*), Reich et al. (1986) reported that increasing ozone levels caused a significant decline in the photosynthesis for both species and growth reductions in maple. Simulated acid rain (pH 5.6 to 3.0) had no effect on growth or photosynthesis and no interactions between the two pollutants were significant.

Skeffington and Roberts (1985) proposed that ozone may cause needles of Scots pine (*Pinus sylvestris* L.) to be more susceptible to leaching by acid rain or that the combined pollutants may damage the photosynthetic apparatus. However, they reported that their investigation did not indicate a good dose-response relationship or any significant interactions between the two pollutants.

Reich and Amundson (1985) measured photosynthetic response of four tree species to ozone and simulated acid rain. Ozone exposure resulted in significant reductions in photosynthesis for all species tested, yet acidic rain produced no negative effects and no interactions were observed.

A review of the studies conducted on the effects of simulated acidic rain and ozone in conjunction indicates that there is no clear-cut effect on the productivity of crops or tree species. More research is necessary to discern the possible mechanisms for the reported reduction in growth and biomass. The combined effects of both ozone and simulated acidic precipitation on vegetation should also be further explored.

Experiment 1

EFFECTS OF OZONE AND WATER STRESS ON FRASER FIR SEEDLING GROWTH AND PHYSIOLOGY

Abstract

Three-year-old Fraser fir (*Abies fraseri* (Pursh) Poir.) seedlings were exposed to two levels of moisture stress conditioning (MSC) for 4 weeks prior to ozone fumigation. Seedlings were either well-watered (control) or watered after pre-dawn needle water potential levels (Ψ) fell below -1.0 MPa. Seedlings were then exposed to 3 levels of ozone (< 0.02, 0.05, and 0.10 ppm) and three

levels of moisture stress for 10 weeks. Control seedlings were well-watered at all times; moderately water-stressed seedlings were watered when Ψ was between -0.8 and -1.0 MPa; severely water-stressed seedlings were watered when Ψ fell below -1.2 MPa. Net photosynthesis (Pn), transpiration (Ts), and needle conductance (Cs) were measured prior to, 5 weeks after, and at the end of the fumigation period following rehydration for at least 24 hours. Seedling dry weight and height were measured at the end of the study. Water stress caused a significant decrease in Fraser fir biomass, Ts, and Cs; MSC caused a significant reduction in root collar diameter. Exposure to ozone resulted in no biomass changes. Photosynthesis decreased significantly in all treatments following fumigation, with the largest decrease occurring at 0.10 ppm ozone. Water stress significantly reduced root and shoot dry weight by at least 20%. An MSC x water stress interaction resulted in a significant reduction in the root weight of moderately stressed seedlings over control seedlings. Moisture stress conditioning also caused a significant reduction in Pn, Ts, and Cs prior to the onset of fumigations. Water-use efficiency improved 30% after 10 weeks of exposure to severe moisture stress. Water stress x ozone interactions were not significant for any variable.

Introduction

Ozone is considered to be the most important air pollutant affecting plant growth and productivity in the United States due to its regional occurrence and strong oxidizing properties (Chevone 1985, Tingey and Hogsett 1985). Ozone is known to cause deleterious effects on a variety of tree species, and visible effects such as foliar damage have been reported (Coyne and Bingham 1982). Most effects of ozone, however, are not immediately apparent. These effects include reductions in relative growth rate and dry weight (Jensen 1985, Kress and Skelly 1982), photosynthesis (Coyne and Bingham 1982, Yang et al. 1983), water-use efficiency, range of leaf conductance, and ability to control water loss and withstand dessication (Reich and Lassoie 1984).

Ozone has been suspected as a contributing factor in tree decline in high elevation spruce and fir sites in the eastern United States (Wargo 1985). Damage to seedlings could affect the quality of regeneration and, in turn, the tree species diversity of these forest ecosystems. However, little research has been conducted to determine the response of these tree species to ozone.

It is generally accepted that water stress is the major environmental factor limiting plant productivity. In high elevation Fraser fir (*Abies fraseri* (Pursh) Poir.) stands, moisture stress often occurs during times of highest ozone concentration. The effect of ozone in combination with drought stress may act to intensify the damage caused by ozone (Heggestad et al. 1985). Tingey and Hogsett (1985) have also suggested that water stress may result in physiological modifications within the plant which result in a decreased sensitivity to ozone. This study was conducted to determine the impact of moisture stress conditioning, ozone, and water stress alone and in combination on the growth and physiology of Fraser fir seedlings.

Materials and Methods

Three-year-old Fraser fir were obtained from a commercial grower (Lamtree Farm, Warrenville, N.C.) and planted in 10 cm diameter plastic pots filled with a natural, unclassified mountain Spodosol collected from under a high elevation (1220 m) spruce stand near Mountain Lake in Giles County, Virginia. The upper 30 cm of mineral soil was collected and thoroughly mixed before placement in the pots. Seedlings were placed in a greenhouse and maintained under a 16-hour photoperiod using supplemental lighting with high pressure sodium vapor lamps. The greenhouse was supplied with charcoal filtered air, and ozone levels were maintained below 0.025 ppm. Greenhouse temperatures ranged from 20 to 35 °C.

After a one week acclimation period, one half of the seedlings were subjected to 4 weeks of moisture stress conditioning (MSC) prior to fumigation. Control seedlings were well watered at all times and MSC seedlings were watered after pre-dawn needle water potential (Ψ) levels fell below -1.0 MPa. Following the 4 weeks of the MSC treatment, seedlings were randomly assigned to a combination of < 0.02, 0.05, or 0.10 ppm ozone concentrations and three moisture stress treatments. The moisture stress treatments used were the following: no stress (seedlings were kept well-watered); moderate stress (seedlings were not watered until pre-dawn needle water potential (Ψ) averaged -0.8 to -1.0 MPa); and severe stress (seedlings were not watered until Ψ was below -1.2 MPa). Pre-dawn Ψ measurements were taken with a pressure bomb (PMS Instruments, Corvallis, OR). Twelve seedlings were allocated to each of the nine treatment combinations (3 ozone levels and 3 water stress levels).

After bud break had occurred, seedlings were exposed to ozone in a continuously stirred tank reactor system (CSTR) (Heck et al. 1978) for four consecutive hours (0900-1300 EST) three days per

week for 10 wks. Seedlings whose pre-dawn needle water potentials fell within the specified stress range were watered following fumigation. Temperatures within the chambers were maintained at 25 ± 1.5 °C. Photosynthetic photon flux density (PPFD) was maintained at 600 ± 20 μMol m^{-2} s^{-1}. Ozone was generated from oxygen exposed to ultraviolet irradiation (Welsbach Laboratory Ozonator Model T-408, Welsbach Ozone Systems Corp., Philadelphia, PA 19129). Ozone concentrations were monitored by a Bendix Chemiluminescent Ozone Analyzer (Model 8002 Bendix Process Instruments Division, Lewisburg, WV 24901) calibrated with a Photocal 3000 Automated Ozone Calibrator (Columbia Scientific Instruments, Austin, TX 78720). The primary transfer standard was a Dasibi UV Ozone Analyzer (Dasibi Environmental Corp, Glendale, CA 91204).

Three seedlings from each of the treatment combinations were used to measure gas exchange prior to, after 5 wks, and at the end of the 10 wk fumigation period. A branch from each seedling was stripped of a small ring of needles, tagged at that point, and emergent needles plucked off to ensure that repeated net photosynthesis (Pn), transpiration (Ts), and needle conductance (Cs) measurements were taken on the same needles. Seedlings were placed within a CSTR and allowed to equilibrate for at least one hour before mesurements began. Pn, Ts, and Cs were measured with a portable photosynthesis system (LI-6000, LI-COR, Inc., Lincoln, NE) using a quarter-liter cuvette. Seedlings were allowed to rehydrate overnight before any measurements were taken. Average environmental variables within the cuvette were 14.9 ± 4.4% RH, 30 ± 0.7 °C, 600 ± 20 μMol m^{-2} s^{-1} PPFD, and 365 ± 20 ppm CO_2.

At the end of the ten week fumigation period, needles used to measure photosynthesis were stripped and projected needle surface area was taken with a LI-3000 Portable Area Meter (LI-COR, Lincoln, NE). The needles used to measure photosynthesis, shoots, and roots were dried in a 60°C drying oven for at least 24 hours before dry weights were measured. Water-use efficiency (WUE) was calculated as Pn (mg CO_2 m^{-2} h^{-1}) divided by Ts (mg H_2O m^{-2} h^{-1}) for each seedling.

The experiment was designed as a split plot replicated three times for fumigation exposures. Three blocks of 3 CSTR's were used. Whole-plot treatments were O_3 concentrations; subplot treatments were the 2 levels of MSC and the 3 levels of water stress. Each treatment combination was replicated 4 times/CSTR. Growth variables were measured on all 12 seedlings, and weight and gas exchange variables were measured on 6 seedlings per treatment combination. A mean separation test (Duncan Multiple Range Test) was used to determine significance among treatments. All data were analyzed for homogeneity of variance, and transformations were not necessary.

Results and Discussion

Ten weeks of ozone exposure resulted in no significant differences in shoot weight, root weight, final root collar diameter, or root to shoot ratio. MSC resulted in no significant reductions in biomass except for a significant decrease of 26% in root collar diameter difference from the initiation of the MSC study to the end of the fumigation period (Table 1). Water stress resulted in significant reductions ($\alpha = .05$) in seedling biomass, stem diameter, and root to shoot ratio (Table 2). Shoot weight and root weight were decreased an average of 22% and 31% for the moderate and severe water stress treatments, respectively, when compared to the control plants. A significant interaction between MSC and water stress on root weight is presented in Table 3. Moisture-stress-conditioning in combination with moderate water stress reduced root weight significantly over the controls, whereas in the control seedlings for the MSC treatment, only severe water stress significantly reduced root weight (Table 3). Root collar diameter was reduced 7% by the moderate treatment and 14% by the severe water stress treatment. The root to shoot ratio was also reduced

Table 1. Diameter increment of 4-yr-old Fraser fir after 4 wks of moisture stress conditioning & 10 wks of water stress[1].

Moisture Stress Conditioning	Diameter Increment (mm)
Control[2]	1.081 a
Stress[3]	0.794 b

[1]Means with the same letter are not significantly different ($\alpha = .05$).
[2]Seedlings well-watered at all times.
[3]Seedlings watered when $\Psi > 1.0$ MPa.

Table 2. Biomass and growth variables of 4-yr-old Fraser fir seedlings exposed to 10 wks of moisture stress[1].

Water Stress	Shoot Wt. (g)	Root Wt. (g)	Diam. (mm)	Root/Shoot Ratio (g/g)
Control	4.50 a	1.83 a	5.11 a	0.39 a
Moderate[2]	3.65 b	1.42 b	4.73 b	0.38 ab
Severe[3]	3.27 b	1.17 c	4.41 c	0.34 b

[1]Means within a column followed by the same letter are not significantly different ($\alpha = .05$)
[2]Seedlings watered when Ψ between -0.8 and -1.0 MPa.
[3]Seedlings watered when Ψ > 1.2 MPa.

Table 3. Root weight of 4-yr-old Fraser fir after 4 weeks of moisture stress conditioning and 10 wks of moisture stress[1].

Water Stress	Root Weight (g)	
	Control	Moisture Stress Conditioning
Control	1.7 a	2.0 a
Moderate[2]	1.6 a	1.3 b
Severe[3]	1.2 b	1.1 b

[1]Means within a column followed by the same letter are not significantly different ($\alpha = .05$).
[2]Seedlings watered when Ψ between -0.8 and -1.0 MPa.
[3]Seedlings watered when Ψ < -1.2 MPa.

significantly as a result of severe water stress. A similar reduction in the root to shoot ratio in response to water stress has been reported in loblolly pine (*Pinus taeda* L.) and white pine (*Pinus strobus* L.) (Kaufman 1968, Seiler 1984, Seiler and Johnson 1985b). It is usually accepted that water stress increases the root to shoot ratio (Kramer 1983). Possibly in coniferous species, the roots become quiescent in response to water stress and growth is not resumed upon rewatering. Lesham (1970) found this to be the case in Aleppo pine (*Pinus halepensis* Mill.).

Moisture-stress-conditioning prior to the intiation of fumigations decreased Pn 17%; however, after 5 weeks of fumigation, MSC seedlings exhibited a higher Pn by 43% (Table 4). Photosynthesis at the end of the 10 week fumigation period showed no significant differences among treatments.

A similar response was observed after 4 weeks of MSC for Cs and Ts. Conductance and Ts were reduced 14 and 20%, respectively, when compared to the controls. Five weeks later, MSC seedlings had a 14% higher Cs and a 37% higher Ts. Conductance for MSC seedlings was not significantly different from controls, and Ts was only 11% higher in the MSC seedlings at the end of the study period. It appears that the effect of MSC on Pn, Ts, and Cs was short-term was no longer evident after exposure to differing moisture stress regimes and ozone.

Ozone exposure did not result in any significant changes in Ts or Cs in Fraser fir (Table 5). Needle conductance increased slightly for the seedlings exposed to 0.10 ppm ozone; however, this difference was not significant.

Following an overnight rehydration period, seedlings exposed to water stress during fumigation failed to achieve control seedling levels of transpiration and needle conductance (Table 6). Moderate and severe moisture stress resulted in significantly reduced Ts and Cs at both time periods

Table 4. Conductance, transpiration, & photosynthesis of 4-yr-old Fraser fir after 4 wks of MSC and 5 & 10 wks of O_3[1].

	Moisture Stress Conditioning (MSC)	
	Control[2]	Stress[3]
Needle Conductance (cm/s)		
0 wks	0.148 a	0.128 b
5 wks	0.095 b	0.110 a
10 wks	0.204 a	0.225 a
Photosynthesis (mg $CO_2 cm^{-2} hr^{-1}$)		
0 wks	0.059 a	0.049 b
5 wks	0.024 b	0.042 a
10 wks	0.035 a	0.036 a
Transpiration (mg H_2O $cm^{-2} hr^{-1}$)		
0 wks	10.77 a	8.56 b
5 wks	6.85 b	10.91 a
10 wks	10.95 b	12.27 a

[1]Means within a row followed by the same letter are not significantly different ($\alpha = .05$).
[2]Seedlings well-watered at all times.
[3]Seedlings watered when $\Psi < -1.0$ MPa.

Table 5. Transpiration and conductance of 4-yr-old Fraser fir after 5 and 10 wks of O_3

Ozone (ppm)	Transpiration ($mg\ cm^{-2}\ hr^{-1}$)		Conductance (cm/s)	
	5 wks	10 wks	5wks	10 wks
< 0.02	8.53	11.57	0.100	0.197
0.05	9.13	11.53	0.106	0.212
0.10	8.98	11.73	0.101	0.235

[1]No means within a column were significantly different ($\alpha = .05$).

Table 6. Transpiration and conductance of 4-yr-old Fraser fir seedlings after 5 and 10 wks of water stress[1].

Water Stress	Transpiration ($mg\ m^{-2}\ hr^{-1}$)		Conductance (cm/s)	
	5 wks	10 wks	5 wks	10 wks
Control	10.42 a	12.92 a	0.124 a	0.270 a
Moderate[2]	8.68 b	11.38 b	0.101 b	0.192 b
Severe[3]	7.55 c	10.54 b	0.083 c	0.181 b

[1]Means within a column followed by the same letter are not significantly different ($\alpha = .05$). Measurements were taken after seedlings were rehydrated for at least 24 hours.
[2]Seedlings watered when Ψ between -0.8 and -1.0 MPa.
[3]Seedlings watered when Ψ < -1.2 MPa.

measured. Seiler and Johnson (1985) also noted a 30% reduction in transpiration for loblolly pine seedlings after an 8 week period of moisture stress.

Due to initial variation in the mean Pn rate among treatments, ozone effects were analyzed on the percent change in Pn prior to fumigation. After 5 weeks of ozone exposure, seedlings from all treatments exhibited a reduction in net photosynthesis. However, the greatest decrease of 41% was observed at the 0.10 ppm level (Table 7). There was no significant difference in photosynthesis among treatments after ten weeks despite a 42% decrease for seedlings fumigated with 0.10 ppm ozone. The decreased photosynthetic rate failed to result in any significant seedling growth reductions. This is possibly due to the fixed growth pattern of Fraser fir. Seedling growth during fumigation was largely determined the previous year in the nursery. Unless ozone had some effect on cell elogation, it is not surprising that no growth reduction was observed. It is possible that growth the year following ozone exposure could be reduced since decreased Pn may result in a smaller bud size.

Another consideration is that photosynthesis measurements were taken on only one branch. This may not accurately reflect the status of the entire seedling. Measurements were taken three times during the fumigation period. These periodic measurements are probably not representative of changes in photosynthesis over the course of the fumigation period.

The effect of water stress on Pn is shown in Table 8. After rehydrating for at least 24 hours, the severe water stress treatment resulted in a 14% decrease at 5 weeks, yet it resulted in the highest photosynthetic rate of 0.041 mg CO_2 cm^{-2} hr^{-1} at 10 weeks. After rehydration, Pn did not differ significantly from the controls as a result of moderate moisture stress. WUE was significantly improved by 30% after 10 weeks of severe water stress during fumigation (Table 8). Upon rehydration, the severe water stress treatment caused a significant reduction in Ts but did not decrease

Table 7. Changes in net photosynthesis of 4-yr-old Fraser fir following exposure to ozone for 5 and 10 wks[1].

Ozone (ppm)	Percent Decrease from Initial Photosynthesis	
	5 wks	10 wks
< 0.02	31.1 b	22.1 a
0.05	20.4 c	22.3 a
0.10	40.5 a	41.7 a

[1]Means within a column followed by the same letter are not significantly different ($\alpha = .05$).

Table 8. Net photosynthesis and water-use efficiency of 4-yr-old Fraser fir seedlings after 5 and 10 wks of water stress[1].

Water Stress	Photosynthesis (mg CO_2 cm^{-2} hr^{-1})		WUE (mg CO_2/mg H_2O)	
	5 wks	10 wks	5 wks	10 wks
Control	.036 a	.036 ab	.0036 a	.0027 b
Moderate[2]	.033 ab	.030 b	.0038 a	.0028 b
Severe[3]	.031 b	.041 a	.0040 a	.0039 a

[1]Means within a column followed by the same letter are not significantly different ($\alpha = .05$). Measurements were taken after seedlings were rehydrated for at least 24 hours.
[2]Seedlings watered when Ψ between -0.8 and -1.0 MPa.
[3]Seedlings watered when Ψ < -1.2 MPa.

photosynthesis. This resulted in an improved WUE. Seiler and Johnson (1985) found the same response in loblolly pine where WUE was improved over 67% in response to 8 weeks of water stress when compared to well-watered seedlings.

In conclusion, MSC and water stress caused a significant decrease in Ts and Cs. MSC also caused a significant reduction in Pn after 4 weeks. Additionally, the ten weeks of water stress resulted in a significant biomass decline in Fraser fir seedlings. These reductions were more significant than those resulting from ozone exposure. A significant increase in WUE was associated with severely water stressed seedlings. Through physiological modifications, seedlings may be able to withstand drought stress for longer periods, although these modifications result in decreased growth.

Fumigation for 10 weeks with ozone levels of 0.05 and 0.10 ppm 4 h d^{-1}, 3 d wk^{-1} did not significantly reduce the growth of Fraser fir seedlings, although a decrease in Pn was observed. Over prolonged exposure, however, this reduction in photosynthesis may result in growth reductions. No interactions between moisture stress and ozone were significant in this study, suggesting that ozone and water stress may act independently in affecting Fraser fir seedling physiology and growth.

Experiment II

THE IMPACT OF OZONE AND ACID RAIN ON FRASER FIR SEEDLING GROWTH AND PHYSIOLOGY

Abstract

Four-year-old Fraser fir (*Abies fraseri* (Pursh) Poir.) seedlings were exposed to ozone levels of < 0.02 (control) and 0.10 ppm and three simulated acid rain treatments (3.0, 4.3, 5.6 pH) for ten weeks. Photosynthesis (Pn), transpiration (Ts), and needle conductance (Cs) were measured prior to, after 5 wks, and at the end of the treatment period. Neither ozone nor simulated acid rain re-

sulted in significant changes in biomass accumulation, height or diameter increment. However, simulated acid rain of pH 3.0 did result in a significantly higher root surface area and root surface area/root wt ratio, as well as significantly decreasing Ts after 5 wks but failed to result in significant decreases at the end of the study. No significant differences among the rain treatments for Pn occurred; however, seedlings exposed to rain pH of 3.0 exhibited a significantly higher water-use efficiency (WUE) at the end of 10 wks. A fertilization effect is hypothesized. Ozone fumigation failed to result in any significant differences for Ts; Cs and Pn were approximately 33% higher for seedlings exposed to 0.10 ppm ozone after 5 wks. Water-use efficiency was improved 17% by 0.10 ppm ozone compared to controls. Ozone x acid rain interactions were not significant for any physiological variable.

Introduction

The potential deterimental impacts of acid rain and gaseous pollutants, such as ozone, on the health and productivity of crops and forest tree stands in the U.S. are complex (Johnson and Richter 1983, McLaughlin 1985, and many others). An increase in the acidity of rain is largely a result of increasing combustion of fossil fuels, specifically the loading of sulfuric and nitric acids from sulfur dioxide (SO_2) and nitrogen oxides in atmospheric water (Lee and Weber 1979). Effects of acid rain, however, have been mixed and, generally, inconclusive (Irving 1983, Amthor 1984). Ozone is a secondary pollutant also resulting from the combustion of fossil fuels. Ozone has been reputed as the most phytotoxic pollutant affecting terrestrial vegetation, affecting the growth and physiology of a variety of species (Barnes 1972, Carlson 1979, Kress and Miller 1983, Reich and Amundson 1985, Chevone 1985).

The apparent decline of red spruce (*Picea rubens* Sarg.) and Fraser fir (*Abies fraseri* (Pursh) Poir.) at high elevations in the southern Appalachians is thought to be the result of, at least in part, increasing ozone levels (Wargo 1985). The low acidity of rain and mist at these sites may also be a contributing factor to the reduction in vigor. A reduction in the productivity and health of these species may significantly alter the stand composition and ecosystem dynamics.

This study was conducted to determine the impact of ozone and simulated acidic rain on the biomass accumulation, growth, and physiology of Fraser fir seedlings. Effects on seedling growth may affect the viability of regeneration under declining stands of Fraser fir and red spruce. Studying the effects of ozone and acidic rain on physiological parameters as well as growth may lead to a greater understanding of how growth may be affected.

Materials and Methods

Three-year-old Fraser fir were obtained from a grower in White Top, Virginia, and planted in 10 cm diameter plastic pots filled with a natural, unclassified mountain Spodosol collected from under a high elevation (1220 m) spruce stand near Mountain Lake in Giles county, Virginia. The upper 30 cm of mineral soil was collected and thoroughly mixed before placement in the pots. Seedlings were placed in a greenhouse and maintained under a 16-hour photoperiod using supplemental lighting with high pressure sodium vapor lamps. The greenhouse was supplied with charcoal filtered air, and ozone levels were maintained below 0.025 ppm. Greenhouse temperatures ranged from 20 to 35 °C.

After a 2 week acclimation period, seedlings were randomly assigned to a combination of < 0.02 or 0.10 ppm ozone concentrations and three acid rain treatments (pH 3.0, 4.3, or 5.6). Simulated acid rain was applied to the seedlings using a simulator developed on the principle of droplet formation from needle tips (Chevone et al. 1984). Ionic concentrations were similar to the average ambient rainfall occurring in southwestern Virginia; solutions were prepared as described by Chevone et al. (1985). The solution pH's were adjusted to 5.6, 4.3, or 3.0 by addition of 1 M NaOH or 1 M H_2SO_4 + 0.5 M HNO_3. Rain was applied to the seedlings at a rate of 1.25 cm/hr for 1 hr, 2 times weekly for ten weeks on days in which ozone fumigations were not occurring.

After bud break had begun, seedlings were exposed to ozone in a continuously stirred tank reactor system (CSTR) (Heck et al. 1978) for four consecutive hours (0900-1300 EST) three days week^{-1} for ten wks. Temperatures and photosynthetic photon flux density (PPFD) within the chambers were maintained at 25 ± 1.5°C and 600 ± 20 μMol m^{-2} s^{-1}, respectively. Ozone was generated from oxygen exposed to ultraviolet irradiation (Welsbach Laboratory Ozonator Model T-408,

Welsbach Ozone Systems Corp., Philadelphia, PA 19129). Ozone concentrations were monitored by a Bendix Chemiluminescent Ozone Analyzer (Model 8002 Bendix Process Instruments Division, Lewisburg, WV 24901) calibrated with a Photocal 3000 Automated Ozone Calibrator (Columbia Scientific Instruments, Austin, TX 78720). The primary transfer standard was a Dasibi UV Ozone Analyzer (Dasibi Environmental Corp, Glendale, CA 91204).

Five seedlings from each of the treatment combinations were used to measure gas exchange prior to, after 5 wks, and at the end of the 10 wk fumigation period. A branch from each seedling was stripped of a small ring of needles, tagged at that point, and emergent needles plucked off to ensure that repeated net photosynthesis (Pn), transpiration (Ts), and needle conductance (Cs) measurements were taken on the same needles. Seedlings were placed within a CSTR and allowed to equilibrate for at least one hour before measurements began. Pn, Ts, and Cs were measured with a portable photosynthesis system (LI-6000, LI-COR, Inc., Lincoln, NE) using a quarter-liter cuvette. Average environmental variables within the cuvette were $20 \pm 5.9\%$ RH, $30 \pm 2°C$, 600 ± 20 μMol m^{-2} s^{-1} PPFD, and 348 ± 22 ppm CO_2.

At the end of the ten week fumigation period, needles used to measure photosynthesis were removed and projected needle surface area was taken with a LI-3000 Portable Area Meter (LI-COR, Lincoln, NE). Root surface area of the same seedlings was also measured with the area meter. The needles used to measure photosynthesis, new growth, shoots, and roots were dried in a 60°C drying oven for at least 24 hours before dry weights were measured. Water-use efficiency (WUE) was calculated as Pn (mg CO_2 m^{-2} h^{-1}) divided by Ts (mg H_2O m^{-2} h^{-1}) for each seedling.

Five soil samples were randomly selected from each pot for six treatment combinations. Each soil sample was analyzed to determine the effect of simulated acid rain on cations, nitrates, organic mater and pH. Cations were prepared with a 0.05N HCl + 0.025 N H_2SO_4 extraction and ana-

lyzed with inductively coupled plasma, similar to the atomic absorption method. Soil nitrate was determined with a 2.5 : 1, 0.02 M $CuSO_4$: soil extraction and measured with a NO_3^- electrode. Soil organic matter was determined using a 0.67 M $Na_2Cr_2O_7 \bullet H_2O$: soil extraction and measured calorimetrically. The pH of the soil was measured using a 1:1 water to soil paste.

The experiment was designed as a split plot replicated five times for fumigation exposures. Five blocks of 2 CSTR's were used. Whole-plot treatments were O_3 concentrations; subplot treatments were the 3 levels of acid rain. Each treatment combination was replicated 5 times/CSTR. Growth variables were measured on all 15 seedlings and gas exchange variables were measured on 5 seedlings per treatment combination. A mean separation test (Duncan Multiple Range Test) was used to determine significance among treatments. All data were analyzed for homogeneity of variance, and transformations were not necessary.

Results

Neither ozone nor simulated acid rain resulted in any significant differences in biomass variables; some trends, however, are apparent (Table 9). Seedlings exposed to a rain pH of 3.0 exhibited a decreasing trend for both total shoot weight and root weight when compared to seedlings exposed to 5.6 pH rain. However, weight of new growth for seedlings exposed to 3.0 pH simulated acid rain appeared to be slightly higher. Root to shoot ratio did not exhibit a trend among rain pH's. An interaction between ozone and acid rain was significant for the ratio of new growth to old shoot growth (Table 10). At pH 4.3, 0.10 ppm ozone resulted in a significant increase in this ratio.

Table 9. Biomass of 4-yr-old Fraser fir after exposure to 10 wks of ozone and acid rain[1].

	Acid Rain (pH)			Ozone (ppm)	
	3.0	4.3	5.6	< 0.02	0.10
Shoot Wt (g)	3.51	3.55	3.81	3.78	2.82
Root Wt (g)	1.66	1.58	1.70	1.58	1.71
New Growth (g)	0.789	0.719	0.782	0.794	0.733
Root/Shoot Ratio	0.680	0.722	0.683	0.678	0.713

[1]No means were significantly different ($\alpha = .05$).

Table 10. Root surface area, weight, and area/wt ratio of 4-yr-old Fraser fir[1].

	Acid Rain (pH)			Ozone (ppm)	
	3.0	4.3	5.6	< 0.02	0.10
Root Wt (g)	1.92 a	1.77 a	1.72 a	1.83 a	1.78 a
Root Surface Area (cm^2)	169.2 a	136.7 b	146.4 ab	152.4 a	149.2 a
Root area/ weight Ratio (cm^2/gm)	88.8 a	77.5 b	85.8 a	83.9 a	84.2 a

[1]Means within the same row followed by the same letter are not significantly different ($\alpha = .05$).

Simulated acidic rain of 3.0 resulted in a significantly higher root surface area and root surface to root weight ratio when compared to the 4.3 pH treatment (Table 11). Ozone did not result in any significant alterations in root weight, root surface area, or surface area/weight ratio.

Ozone appears to have caused a slight increase in root weight, but a slight reduction in total shoot weight and new shoot weight is indicated (Table 9). Root to shoot ratio appears to be slightly higher in seedlings exposed to 0.10 ppm ozone.

Although no significant differences among treatments for biomass and growth variables occurred for either simulated acid rain or ozone treatments, with the exception of the rain effect of root surface area, significant differences did occur for both treatments for physiological parameters measured. Transpiration (Ts) was significantly lower at pH 3.0 than pH 5.6, although pH 4.3 resulted in an even lower Ts (Table 12). At the end of 10 wks, Ts was not significantly different among treatments despite a decreasing trend with increasing rain acidity. Seedlings exposed to a rain pH of 5.6 exhibited a significantly higher conductance (Cs) than seedlings exposed to 4.3 pH rain, but this was not significantly higher than pH 3.0. Following the end of the study, Cs was not significantly different among treatments, exhibiting a similar response as observed in Ts.

Photosynthesis was not significantly affected by the simulated acid rain treatments (Table 12); however, a significant increase in WUE at a pH of 3.0 was observed as compared to pH 5.6 treatments after 10 wks.

Transpiration was not significantly different after either 5 wks or 10 wks of ozone fumigation (Table 13). After 5 weeks, Cs was not significantly higher for seedlings exposed to 0.10 ppm, but an increasing trend is indicated. Conductance at the culmination of the study was significantly higher by 33% for seedlings exposed to 0.10 ppm ozone. Photosynthesis at the end of the study

Table 11. Ratio of new shoot to old shoot for 4-yr-old Fraser fir after 10 wks of acid rain and O_3[1].

Ozone (ppm)	Ratio of New to Old Shoot Acid Rain (pH)		
	3.0	4.3	5.6
< 0.02	0.192 a	0.165 b	0.222 a
0.10	0.217 a	0.208 a	0.145 a

[1]Means within the same column followed by the same letter are not significantly different ($\alpha = .05$).

Table 12. Transpiration, conductance, photosynthesis, and WUE of 4-yr-old Fraser fir after 5 and 10 wks of simulated rain[1].

	Acid Rain (pH)		
	3.0	4.3	5.6
Needle Conductance (cm/s)			
5 wks	0.784 ab	0.673 b	0.953 a
10 wks	0.352 a	0.368 a	0.375 a
Photosynthesis (mg CO_2 cm^{-2} h^{-1})			
5 wks	0.097 a	0.090 a	0.097 a
10 wks	0.067 a	0.066 a	0.057 a
Transpiration (mg H_2O cm^{-2} h^{-1})			
5 wks	24.15 b	20.67 c	27.50 a
10 wks	17.89 a	19.71 a	21.47 a
WUE (mg CO_2/mg H_2O)			
5 wks	0.0040 a	0.0043 a	0.0036 a
10 wks	0.0037 a	0.0034 ab	0.0027 b

[1]Means within the same row followed by the same letter are not significantly different ($\alpha = .05$).

Table 13. Transpiration and conductance of 4-yr-old Fraser fir seedlings after 5 and 10 wks of O_3 fumigation[1].

	Ozone (ppm)	
	< 0.02	0.10
Needle Conductance (cm/s)		
5 wks	0.695 a	0.911 a
10 wks	0.294 b	0.436 a
Photosynthesis (mg CO_2 cm^{-2} h^{-1})		
5 wks	0.087 a	0.103 a
10 wks	0.050 b	0.077 a
Transpiration (mg H^2O cm^{-2} h^{-1})		
5 wks	22.80 a	25.42 a
10 wks	17.61 a	21.77 a
Water-use Efficiency (mg CO_2/mg H_2O)		
5 wks	0.0039 a	0.0040 a
10 wks	0.0030 b	0.0036 a

[1]Means within a row followed by the same letter are not signifcantly different ($\alpha = .05$).

was significantly higher for seedlings exposed to 0.10 ppm ozone (Table 13). WUE was 17% higher than that of the controls.

Simulated rain of 3.0 significantly decreased the pH of the soil (Table 14). A reduction in the amount of potassium and magnesium is also significant.

Discussion

The lack of any significant effects on growth and biomass accumulation due to short-term simulated acidic rain was observed in this study and has been reported by various other authors (Neufeld et al. 1985, Irving 1983, Amthor 1984). Lee and Weber (1979) indicated varying responses of eleven woody species to the effects of simulated acidic rain, yet none of the species exhibited significant inhibition of top growth. Wood and Bormann (1977) reported a short-term increase in the productivity of eastern white pine (*Pinus strobus* L.) after exposure to simulated rain of pH 2.3. MacDonald et al. (1986) found increases in the shoot biomass of jack pine (*Pinus banksiana* Lamb.) when exposed to decreasing simulated rain pH. Our studies show a similar reponse in the slight stimulation of new growth in response to increasing rain acidity (Table 9). This may indicate a fertilization effect at the lower pH due to the use of nitrates in preparation of the rain solution. Although the soil analysis did not indicate significant differences among rain treatments for NO_3, a slight increase in NO_3 concentration is observed (Table 14). Fraser fir typically grows in nutrient-poor soils which are characteristically acidic and have been subject to leaching of iron,

Table 14. Analysis of soil components after 10 wks of simulated acid rain[1].

	Rain pH		
	3.0	4.3	5.6
pH	4.23 b	4.62 a	4.57 a
P (ppm)	3.0 a	3.0 a	2.4 b
K (ppm)	17.6 b	20.5 a	19.1 a
Ca (ppm)	81.6 a	86.4 a	82.8 a
Mg (ppm)	10.7 b	13.7 a	12.8 ab
OM (%)	3.34 a	3.13 b	3.40 a
NO_3 (ppm)	9.1 a	8.4 a	8.2 a

[1]Means within the same row followed by the same letter are not significantly different ($\alpha = .05$).

aluminum, and organic matter (Brady 1974). Additions of nitrate may enhance the productivity of high-elevation Fraser fir.

Ozone also failed to affect significantly any biomass or growth variables, with the exception of the increase of new shoot weight to initial shoot weight at rain pH 4.3 (Table 10). Kress and Skelly (1982) did not find any significant adverse growth effects in yellow-poplar (*Liriodendron tulipifera* L.) or Virginia pine (*Pinus virginiana* Mill.) due to ozone concentrations of 0.15 ppm. In contrast, Jensen (1985) reported a decrease in the growth of yellow-poplar after exposure to 1.2 ml l^{-1} hr^{-1} per week applied once a week after 20 wks, although these fumigation levels far exceed levels applied in this study. Exposure of green ash (*Fraxinus pennsylvanica* Marsh.) to 6 weeks of 0.5 ppm O_3 resulted in a decrease in new stem weight but not in original stem or root dry weight (Jensen 1981). Although not statistically significant, new growth and shoot weight appears to have been suppressed slightly after exposure to 0.10 ppm O_3 for 10 wks. Root to shoot ratio for seedlings exposed to 0.10 ppm O_3 appeared to be higher than that of the controls. Wang et al. (1986) reported a similar, although not statistically significant, increase in the root/shoot ratio of *Populus tremuloides* exposed to ambient air over a 3 yr period.

After 5 weeks of simulated acid rain treatments, seedling Ts was significantly decreased from the 5.6 pH treatment. Rain pH of 4.3 decreased Ts more than rain pH of 3.0 (Table 12). A decreased transpiration rate may actually enhance productivity, if photosynthesis is maintained, due to decreases in water loss. This reduction in transpiration was not observed after 10 weeks of treatment despite the presence of a similar decreasing trend.

Needle conductance was also significantly lower for seedlings treated with 4.3 pH rain after 5 wks. Neufeld et al. (1985) reported a decrease in the stomatal conductance of *Liquidambar styraciflua* L. only after exposure to rain treatments below 2.0 pH. Leaf conductance for yellow-poplar,

sycamore (*Platanus occidentalis* L.) and black locust (*Robinia pseudoacacia* L.) was not affected. A lowering of conductance at lower pH rain levels may be due to a loss of turgor by guard or subsidiary cells, a disruption of the ionic balance in the guard cells caused by excess H^+ ions, leaching of nutrients (especially K^+) from guard cells, or a reduction in photosynthetic capacity (Neufeld et al. 1985).

No significant reductions in photosynthesis were observed in this study due to simulated acid rain (Table 12); however, simulated rainfall of 3.0 pH resulted in the highest WUE of 0.0037 mg CO_2/mg H_2O after 10 wks. A higher WUE may also be the result of the hypothesized fertilization effect noted earlier. An improved WUE would certainly decrease the susceptibility of Fraser fir to periodic drought stress.

Transpiration was not significantly affected by ozone exposure (Table 13) at either 5 or 10 wks. An apparent increase in Ts due to 0.10 ppm O_3 is indicated. Keller and Hassler (1984) reported an increase in transpiration in Norway spruce (*Picea abies* (L.) Karst.) after exposure to 0.15 ppm ozone, although Ts was depressed in white fir (*Abies alba* Mill.).

Conductance was significantly higher in seedlings exposed to 0.10 ppm ozone (Table 13) at the end of the study. Needle conductance was higher in the 1- and 2-year needles in the slightly injured class of ponderosa pine and the current-year needles of severely injured trees (Coyne and Bingham 1982). In another study conducted on ponderosa pine trees in the San Bernardino National Forest, Coyne and Bingham (1981) also noted that conductance tended to be larger in the ozone sensitive trees. However, they found that stomatal function recovered during the relatively O_3-free winter months. Keller and Hasler (1984) reported that conductance of spruce grafts (*Picea abies* (L.) Karst.) fumigated with 0.15 ppm ozone had higher conductance values than those of the control. Reich and Amundson (1985) suggested that an increase in conductance may increase uptake of ozone that

would result in increased internal damage. A higher conductance caused by increasing levels of ozone may also result in an increase in the rate of water loss from Fraser fir and contribute to water stress during period of drought.

Photosynthesis after 5 weeks of ozone exposure was significantly lower in the control treatment as was WUE (Table 13). Barnes (1972) reported that for *P. strobus* seedlings, 0.05 ppm ozone caused a slight, but insignificant, increase in Pn; however, 0.15 ppm ozone resulted in a suppression of Pn; however, photosynthetic response to 0.15 ppm O_3 was measured on *P. elliottii, P. serotina*, and *P. taeda* and was not statistically different than controls. Ozone of 0.05 ppm ozone resulted in a stimulatory effect on photosynthesis. Reich and Amundson (1985) found that ozone exposure resulted in lower Pn values for four tree species tested.

No ozone x acid rain interactions were significant except for the ratio of new to old shoot growth. Reich et al. (1986) reported no significant interactions between ozone and rain treatments on northern red oak (*Quercus rubra*) or sugar maple (*Acer saccharum* L.). In a study of greenhouse-grown soybeans (*Glycine max* (L.) Merr. cv 'Davis') exposed to simulated acid rain and gaseous pollutants (SO_2 + O_3), Norby et al. (1983) failed to find any significant pH X air pollutant interactions.

Conclusions

The failure of either ozone or simulated acid rain to result in significant reductions in the growth and biomass variables over a 10 wk period may not be indicative of field situations. Slight reductions in biomass in a short span of time could, over the long-range nature of ecosystem devel-

opment, result in significant alterations in the productivity and species composition of the spruce/fir ecotype. Estimates of physiological parameters can be a constructive tool in determining the impact of air pollution and other stresses on the potential development of individual trees. However, gas exchange and photosynthetic variables are typically measured at short-term intervals and may not be sensitive enough to detect degrees of stress (Evans 1982). Diurnal and seasonal fluctuations may significantly impact and add to variations observed among treatments. In addition, gas exchange measurements were taken on the previous year's foliage in this study. These needles were probably not operating at full photosynthetic efficiency. Gas exchange measurements taken on current year's foliage may be more indicative of pollutant stress effects. However, some means of measuring the leaf area at the time of measurement is necessary as new leaf tissue is expanding over the treatment period.

The lack of any significant interactions between ozone and acid rain implies that the two pollutants may be independently affecting Fraser fir. Under naturally occurring stands, a variety of stresses and environmental factors affect growth and development of trees. The long-term indirect effect of acidification of the soil due to decreasing rain pH may also have a significant effect on the buffering capacity of the soil. The manifestation of all the combined factors is most clearly expressed in the growth and vigor of that tree. Clearly, more thorough research is needed, both in controlled environments and in the field, to discern the potential direct and indirect effects that each stress component has on the overall health of the forest ecosystem.

Experiment III

THE EFFECT OF OZONE AND WATER STRESS ON THE FOLLOWING YEAR'S GROWTH AND PHYSIOLOGY IN FRASER FIR

Abstract

The effect of ozone and water stress during the previous year on subsequent growth and physiology of Fraser fir (*Abies fraseri* (Pursh) Poir.) seedlings were investigated. Four-year-old Fraser fir were fumigated with three levels of ozone (< 0.02, 0.05, and 0.10 ppm) and exposed to three levels of water stress (control, moderate, and severe). Control seedlings were kept well-watered at all times;

moderately stressed seedlings were not watered until pre-dawn needle water potential (Ψ) levels fell between -0.8 to -1.0 MPa; severely stressed seedlings were watered after Ψ levels fell below -1.2 MPa. Seedlings were then allowed to go dormant and were chilled outdoors until late November. Seedlings were brought indoors and allowed to break bud while being fumigated a second time with ozone (< 0.02, 0.10 ppm). Analysis of data indicate that severe moisture stress caused a significant 27% and 29% decrease in shoot and root weight, respectively. Previous ozone exposure did not significantly alter growth or physiological variables but decreasing trends in growth are apparent. Current year ozone of 0.10 ppm did not significantly decrease growth or physiology; however, trends were again apparent. Cumulative effects of these stresses may, over the course of forest ecosystem development, alter forest stand productivity and species composition.

Introduction

The deleterious effects of ozone have been cited in a number of studies on a variety of plant species (Kress and Skelly 1982, Kress and Miller 1983, Jensen 1985, and others). Many studies indicate that ozone can significantly affect growth and physiological parameters without evidence of foliar damage. Tree species at high elevation sites are in an apparent state of decline (Bruck 1985, Wargo 1985). The concomitant occurrence of this decline with increasing levels of atmospheric ozone has created the need for studying the effects of ozone on trees within this area. Another possible causal factor to the decline in these areas may also be water stress. Periods of drought are common within this area and are often most severe during high occurrences of ozone (Heggestad et al. 1985).

Most studies have only examined the effects of ozone and water stress on current year's growth. Fraser fir, however, exhibits fixed growth with all bud primordia being developed for the current year during the preceding year. Therefore, the effect of ozone on Fraser fir's fixed growth pattern may be more evident the year after ozone exposure. In an attempt to determine the impact of ozone and moisture stress on Fraser fir seedling growth and physiology over two growing seasons, seedlings were exposed to three levels of ozone and two levels of moisture stress during the spring of 1986 and then subsequently exposed to additional ozone during budbreak the following year.

Materials and Methods

Three-year-old Fraser fir were obtained form a commercial grower (Lamtree Farm, Warrenville, NC) and planted in 10 cm diameter plastic pots filled with a natural, unclassified mountain Spodosol collected from under a high elevation (1220 m) spruce stand near Mountain Lake in Giles County, Virginia. The upper 30 cm of mineral soil was collected and thoroughly mixed before placement in the pots. Seedlings were placed in a greenhouse and maintained under a 16-hour photoperiod using supplemental lighting with high pressure sodium vapor lamps. The greenhouse was supplied with charcoal filtered air, and ozone levels were maintained below 0.025 ppm. Greenhouse temperatures ranged from 20 to 35 °C.

After a four week acclimation period, seedlings were randomly assigned to one of two ozone treatments to take place the following year (control or 0.10 ppm). For the current year, seedlings were randomly assigned to a combination of < 0.02, 0.05, or 0.10 ppm ozone concentrations and three moisture stress treatments. The moisture stress treatments used were the following: no stress (seedlings were kept well-watered); moderate stress (seedlings were not watered until pre-dawn needle water potential (Ψ) averaged -0.8 to -1.0 MPa); and severe stress (seedlings were not watered until Ψ was below -1.2 MPa). Pre-dawn Ψ measurements were taken with a pressure bomb (PMS Instruments, Corvallis, OR). Six seedlings were allocated to each of the 18 treatment combinations (3 ozone levels and 3 water stress levels in the current year and 2 ozone levels the following year).

After bud break had occurred, seedlings were exposed to ozone in a continuous stirred tank reactor (CSTR) system (Heck et al. 1978) for four consecutive hours (0900-1300 EST) three days week^{-1} for 10 wks. Seedlings whose pre-dawn needle water potentials fell within the specified stress range were watered following fumigation. Temperatures within the chambers were maintained at 25 ±

1.5°C. Photosynthetic photon flux density (PPFD) was maintained at 600 ± 20 μMol m^{-2} s^{-1}. Ozone was generated from oxygen exposed to ultraviolet irradiation (Welsbach Laboratory Ozonator Model T-408, Welsbach Ozone Systems Corp., Philadelphia, PA 19129). Ozone concentrations were monitored by a Bendix Chemiluminescent Ozone Analyzer (Model 8002 Bendix Process Instruments Division, Lewisburg, WV 24901) calibrated with a Photocal 3000 Automated Ozone Calibrator (Columbia Scientific Instruments, Austin, TX 78720). The primary transfer standard was a Dasibi UV Ozone Analyzer (Dasibi Environmental Corp., Glendale, CA 9204).

Following the ten week water stress and fumigation period, the seedlings were moved outside to complete bud formation and kept well-watered. Seedlings had finished elongation during the fumigation but had not entirely finished bud development. In late November of the same year, following bud set and adequate chilling, the seedings were brought inside. The two levels of subsequent fumigations began after bud break. Two seedlings from each of the treatment combinations were used to measure gas exchange prior to and at the end of the 10 wk fumigation period. A branch from each seedling was stripped of a small ring of needles, tagged at that point, and emergent needles plucked off to ensure that repeated photosynthesis (Pn), transpiration (Ts), and needle conductance (Cs) measurements were taken on the same needles. Seedlings were placed within a CSTR and allowed to equilibrate for at least one hour before measurements began. Pn, Ts, and Cs were measured with a portable photosynthesis system (LI-6000, LI-COR, In., Lincoln, NE) using a quarter-liter cuvette. Average environmental variables within the cuvette were 17.7± 5.5 %RH, 27.4± 2.5 °C., 572± 22 μMol m^{-2} s^{-1} PPFD, and 349± 32 ppm CO_2 concentration.

At the end of the ten week fumigation period, needles used to measure photosynthesis were removed and projected needle surface area was taken with a LI-3000 Portable Area Meter (LI-COR, Lincoln, NE). The needles used to measure photosynthesis, shoots, new shoots and roots were

dried in a 60°C drying oven for at least 24 hours before dry weights were measured. Water-use efficiency (WUE) was calculated as Pn (mg CO_2 m^{-2} h^{-1}) divided by Ts (mg H_2O m^{-2} h^{-1}) for each seedling.

The experiment was designed as a split, split plot replicated three times for fumigation exposures. Three blocks of 3 CSTR's were used the first fumigation period, and three blocks of two CSTR's were used during the second fumigation. The 3 ozone treatments constituted the main plot (yr 1). Each ozone treatment was then split into a subplot of the three levels of moisture stress. The sub-subplots were the two levels of ozone during the second year. The same blocks were maintained over both growing seasons. Significant treatment effects were analyzed using Duncan's Multiple Range test. All data were analyzed for homogeneity of variance and no transformations were necessary.

Results and Discussion

The effect of ozone during the previous year on the subsequent year's biomass is shown in Table 15. Although ozone during the previous year failed to result in any significant differences among treatments, trends are apparent. Increasing levels of ozone resulted in reductions of 8% and 31% for mean total shoot and new shoot weight, respectively. In measuring the effect of ozone on the biomass production of trembling aspen (*Populus tremuloides* Michx.), Wang et al. (1986) reported that ambient ozone levels were significant in reducing the shoot weight of only 2 of the 4 clones studied. Most studies have found that root growth is more severely impacted by pollution stresses (Hogsett et al. 1985, Jensen 1981). Wang et al. (1986) reported a decrease in root weight

Table 15. Effect of ozone in the previous year on the biomass of 5-yr-old Fraser fir seedlings in the following yr[1].

	Ozone (ppm)		
	< 0.02	0.05	0.10
Shoot Wt (g)	5.41	5.31	4.98
Root Wt (g)	4.01	4.12	3.95
New Growth (g)	0.620	0.554	0.441
Root/Shoot Ratio	0.91	0.88	0.97
New growth/ Old growth	0.10	0.09	0.07

[1]No means were significantly different ($\alpha = .05$).

of aspen exposed to unfiltered versus filtered chambers after 3 years; however, this difference was not significant. Wang et al. (1986) also noted that root to shoot ratios were not significantly different at $\alpha = .05$, yet the clones in the unfiltered ozone chamber did exhibit a higher root/shoot ratio. In this study, the root/shoot ratios also exhibited a similar trend.

The ratio of new shoot growth to old shoot growth was also lower for both 0.05 ppm and 0.10 ppm ozone when compared to the control treatment; however, this was not significant. This decrease in the ratio of new growth to old growth may be due to the fixed growth pattern of Fraser fir. In fixed growth species, ozone may affect the subsequent year's productivity more than the current year's growth because shoot primordia is determined in the preceding year.

Water stress during the previous year did result in significant differences for shoot and root weight that were still evident in the current year (Table 16). Both total shoot and root weight were approximately 27% lower in the severely stressed seedlings than in the control seedlings. This implies a long-term effect on subsequent seedling productivity following a period of severe moisture stress, despite relief from moisture stress. The decrease in root to shoot ratio from moisture stress may be the result of an induced quiescence or dormancy in the roots in response to severe water stress during the previous year (Kaufmann 1967, Lesham 1965, Oppenheimer and Kessler 1965).

The effect of last year's moisture stress was not as apparent on the new growth, although a trend of decreasing growth in response to stress is evident. The ratio of new growth to old growth was lower in the controls than in either stress treatment; however, this again was not significant.

Second year exposure to ten weeks of 0.10 ppm ozone fumigation did not result in any significant changes in the biomass of Fraser fir (Table 17). However, shoot, root, and new growth weight, and the ratio of new growth to old growth was lower at 0.10 ppm ozone than the control treatment.

Table 16. Effect of moisture stress during the previous year on the growth of 5-yr-old Fraser fir seedlings[1].

	Moisture Stress		
	Control	Moderate	Severe
Shoot Wt (g)	6.15 a	5.07 ab	4.50 b
Root Wt (g)	4.73 a	3.99 ab	3.38 b
New Growth (g)	0.584 a	0.519 a	0.515 a
Root/Shoot Ratio	1.02 a	0.92 a	0.83 a
New growth/ Old growth	0.07 a	0.10 a	0.09 a

[1]No means within the same row followed by the same letter are significantly different ($\alpha = .05$).

Table 17. The effect of current year ozone fumigation on the growth of 5-yr-old Fraser fir[1].

	Ozone (ppm)	
	< 0.02	0.10
Shoot Wt. (g)	5.57	4.89
Root Wt. (g)	4.06	3.99
New Growth (g)	0.554	0.526
Root/shoot Ratio	0.86	0.98
New growth/ Old growth	0.11	0.07

[1]No means were significantly different ($\alpha = .05$).

These results are similar to those reported for the effects of ozone in the previous year (Table 15). Ozone may be influencing the amount of new growth available for photosynthesis. Continual decreases in the amount of photosynthetic materials would, over the long-term, decrease the overall productivity of the forest ecosystem. The observed increase in root to shoot ratio, although not statistically significant, is also similar to that reported in Table 15.

Ozone fumigations during the previous year did not result in any significant differences among treatments for Cs, Pn, Ts, or WUE (Table 18). The data suggest a slight increase in the Cs, Ps, and Ts of seedlings. In a study conducted on water-stressed cotton (*Gossypium hirustum* L.), Temple (1986) reported that maximum stomatal conductance and transpiration decreased with increasing levels of ozone; however, maximum transpiration was less affected than conductance. Varying results as to the effect of ozone on the photosynthesis of white pine was reported by Barnes (1972). After 77 days of exposure to 0.05 and 0.15 ppm ozone, white pine seedlings did not exhibit a significant reduction in Pn when compared to the controls. He hypothesized a possible stimulatory effect of 0.05 ppm on Pn. Our study also indicates the same stimulatory effect on Pn for seedlings fumigated with 0.05 ppm ozone and a suppression at 0.10 ppm ozone. Mean WUE was lowest for seedlings exposed to 0.10 ppm ozone.

Moisture stress during the previous year did not have any significant long-term effect on Cs, Pn, or Ts (Table 19). This is most likely due to the 10 months of well-watered conditions prior to second-year fumigations. Needle conductance does appear to be lower in the severe treatment at both 0 and 10 wks when compared to the control treatment. Seedlings exposed to moderate levels of moisture stress and 10 wks of additional ozone exhibited a significantly lower water-use efficiency than with the control or the severely water-stressed seedlings.

Table 18. Effect of ozone in the previous year on conductance, photosynthesis, and transpiration the following year[1].

	Ozone (ppm)		
	< 0.02	0.05	0.10
Needle Conductance (cm/s)			
0 wks	0.240	0.232	0.255
10 wks	0.340	0.430	0.307
Photosynthesis (mg CO_2 cm^{-2} h^{-1})			
0 wks	0.097	0.123	0.073
10 wks	0.083	0.101	0.058
Transpiration (mg H_2O cm^{-2} h^{-1})			
0 wks	10.20	11.70	8.60
10 wks	15.67	22.40	13.49
Water-use Efficiency (mg CO_2/mg H_2O)			
0 wks	.0092	.0092	.0086
10 wks	.0054	.0049	.0046

[1]No means within the same row were significantly different ($\alpha = .05$).

Table 19. Effect of last year's moisture stress on conductance, photosynthesis, transpiration and WUE of Fraser fir[1].

	Moisture Stress		
	Control	Moderate	Severe
Needle Conductance (cm/s)			
0 wks	0.287	0.249	0.190
10 wks	0.402	0.347	0.327
Photosynthesis (mg CO_2 cm^{-2} h^{-1})			
0 wks	0.097	0.101	0.095
10 wks	0.077	0.076	0.089
Transpiration (mg H_2O cm^{-2} h^{-1})			
0 wks	10.12	10.36	10.03
10 wks	16.28	18.15	17.13
Water-use Efficiency (mg CO_2/mg H_2O)			
0 wks	.0091	.0089	.0089
10 wks	.0052 a	.0041 b	.0056 a

[1]Means within the same row followed by the same letter are not significantly different (&alp = .05).

Table 20. Effect of current year ozone on conductance, photosynthesis, and transpiration of 5-yr-old Fraser fir[1].

	Ozone (ppm)	
	< 0.02	0.10
Needle Conductance (cm/s)		
0 wks	0.258	0.225
10 wks	0.381	0.334
Photosynthesis ($mg\ CO_2\ cm^{-2}\ h^{-1}$)		
0 wks	0.098	0.097
10 wks	0.087	0.074
Transpiration ($mg\ H^2O\ cm^{-2}\ h^{-1}$)		
0 wks	10.76	9.50
10 wks	18.02	16.25
Water-use Efficiency ($mg\ CO_2/mg\ H_2O$)		
0 wks	.0083 b	.0097 a
10 wks	.0049 a	.0050 a

[1]No means were significantly different ($\alpha = .05$).

Current year ozone fumigation did not significantly alter Cs, Pn, or Ts of the seedlings, although these variables are lower for the 0.10 ppm ozone treatments (Table 20). Water-use efficiency was significantly lower prior to fumigation for the < 0.02 ppm ozone treatment, but by 10 wks, WUE failed to be significantly different. The cumulative chronic effect of ozone exposure may result in significant decreases in physiological variables over time and may be manifest as reductions in productivity.

Conclusions

This study is the first greenhouse test that addresses the influence of previous year's ozone and water stress in conjunction with a current year ozone fumigation on Fraser fir seedlings growth and physiology. Although neither ozone treatment significantly affected the growth and biomass of the seedlings, moisture stress during the previous year continued to have a significant effect in reducing the shoot and root weight growth. The effect of previous year's stresses on the physiological parameters of seedlings may be less apparent; however, growth variables are sensitive to these stresses throughout their development cycle. No interactions for this study were significant which implies that the stresses may act independently in their impacts on seedling growth and physiology.

Continual studies on the effects of both ozone and moisture stress on tree species conducted in a greenhouse may aid in the assessment of long-term effects of both of these stresses. Most studies are performed for only a very short period of time relative to the life cycle of a tree. The cumulative

effects of environmental stresses may act to impact severely the overall productivity of the forest ecosystem.

Summary and Conclusions

In reviewing these three studies, one can discern the complexity in analyzing the impacts of air pollution on tree growth and physiology. Trends for many of the ozone treatments are suggested. In Experiment I, ozone significantly reduced photosynthesis after 5 wks but was not significantly different from the controls at 10 wks. Second year ozone of 0.10 ppm also resulted in decreasing trends for all growth variables and physiological parameters in Experiment III. However, none of these trends were significant.

The most significant effects were those caused by moisture stress. Moisture stress significantly reduced transpiration and conductance and affected shoot and root development. Severe moisture stress during the previous year seemed to depress the Pn of the current year, but this was not statistically significant. Although the impact of moisture stress is relatively severe, we can do little to control this environmental occurrence.

The effects of simulated acid rain of pH 3.0 indicate a stimulatory effect due to added nitrates to the soil. Simulated acid rain of 3.0 pH significantly increased root surface area and increased the

root surface area to weight ratio when compared to the 4.3 pH treatment. Regions that are characteristically low in nitrates may benefit somewhat by additional nitrates due to acidic deposition. The long-term effects of this increase are not known. Increasing levels of nitrates may decrease frost resistance or alter species composition, especially in areas that are already delicately balanced.

Decreasing trends in growth and physiological variables resulting from exposure to ozone may cause real changes in forest ecosystem composition and productivity. Although effects on an individual tree may not result in drammatic stand changes, the impact of these stress effects on the entire forest may result in signfiicant declines. Further research is a necessary component in the assessment of the potential deterimental effects of ozone, acid rain, and water stress. Studies that encompass a larger time scale may be of great importance in determining the long-term effects of such stress factors. Under a natural forest ecosystem, the combined effects of each stress will be manifest as the overall health of the vegetation.

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TITLE OSMOTIC ADJUSTMENT;
DATA FIR;
COMMENT ********************FILE:OSMOTIC FIR**********************
THESE DATA WERE COLLECTED AFTER THE MOISTURE STRESS PRE-CONDITIONING
PHASE OF EXPERIMENT I, PRIOR TO FUMIGATION WITH OZONE AND SUBSEQUENT
MOISTURE STRESS.  THESE MEASUREMENTS WERE TAKEN DURING THE EARLY
SPRING OF 1986.  ALL MEASUREMENTS ARE GIVEN IN MPA.
SEE JOHN R. SEILER FOR MORE INFORMATION.
STRESS = PRE-CONDITIONING OR NOT (1 OR 0)
WATPOT = WATER POTENTIAL (MEASURED WITH A PRESSURE BOMB)
OSMPOT = OSMOTIC PRESSURE MEASURED WITH A THERMOCOUPLE PSYCHROMETER
TURGOR = TURGOR PRESSURE, CALCULATED BY SUBTRACTION.
**************************************************;
INPUT STRESS WATPOT OSMPOT TURGOR;
CARDS;
0 -.35 -18.3 18.0
1 -.65 -23.9 24.6
0 -.70 -21.5 20.8
1 -1.2 -20.1 18.9
0 -1.0 -22.1 21.1
1 -.50 -18.2 17.7
0 -.70 -13.2 12.5
1 -.75 -23.6 22.9
0 -1.2 -19.5 18.3
1 -.75 -19.3 18.6
PROC ANOVA; CLASS STRESS;
MODEL WATPOT OSMPOT TURGOR=STRESS;
MEANS STRESS/DUNCAN;
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DATA GROWTH;
COMMENT****FILE:DIAMDIF SAS **************************
THESE ARE DIAMETER AND HEIGHT GROWTH DATA FROM EXPERIMENT I IN WHICH
SEEDLINGS WERE EXPOSED TO
2 LEVELS OF PRE-CONDITIONING (PCON), 3 LEVELS OF OZONE, AND 3 LEVELS OF
WATER STRESS.
SEE JOHN R. SEILER FOR MORE INFORMATION.
STUDY WAS A SPLIT PLOT FACTORIAL WITH THE 2 X 3 X 3 FACTORS LISTED ABOVE.
OZONE WAS THE MAIN PLOT AND PRE-CONDITIONING AND WATER STRESS WERE THE
SUBPLOTS.
TREE = TREE #
PCON = PRE-CONDITIONING, NONE OR MSC (0,1)
BLK = BLOCK
REP = REPLICATEION
OZ = OZONE LEVELS (0, 0.05, OR 0.10 PPM)
STRS = WATER STRESS LEVELS CONTROL, MODERATE OR SEVERE (0, 1, 2)
DIAM1 = INITIAL DIAMETER IN MM
DIAM2 = FINAL DIAMETER IN MM
YR1 = HEIGHT AT YEAR 1 IN CM
YR2 = HEIGHT AT YEAR 2 IN CM
YR3 = HEIGHT AT YEAR 3 IN CM
YR4 = HEIGHT AT YEAR 4 IN CM
HT1, HT2, HT3, HT4, HT5, AND HT6 WERE THE HEIGHT GROWTH OF SEEDLINGS MEASURED
EVERY OTHER WEEK DURING THE COURSE OF THE STUDY.
**********************************************;
INPUT TREE 1-4 PCON 6 BLK 8 REP 9 OZ 11 STRS 13 DIAM1 15-18
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DIAM2 20-23 YR1 25-27 YR2 29-32 YR3 34-37 YR4 39-42
HT1 44-45 HT2 47-48 HT3 50-51 HT4 53-54 HT5 56-57 HT6 59-60;
CHT1 = HT1 * .1; CHT2 = HT2 * .1; CHT3 = HT3 * .1; CHT4 = HT4 * .1;
CHT5 = HT5 * .1; CHT6 = HT6 * .1;
 HT61 = CHT6 - CHT1; HT51 = CHT5 - CHT1; HT41 = CHT4 - CHT1;
 HT31 = CHT3 - CHT1; HT21 = CHT2 - CHT1;
  DIAMDIF = DIAM2 - DIAM1;
CARDS;
1    0 22 1 1 3.68 4.18 1.1  6.5 19.2 22.3 08 27 40 42 42 42
2    0 11 1 1 3.30 4.07 2.3 10.3 23.4 25.1 08 20 25 27 27 27
3    0 11 1 0 4.63 5.63 2.0  7.0 19.5 19.7 05 15 21 22 22 22
4    0 12 5 1 4.20 4.62 2.5 11.8 28.3 32.8 15 29 47 48 49 49
5    0 21 5 0 4.52 5.61 4.0  9.9 25.0 29.1 12 36 55 55 57 58
6    0 22 1 2 4.37 4.78 1.1 11.1 23.8 26.6 10 21 35 37 37 37
7    0 21 0 1 4.86 5.04 1.0  9.0 21.6 23.2 00 09 15 17 18 18
8    0 21 5 1 4.47 4.76 2.6  8.6 20.4 23.3 00 12 18 31 34 34
9    0 31 0 2 3.44 4.50 1.3  5.3 16.0 18.7 08 30 38 38 38 38
10   0 32 1 1 3.82 5.81 1.8  7.5 20.8 24.4 09 31 40 42 42 42
11   0 12 5 2 3.97 4.58 4.6  8.6 20.0 23.0 08 21 35 40 40 40
12   0 12 0 2 3.20 4.82 2.8  8.3 19.3 21.1 06 24 32 33 33 33
13   0 32 0 1 3.20 4.90 1.6  6.7 21.7 24.9 07 23 34 39 39 39
14   0 31 5 1 4.88 5.08 0.9 10.6 25.0 25.0 00 00 00 00 00 00
15   0 22 0 1 4.23 5.34 2.2 10.9 26.8 29.5 10 25 33 36 36 36
16   0 11 0 0 3.10 5.56 2.9 14.5 27.7 31.2 10 33 40 40 41 41
17   0 11 5 1 4.11 5.47 2.7 12.0 29.0 31.9 08 20 28 34 34 34
18   0 12 1 1 3.50 4.83 2.4  7.3 19.2 22.9 22 29 50 52 52 52
19   0 31 1 0 4.11 5.32 1.5  9.8 22.4 25.0 06 30 33 35 35 35
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DATA WEIGHT;
COMMENT ***********FILE:PCONTRANS SAS **********************
THESE ARE THE WEIGHT DATA FROM EXPERIMENT I, OZONE AND WATER STRESS
WITH PRE-CONDITIONING.
SEE JOHN R. SEILER FOR MORE INFORMATION.
SEEDLINGS WERE DESTRUCTIVELY SAMPLED FOLOWING A 10 WK PERIOD OF
OZONE FUMIGATION (0, 0.05, 0.10 PPM) AND WATER STRESS (CONTROL,
MODERATE, SEVERE) AND A PREVIOUS MOISTURE STRESS PRE-CONDITIONING
PERIOD (WELL-WATERED OR STRESSED).
THE EXPERIMENT WAS DESIGNED AS A SPLIT PLOT FACTORIAL.  THE MAIN
PLOT BEING THE OZONE TREATMENTS AND SUBPLOTS WERE THE 3 LEVELS OF
MOISTURE STRESS AND THE TWO LEVELS OF PRE-CONDITIONING.
PCON = PRE-CONDITIONING (0, 1)
BLK = BLOCK (1-3)
REP = REPLICATION
OZ = 3 LEVELS OF OZONE (0, 1, 2)
STRS = 3 LEVELS OF MOISTURE STRESS (0, 1, 2)
ROOTWT = ROOT WEIGHT (G) TO 2 DECIMALS
TOPWT = SHOOT WEIGHT (G) TO 2 DECIMALS
TOP2WT = STEM WEIGHT THAT WAS ATTACHED TO ROOT WHEN SLIDES WERE TAKEN
  (G), TO 2 DECIMALS
PSWT = WEIGHT OF NEEDLES USED TO MEASURE PHOTOSYNTHESIS
*************************************************************;
INPUT PCON 1 BLK 3 REP 4 OZ 6 STRS 8 ROOTWT 10-13
 TOPWT 15-18 TOP2WT 20-24 PSWT 25-29;
TOTOP = TOPWT + TOP2WT + PSWT;
TOTWT = ROOTWT + TOTOP;
RTST = ROOTWT/TOTOP;
CARDS;
0 11 0 0 1.72 3.86 0.17 0.204
0 12 0 0 1.22 2.36  .   0.135
0 11 5 0 1.18 2.89 0.19 0.252
0 12 5 0 0.83 2.38  .   0.124
0 11 1 0 1.83 3.85 0.31 0.202
0 12 1 0 1.76 5.14  .   0.133
0 11 0 1 1.06 2.23 0.26 0.098
0 12 0 1 1.33 3.44  .   0.177
0 11 5 1 2.31 7.09 0.26 0.237
0 12 5 1 1.29 3.87  .   0.229
0 11 1 1 1.04 2.79 0.14 0.158
0 12 1 1 1.29 3.59  .   0.113
0 11 0 2 0.93 2.21 0.23 0.131
0 12 0 2 1.16 3.06  .   0.128
0 11 5 2 1.36 3.73 0.28 0.193
0 12 5 2 1.20 3.72  .   0.190
0 11 1 2 1.29 3.04 0.26 0.202
0 12 1 2 1.56 4.56  .   0.179
0 21 0 0 1.06 3.11 0.23 0.189
0 22 0 0 2.12 4.68  .   0.225
0 21 5 0 2.06 5.01 0.26 0.207
0 22 5 0 1.66 3.26  .   0.159
0 21 1 0 2.03 3.97 0.28 0.190
0 22 1 0 1.28 2.82  .   0.207
0 21 0 1 1.79 3.96 0.33 0.188
0 22 0 1 2.13 4.65  .   0.185
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DATA PHOTO;
COMMENT ******************** FILE:PHW1 SAS **********************
THESE PHOTOYSNTHETIC MEASUREMENTS WERE TAKEN ON FRASER FIR
SEEDLINGS USED IN THE FIRST EXPERIMENT - OZONE AND WATER STRESS.
SEE JOHN R. SEILER FOR MORE INFORMATION.
THE EXPERIMENT WAS DESIGNED AS SPLIT PLOT FACTORIAL WITH OZONE
AS THE MAIN PLOT AND SUBPLOTS OF WATER STRESS AND MOISTURE STRESS
PRE-CONDITIONING.  MEASUREMENTS WERE TAKEN DURING THE SPRING OF 1986
AT THREE SEPARATE TIME PERIODS FOR EACH LEVEL OF PRE-CONDITIONING.
MEASUREMENTS WERE TAKEN ON A TAGGED BRANCH THROUGHOUT THE STUDY.
PCON = PRE-CONDITIONING (0 OR 1) (NO OR YES)
BLK = BLOCK (1-3)
REP = REPLICATION
OZ = 0, 5, OR 1 OZONE (0.00, 0.05, OR 0.10 PPM)
STRS = CONTROL, MODERATE, OR SEVERE STRESS ( 0, 1, 2)
TIME = INITIALLY, 5 WKS INTO STUDY, AT THE END OF THE STUDY (0, 1, 2)
RH = RELATIVE HUMIDITY (%)
CT = CHAMBER TEMPERATURE (C)
CO2 = CO2 CONCENTRATION
CS = CONDUCTANCE (CM/S)
PHOTO = PHOTOSYNTHESIS (MG CO2 M-2 H-1)
TRANS = TRANSPIRATION (MG H2O M-2 H-1)
CO2INT = CO2 CONCENTRATION INTERNALLY (PPM)
AREA = SURFACE AREA OF NEEDLES USED TO MEASURE PHOTOSYNTHESIS
WT = WT OF NEEDLES USED TO MEASURE PHOTOSYNTHESIS
**********************************************************************;
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TO       DATASETS A1  06/09/87 11:15 OZONE       F 80     524 RECS    VA TECH    PRINTED 06/10/87 10:30    PAGE 00010

INPUT PCON 1 BLK 3 REP 4 OZ 6 STRS 8 TIME 10
RH 12-15 CT 17-20 CO2 22-24 CS 26-29 PHOTO 31-34
TRANS 36-38 CO2INT 40-42 AREA 44-48 WT 50-54;
CARDS;
0 11 0 0 0 13.0 32.5 310 1.43 1.58 325 169 11.01 0.204
0 12 0 0 0 10.7 32.7 385 0.84 1.24 211 156  7.48 0.135
0 11 5 0 0 19.5 31.7 308 2.59 2.66 455 179 15.80 0.252
0 12 5 0 0 07.1 32.6 374 0.43 0.54 124 294  6.16 0.124
0 11 1 0 0 16.9 31.1 346 2.04 2.59 387 128 10.85 0.202
0 12 1 0 0 09.8 30.9 348 0.79 1.38 189 173  8.20 0.133
0 11 0 1 0 07.5 32.8 409 0.51 1.24 141 -66  6.66 0.098
0 12 0 1 0 14.2 31.0 362 1.33 2.21 277 153 11.84 0.177
0 11 5 1 0 12.6 32.8 345 1.25 0.54 309 380 13.46 0.237
0 12 5 1 0 17.3 32.3 329 2.17 2.45 430 137 13.43 0.229
0 11 1 1 0 11.8 32.2 395 1.07 1.29 254 226  8.98 0.158
0 12 1 1 0 09.7 32.2 375 0.77 1.21 207 192  6.11 0.113
0 11 0 2 0 08.4 32.6 394 0.71 1.41 195 162  9.39 0.131
0 12 0 2 0 08.1 33.0 345 0.60 0.91 165 089  8.48 0.128
0 11 5 2 0 16.0 31.9 326 0.81 2.37 372 139 11.96 0.193
0 12 5 2 0 09.2 32.4 350 0.65 1.00 181 163 10.63 0.190
0 11 1 2 0 17.7 32.1 332 2.16 2.54 420 102 15.05 0.202
0 12 1 2 0 14.5 32.7 387 1.66 2.03 379 246 10.84 0.179
0 21 0 0 0 14.8 31.8 369 1.57 1.83 335 245 11.38 0.189
0 22 0 0 0 16.9 31.5 375 1.88 1.92 381 212 11.93 0.225
0 21 5 0 0 18.7 29.4 357 2.08 2.52 355 200 10.90 0.207
0 22 5 0 0 15.0 28.4 353 1.31 1.68 241 133  9.94 0.159
0 21 1 0 0 10.7 32.0 351 2.38 2.54 660 107 11.65 0.109
```

```
DATA GROWTH;
COMMENT **************FILE: RAINDIAM SAS ***************************
THESE ARE THE DIAMETER AND HEIGHT GROWTH DATA FOR FRASER FIR
SEEDLINGS USED IN EXPT II - OZONE AND ACID RAIN (10 WKS).
SEE JOHN R. SEILER FOR MORE INFORMATION
THIS EXPERIMENT WAS DESIGNED AS A SPLIT PLOT WITH OZONE (0, 0.10 PPM)
AS THE MAIN PLOT AND SIMULATED ACID RAIN (3.0, 4.3, 5.6 PH) AS THE SUBPLOT.
BLK = BLOCK (1-5)
REP = REPLICATION
RAIN = RAIN PH (3, 4, 5)
DIAM1 = INITIAL DIAMETER (CM) TO 2 DECIMALS
DIAM2 = DIAMETER AT THE END OF THE STUDY (CM) TO 2 DECIMALS
HT3 = HEIGHT OF SEEDLING BEFORE CURRENT YEAR'S GROWTH  (CM) TO 1 DECIMAL
HT4 = TOTAL HEIGHT OF SEEDLING AT THE END OF THE STUDY (CM) TO 1 DECIMAL
*************************************************************;
INPUT BLK 1 REP 2 OZ 4-6 RAIN 8-10 DIAM1 12-15 DIAM2 17-20
HT3 22-25 HT4 27-30;
HTRATIO=HT3/HT4;
DIAMDIF = DIAM2 - DIAM1;
HTDIF = HT4 - HT3;
CARDS;
11 0.0 3.0 3.32 5.09 15.1 17.0
12 0.0 3.0 3.96 5.02 23.2 27.9
13 0.0 3.0 3.90 5.61 23.2 25.6
14 0.0 3.0 3.75 5.27 23.1 25.2
15 0.0 3.0 3.72 5.51 23.6 24.2
11 0.0 4.3 2.61 3.84 17.0 19.1
12 0.0 4.3 3.23 4.56 21.9 24.5
13 0.0 4.3 2.76 4.05 19.3 20.9
14 0.0 4.3 3.32 4.56 16.3 18.1
15 0.0 4.3 3.75 5.00 21.0 24.9
11 0.0 5.6 3.46 4.57 20.2 25.7
12 0.0 5.6 4.08 5.55 21.1 26.0
13 0.0 5.6 2.92 4.65 18.4 21.4
14 0.0 5.6 2.80 4.31 22.3 24.9
15 0.0 5.6 3.05 3.86 16.2 19.2
11 0.1 3.0 3.60 5.40 22.8 26.2
12 0.1 3.0 3.43 5.15 21.7 25.5
13 0.1 3.0 3.79 5.53 20.4 24.0
14 0.1 3.0 3.05 4.96 19.5 21.9
15 0.1 3.0 3.68 5.49 21.6 23.8
11 0.1 4.3 2.75 4.64 19.5 20.9
12 0.1 4.3 3.13 4.98 22.2 23.7
13 0.1 4.3 3.86 5.78 15.6 19.4
14 0.1 4.3 3.72 5.83 19.5 22.4
15 0.1 4.3 3.05 5.06 24.2 27.0
11 0.1 5.6 4.42 6.03 22.7 24.0
12 0.1 5.6 3.26 6.78 18.6 22.8
13 0.1 5.6 3.09 4.14 21.6 23.9
14 0.1 5.6 3.75 5.17 16.4 19.3
15 0.1 5.6 4.02 5.75 16.8 20.5
21 0.0 3.0 3.26 4.70 24.0 25.0
22 0.0 3.0 3.84 5.12 17.2 18.3
23 0.0 3.0 2.90 4.20 17.7 19.3
24 0.0 3.0 3.58 5.22 19.2 22.2
```

```
DATA GROWTH;
COMMENT ********** FILE: NEWSHOOT SAS **********************
THESE DATA ARE THE FINAL WEIGHT MEASUREMENTS TAKEN ON FRASER FIR
SEEDLINGS USED IN EXPERIMENT II - OZONE AND ACID RAIN STUDY.
SEE JOHN R. SEILER FOR INFORMATION
THE NEW GROWTH OF THESE SEEDLINGS ( IE. CURRENT YEAR) WAS CLIPPED
AND WEIGHED SEPARATELY.
BLK = BLOCK (1-5)
REP = REPLICATION
OZ = OZONE LEVEL (0, 0.10 PPM)
RAIN = RAIN PH (3.0, 4.3, 5.6)
SHOOT = TOTAL WEIGHT OF SHOOT BEFORE THIS YEAR'S GROWTH (G) TO 2 DECIMALS
NEW = WEIGHT OF NEW SHOOT GROWTH ( GM) TO 3 DECIMALS
ROOT = ROOT WEIGHT (G) TO 2 DECIMALS
NEEDLES = WEIGHT OF NEEDLES USED TO MEASURE PHOTOSYNTHESIS
**************************************************;
INPUT BLK 1 REP 2 OZ 4 RAIN 6-8 SHOOT 10-13 NEW 15-19
ROOT 21-25 NEEDLE 27-31;
TOP = SHOOT + NEEDLE + NEW; ALL = TOP + NEW + ROOT;
NSTRAT = NEW/TOP;
RTSHT = ROOT/SHOOT;
CARDS;
11 0 3.0 1.98 0.710 1.257  .
12 0 3.0 2.31 0.558 1.227  .
13 0 3.0 3.48 0.897 1.681  .
14 0 3.0 2.67 0.677 1.71  0.188
```



```
15 0 3.0 2.56 0.783 1.848  .
11 0 4.3 1.43 0.510 0.946  .
12 0 4.3 2.34 0.444 1.56  0.201
13 0 4.3 1.94 0.557 1.235  .
14 0 4.3 1.72 0.444 1.163  .
15 0 4.3 2.98 1.064 1.694  .
11 0 5.6 2.05 1.112 1.258  .
12 0 5.6 3.40 1.069 1.929  .
13 0 5.6 2.12 0.821 1.59  0.177
14 0 5.6 1.78 0.635 1.203  .
15 0 5.6 1.58 0.666 0.985  .
11 1 3.0  .  0.715 1.64  0.149
12 1 3.0 2.94 0.870 1.838  .
13 1 3.0 3.26 0.842 2.106  .
14 1 3.0 1.60 0.522 1.253  .
15 1 3.0 2.58 0.797 1.676  .
11 1 4.3 2.04 0.754 1.402  .
12 1 4.3 1.49 0.690 0.995  .
13 1 4.3 3.12 1.420 2.424  .
14 1 4.3 3.94 1.477 3.776  .
15 1 4.3 2.91 0.867 2.23  0.144
11 1 5.6 3.31 0.609 1.97  0.166
12 1 5.6  .  1.529 1.720  .
13 1 5.6 2.04 0.884 1.155  .
14 1 5.6 2.27 1.037 1.545  .
15 1 5.6 2.85 1.130 1.738
```

```
DATA ROOT;
COMMENT ***************** FILE:RAINROOT SAS ****************************
THESE DATA WERE COLLECTED ON FRASER FIR SEEDLINGS USED DURING EXPT II-
OZONE AND ACID RAIN STUDY.  MEASUREMENTS WERE TAKEN ONLY ON SEEDLINGS
USED FOR PHOTOSYNTHESIS MEASUREMENTS.
SEE JOHN R. SEILER FOR MORE INFORMATION.
ROOT SURFACE AREA WAS TAKEN WITH A LI-COR LEAF AREA METER.  VALUES
SHOULD BE MULTIPLIED BY PI (3.1415) IN ORDER TO GET ACTUAL SURFACE AREA.
BLK = BLOCK (1-5)
REP = REPLICATION
OZ = OZONE LEVELS (0, 0.10 PPM) (0,1)
RAIN = ACID RAIN LEVELS, 3.0, 4.3, 5.6 PH (3, 4, 5)
ROOTSA = ROOT SURFACE AREA (CM2) TO 2 DECIMAL PLACES
ROOTWT = ROOT WEIGHT IN GRAMS TO 2 DECIMAL PLACES
**************************************************************;
INPUT BLK 1 REP 2 OZ 4 RAIN 6-8 ROOTSA 10-14 ROOTWT 16-19;
ROOTSAP = ROOTSA * 3.1415;
ROOTRAT = ROOTSAP/ROOTWT;
CARDS;
14 0 3.0 45.97 1.71
12 0 4.3 41.29 1.56
13 0 5.6 55.12 1.59
11 1 3.0 48.11 1.64
15 1 4.3 60.77 2.23
11 1 5.6 43.86 1.97
21 0 3.0 65.46 2.17
24 0 4.3 47.52 2.17
25 0 5.6 52.31 1.93
23 1 3.0 40.52 1.30
23 1 4.3 38.41 1.80
24 1 5.6 47.09 1.55
33 0 3.0 55.38 2.09
33 0 4.3 37.25 1.59
32 0 5.6 46.49 1.66
32 1 3.0 54.96 1.73
35 1 4.3 33.07 1.26
33 1 5.6 51.16 1.81
43 0 3.0 65.76 2.61
43 0 4.3 50.55 2.17
41 0 5.6 42.41 1.65
43 1 3.0 60.25 2.04
44 1 4.3 48.23 1.79
44 1 5.6 44.59 1.75
51 0 3.0 42.86 1.71
52 0 4.3 43.32 1.56
51 0 5.6 35.77 1.26
52 1 3.0 59.23 2.18
52 1 4.3 34.83 1.58
51 1 5.6 47.09 2.06
PROC GLM;
CLASS BLK OZ RAIN;
MODEL ROOTWT ROOTSAP ROOTRAT = BLK OZ RAIN BLK*OZ OZ*RAIN;
TEST H = BLK OZ E=BLK*OZ;
MEANS RAIN/DUNCAN;
MEANS BLK OZ/DUNCAN E = BLK*OZ;
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DATA PHOTO;
COMMENT***** FILE: FIRPHOTO SAS **********************************
THESE ARE PHOTOSYNTHESIS MEASUREMENTS TAKEN ON THE LI-COR
PHOTOSYNTHESIS METER FOR EXPERIMENT II - THE OZONE AND ACID RAIN
STUDY.
SEE JOHN R. SEILER FOR INFORMATION.
STUDY WAS A SPLIT DESIGN WITH OZONE AS THE MAIN PLOT AND ACID RAIN AS
THE SUBPLOT.  THESE MEASUREMENTS WERE TAKEN ON 11/10/86, 12/19/87, AND
1/28/87 ON ONE TAGGED BRANCH OF THE FRASER FIR SEEDLING.
BLK = BLOCK (1-5)
REP = REPLICATION
OZ = OZONE LEVEL (0, 0.10 PPM)
RAIN = RAIN PH LEVELS (3.0, 4.3, 5.6)
CS1, CS2, CS3 = CONDUCTANCE MEASUREMENTS AT THE 3 TIMES (CM/S)
PS1, PS2, PS3 = PHOTOSYNTHESIS MEASUREMENTS AT THE 3 TIMES (MGCO2 M-1 H-1)
TS1, TS2, TS3 = TRANSPIRATION MEASUREMENTS AT THE 3 TIMES (MGH20 M-1 H-1)
**********************************************************************;
SA = NEEDLE SURFACE AREA OF BRANCH USED TO MEASURE PHOTOSYNTHESIS
INPUT BLK 1 REP 2 OZ 4 RAIN 6-8 CS1 10-13 PS1 15-18 TS1 20-22
CO21 24-26 CS2 28-31 PS2 33-36 TS2 38-40 CO22 42-44 CS3 46-49 PS3 51-54
TS3 56-58 CO23 60-62 SA 64-67;
CS1SA = CS1/SA; CS2SA = CS2/SA; CS3SA = CS3/SA;
PS1SA = PS1/SA*.36; PS2SA = PS2/SA*.36; PS3SA = PS3/SA*.36;
TS1SA = TS1/SA*.36; TS2SA = TS2/SA*.36; TS3SA = TS3/SA*.36;
CARDS;
13 0 5.6 4.10 3.30 375 212 7.41 2.15 609 266 2.30 1.16 412 279 8.10
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III

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11 1 3.0 5.00 2.80 409 246 6.23 2.18 604 245 [illegible] 2.26 558 261 7.25
12 0 4.3 3.46 2.95 368 212 2.88 2.38 424 282 3.29 1.84 509 243 8.61
14 0 3.0 0.52 0.71 111 100 6.59 2.14 603 270 2.87 0.57 435 187 8.35
15 1 4.3 2.39 2.51 320 238 5.37 1.84 558 255 2.83 1.36 432 269 8.04
11 1 5.6 1.42 1.71 280 279 4.67 2.26 524 264 2.51 0.90 421 157 7.47
23 1 4.3 3.76 2.83 402 219 4.53 2.60 516 228 3.03 1.88 492 275 8.04
23 1 3.0 4.17 3.05 419 233 8.72 2.75 626 319 4.55 2.30 527 245 8.76
24 1 5.6 1.06 1.37 193 279 8.47 2.63 670 239 2.93 1.31 498 244 8.48
25 0 5.6  .    .    .   .  6.24 1.32 572 329 1.39 0.97 328 260 7.62
21 0 3.0 6.27 4.18 499 227 7.46 2.12 592 233 4.10 1.84 483 199 11.2
24 0 4.3 1.17 1.09 215 272 1.62 1.35 331 228 1.56 0.60 353 254 8.35
33 1 5.6 1.80 1.80 293 185 15.1 1.84 731 258 5.18 2.25 609 234 8.75
32 1 3.0 3.93 2.66 417 215 5.06 2.32 489 243 2.59 1.77 422 281 6.35
33 0 4.3 0.80 0.90 169 264 2.65 1.05 430 289 2.02 1.51 367 261 10.3
35 1 4.3 0.22 0.12  54 644 12.3 2.89 606 250 4.16 1.35 550 266 8.04
32 0 5.6 1.14 1.56 212 282 4.43 1.69 517 298 1.92 0.60 365 245 5.39
33 0 3.0 2.74 2.89 363 188 4.03 1.88 524 241 2.42 1.40 398 224 7.73
43 0 4.3 2.27 2.66 334 158 8.92 3.26 565 232 4.32 2.63 558 241 10.9
44 1 5.6 13.3 4.39 572 243 10.6 2.76 632 243 3.16 1.69 486 223 11.9
44 1 4.3 3.99 2.54 440 216 6.67 2.39 526 250 4.05 2.44 542 222 8.61
41 0 5.6 6.34 2.30 544 224 4.96 1.86 507 290 2.41 0.60 434 287 5.77
43 0 3.0 3.98 2.48 464 245 7.88 2.43 541 208 1.73 1.58 318 224 7.51
43 1 3.0 2.65 2.14 381 236 12.0 3.44 626 232 1.96 1.73 356 189 9.56
52 1 3.0 1.31 1.24 258 227 3.22 1.13 440 286 1.22 0.98 264 205 6.87
51 1 5.6 1.37 1.32 271 253 3.48 1.44 433 258 4.35 1.73 542 281 5.37
52 1 4.3 1.07 1.24 218 175 8.29 2.69 587 231 3.44 1.65 528 244 9.59
52 0 4.3 4.81 2.02 489 263 6.45 1.74 540 227 3.92 1.28 518 238 8.94
51 0 5.6 0.35 0.43  96 203 11.7 2.92 608 254 1.51 0.86 316 258 9.58
51 0 3.0 1.68 1.96 248 187 5.32 2.30 543 229 1.94 0.75 349 208 12.2
```

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DATA SOIL;
COMMENT ****************** FILE:SOIL SAS ****************************
THESE DATA ARE FROM THE SOIL TESTING LAB. 6 SAMPLES WERE TAKEN FROM EACH
TREATMENT COMBINATION OF EXPERIMENT II - OZONE (0, 0.10 PPM) AND ACID
RAIN (3.0, 4.3, 5.6 PH).
SEE JOHN R. SEILER FOR MORE INFORMATION
BLK = BLOCK (1-5)
OZ = OZONE LEVELS (0,1)
RAIN = RAIN LEVELS (3, 4, 5)
PH = PH OF SOIL
P = PHOSPHORUS (PPM)
K = POTASSIUM (PPM)
CA = CALCIUM (PPM)
MG = MAGNESIUM (PPM)
OM = % ORGANIC MATTER
NO3 = NITRATES (PPM)
*************************************************************************;
INPUT BLK 1 OZ 2 RAIN 3 PH 5-7 P 9 K 11-12 CA 14-16 MG 18-19 OM 21-23
NO3 25-26;
MEQ=10**(-PH);
CARDS;
103 4.3 4 20 120 12 3.4 13
104 4.8 3 22  96 15 3.0 10
105 4.6 2 22  84 12 3.4  8
113 4.2 3 17  72  8 3.6  8
114 4.7 3 18  72 11 3.6  8
115 4.5 2 22  96 15 3.6 10
203 4.2 3 17  60  7 3.6 10
204 4.6 3 18  84 13 3.0  8
205 4.5 2 20  84 13 3.8  8
213 4.3 3 20 108 20 3.0 10
214 4.5 3 22 108 20 3.0  8
215 4.5 2 18  84 13 3.4  8
303 4.3 3 18  84 11 3.1  8
304 4.7 3 23  96 12 3.0  8
305 4.6 3 18  84 13 3.0  8
313 4.3 3 17  72  9 3.0  8
314 4.6 3 22  84 15 3.0 10
315 4.6 3 20  84 13 3.6  8
403 4.2 2 17  72  8 3.4  5
404 4.6 3 23  84 12 3.1  8
405 4.5 2 17  72 12 3.6  8
413 4.2 3 15  72  8 3.7  8
414 4.5 3 20  72 12 3.4  8
415 4.9 2 20  84 13 3.4  8
503 4.1 3 20  72 11 3.6 13
504 4.6 3 17  84 14 3.0  8
505 4.6 3 14  72 11 3.0  8
513 4.3 3 15  84 13 3.0  8
514 4.7 3 20  84 13 3.2  8
515 4.5 3 20  84 13 3.2  8
PROC GLM;
CLASS BLK OZ RAIN;
MODEL MEQ P K CA MG OM NO3 = BLK OZ RAIN BLK*OZ OZ*RAIN;
TEST H = BLK OZ E=BLK*OZ;
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DATA PHYSIO;
COMMENT ************ FILE:PS2FIR SAS ******************************
THESE MEASUREMENTS WERE TAKEN ON FRASER FIR SEEDLINGS FOR EXPERIMENT III,
IN WHICH SEEDLINGS WERE EXPOSED TO OZONE AND MOISTURE STRESS DURING THE
PREVIOUS YEAR AND THEN EXPOSED TO ANOTHER 10 WK PERIOD OF OZONE DURING
THE CURRENT YEAR (1986-87).
SEE JOHN R. SEILER FOR MORE INFORMATION.
THIS EXPERIMENT WAS DESIGNED AS A SPLIT SPLIT PLOT WITH THE WHOLE PLOT
BEING THE 3 LEVELS OF OZONE (0, 0.05, AND 0.10 PPM) DURING THE 1985-86
YEAR, AND THE SUBPLOTS BEING THE THREE LEVELS OF MOISTURE STRESS (CONTROL,
MODERATE, AND SEVERE DURING THE 1985-86 YEAR, AND A SUBSEQUENT OZONE
FUMIGATION (0, 0.10 PPM) DURING THE CURRENT YEAR (1986-87).
PHOTOSYNTHESIS MEASUREMENTS WERE TAKEN WITH A LI-COR PHOTOSYNTHESIS
METER.  MEASUREMENTS WERE MADE ON THE SAME TAGGED BRANCH PRIOR TO,
AND AT THE END OF THE 10 WK FUMIGATION PERIOD.
MEASUREMENTS WERE TAKEN ON 11/13/86 AND 2/14/87.
PSTR = MOISTURE PRE-CONDITIONING DURING THE PREVIOUS YEAR
BLK = BLOCK (1-3)
REP = REPLICATION
O3 = OZONE FUMIGATION LAST YEAR (0, 5, 1)
STR = MOISTURE STRESS LAST YEAR (0, 1, 2)
OZ2 = OZONE FUMIGATION DURING THE CURRENT YEAR (0, 1)
CS1, CS2 = CONDUCTANCE MEASUREMENTS INITIALLY AND AT THE END OF THE
  STUDY (CM/S)
PS1, PS2 = PHOTOSYNTHESIS MEASUREMENTS INITIALLY AND AT THE END OF THE
  STUDY (MG CO2 M-2 H-1)
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TS1, TS2 = TRANSPIRATION MEASUREMENTS INITIALLY AND AT THE END OF THE
  STUDY (MG H2O M-2 H-1)
SA = SURFACE AREA OF NEEDLES USED TO MEASURE PHOTOSYNTHESIS
**************************************************************;
INPUT PSTR 1 BLK 3 REP 4 O3 6 STR 8 OZ2 10
CS1 12-15 PS1 17-20 TS1 22-24 CS2 26-29 PS2 31-34 TS2 36-38 SA 40-44;
CS1SA=CS1/SA; CS2SA=CS2/SA; PS1SA=PS1/SA*.36; PS2SA=PS2/SA*.36;
TS1SA=TS1/SA*.36; TS2SA=TS2/SA*.36;
PSRAT=PS1SA-PS2SA/PS1SA; CSRAT=CS2SA-CS1SA/CS2SA;
TSRAT=TS2SA-TS1SA/TS2SA;
WUE1=PS1SA/TS1SA; WUE2=PS2SA/TS2SA;
WUE=WUE2-WUE1/WUE2;
LCS1=LOG(CS1SA); LCS2=LOG(CS2SA); LPS1=LOG(PS1SA); LPS2=LOG(PS2SA);
LTS1=LOG(TS1SA); LTS2=LOG(TS2SA);
CARDS;
1 11 0 0 1 10.2 4.22 401 10.6 3.74 538 21.31
0 12 0 0 0 1.58 2.97 293 4.30 2.14 411  7.08
1 11 5 0 1 0.93 1.68 137 1.92 0.96 278  1.35
0 12 5 0 0 4.52 2.77 260 1.76 1.49 282 14.08
1 12 1 0 1 1.66 2.32 172 1.82 0.99 259  5.90
1 12 1 0 0 2.13 1.07 190 1.88 0.99 279  5.03
0 11 0 1 1 1.76 1.61 176 1.03 0.27 199  7.24
1 12 0 1 0 2.31 1.83 208 2.71 1.68 345  4.08
0 11 5 1 0 0.60 1.61  98 1.53 1.22 256  1.22
1 12 5 1 1 1.55 1.63 168 1.86 0.73 287  5.27
```