EFFECTS OF ACID FOG AND OZONE ON CONIFERS

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ABSTRACT

The following report describes the findings of the California Air Resources Board-sponsored study entitled, "Effects of Acid Fog and Ozone on Conifers." The objectives of the study were to evaluate the effects of acidic fog (pH 2.0, 3.0, or 4.0) on the physiological, biochemical, and growth responses of two coniferous tree species (Pinus ponderosa and Abies concolor), and to determine if exposure to acidic fog predisposed the tree seedlings to the phytotoxic effects of ozone (O_3). The present study was conducted as two sequential experiments. The first experiment was conducted in the spring of 1987, and focused on determining the metabolic basis of acidic fog-induced alterations in seedling growth. The second experiment was conducted in the summer of 1987, and focused on determining whether the prior exposure to acidic fog altered the sensitivity of the seedlings to O_3-induced effects on growth and injury. The criteria used to assess seedling metabolic responses to acidic fog and O_3 were measures of gas exchange (i.e., net photosynthesis, transpiration, stomatal conductance of water vapor), metabolite levels (i.e., foliar pigment concentrations, needle starch content), membrane permeability and mycorrhizal establishment. Additionally, measures of growth (i.e., organ dry weights, stem dimensions), and foliar injury were also assessed.

The spring fog experiment was conducted in open plots located on field 8C on the University of California at Riverside (UCR) Agricultural Field Experiment Station. Acidic fog was applied three times per week over a six week period (16 fog exposures in total).

Injury symptoms developed on newly emerging needles in both tree species exposed to pH 2.0 fog, but did not occur in plants in the other fog treatment groups (pH 3.0 or 4.0, and no fog). For white fir, significant injury at pH 2.0 was also found for old needles. While white fir exhibited a greater amount of foliar injury than ponderosa pine, fog did not cause significant alterations in growth in either species.

Membrane permeability, measured as changes in the conductivity of needle segment extracts and K^+ leakage, were significantly increased in ponderosa pine following exposures to pH 2.0 fog. In contrast, fog treatments did not significantly alter membrane permeability responses in white fir.

On selected dates gas exchange rates in ponderosa pine were reduced by pH 2.0, and in white fir by pH 2.0 and 3.0 compared to plants exposed to pH 4.0 fog. Despite these reductions, whole-study average photosynthesis rates were not significantly inhibited. Needle starch levels were significantly decreased in white fir by pH 2.0 fog.

Ozone treatments were applied during the summer utilizing the open-top chambers in field 8C maintained by the California Air Resources Board (ARB). The seedlings were exposed to either charcoal-filtered (CF) or nonfiltered (NF) air from 22 May 1987 to 14 September 1987. The 12 h average O_3 concentration in NF and CF chambers was 73 and 18 nL L^{-1}, respectively.

After 115 days of exposure to NF air, there were no significant effects of O_3 on injury development in either species, or on growth in ponderosa pine. On the other hand, white fir exposed to NF air exhibited higher stem and needle dry weights than trees grown in CF air.

Conductivity and K^+ leakage were increased by exposure to NF air in ponderosa pine. On a percentage basis, old ponderosa pine needles were affected more than young needles. In contrast, membrane permeability in young needles of white fir was decreased by exposure to NF air, but old needles were not affected.

Air quality or fog pretreatment effects on foliar pigment concentrations were variable in both tree species. While significant treatment effects were sporadically observed during the study, carotenoid levels in young needles of ponderosa pine and chlorophyll levels in old needles of white fir were altered most often by exposure to O_3.

Mycorrhizal colonization responses were significantly higher in ponderosa pine seedlings exposed to NF air compared to plants in CF air. In white fir, no significant treatment effects were observed.

The results of the present study provide evidence that the growth and metabolic responses of two coniferous tree species could be altered by multiple applications of acidic fog, and by exposure to ambient O_3. In general, the alterations were slight to modest, which may be attributed to the low degree of stress severity, and the slow rate of tree growth. In the spring, significant treatment effects were found only in seedlings exposed to pH 2.0 fog. Affected seedlings in both species exhibited increased injury to young needles, and increased membrane permeability in old needles. In the summer, exposure to O_3 had sporadic effects, and no clear trends were evident. The findings of the present study indicate that exposure to acidic fog followed by O_3 does not cause detectable changes in conifer seedling growth within a single-growing season. Nevertheless, it is clear that acidic fog and O_3 cause temporal alterations in seedling physiology and biochemistry. Additional research is needed to determine if these data provide a reliable indication of air pollution effects on conifer seedling growth within a single-growing season, and to provide baseline information for making accurate assessments of air pollution effects on forest ecosystems in the Sierra Nevada.

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DISCLAIMER

The statements and conclusions in this report are not necessarily those of the California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

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LIST OF ABBREVIATIONS

ANOVA:	Analysis of Variance
AQ:	Air Quality
ARB:	Air Resources Board
C:	Temperature in Degrees Celsius
Ca^{2+}:	Calcium Ion(s)
CF air:	Charcoal-Filtered Air
Cl^{-}:	Chloride Ions(s)
cm:	Centimeter(s) (cm^{-1} denotes "per centimeter")
Cs:	Stomatal Conductance of Water Vapor
DI:	Degree of Foliar Injury
DMSO:	Dimethylsulfoxide
ERL:	Environmental Research Laboratory
FP:	Fog Pretreatment
g:	Gram(s)
h:	Hour(s)
H^{+}:	Hydrogen Ion(s)
K^{+}:	Potassium Ion(s)
kPa:	Kilopascal(s)
L:	Liter(s) (L^{-1} denotes "per liter")
m:	Meter(s) (m^{-2} and m^{-3} denote "per square meter" and "per cubic meter," respectively)
M:	Molarity
mg:	Milligram(s)
Mg^{2+}:	Magnesium Ion(s)
mil:	One-thousandth of an inch
min:	Minute(s) (min^{-1} denotes "per minute")
mL:	Milliliter(s)
mm:	Millimeter(s)
μeq:	Microequivalent(s)
μg:	Microgram(s)
μmhos:	Micromho(s)
μmol:	Micromolarity
NH_4^{+}:	Ammonium Ion(s)
nL:	Nanoliter(s) (nL L^{-1} denotes "nanoliters per Liter" equal to 1 ppb)

LIST OF ABBREVIATIONS
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nm: Nanometer(s)

NO_3^-: Nitrate Ion(s)

NF air: Nonfiltered air

NF/No Fog: Nonfiltered Air and No Fog Pretreatment

O_3: Ozone

pH: -Log $[H^+]$ (negative logarithm of the hydrogen concentration in moles L^{-1})

PI: Percentage of Needles Injured on a Seedling

Pn: Net Photosynthesis

ppb: Parts per Billion

psi: Pounds per Square Inch

PST: Pacific Standard Time

PVC: Polyvinylchloride

s: Seconds(s) (s^{-1} denotes "per second")

SAPRC: Statewide Air Pollution Research Center

$SO4_2^-$: Sulfate Ion(s)

TI: Total Foliar Injury on a Seedling

Ts: Transpiration

UCR: University of California at Riverside

v/v: Volume to Volume Basis

GLOSSARY OF SELECTED TERMS

Acidic Fog:	Fog more acidic than pH 5.6.
Carotenoid(s):	The yellow or orange pigments in leaves of plants; lipids that function as accessory pigments to protect chlorophyll molecules from photo-oxidation, and absorb and transfer light energy to chlorophyll a.
Chlorophyll(s):	The green pigments of plant leaves; lipids that are the primary light absorbing molecules in the light reactions of photosynthesis.
Conifer:	A cone-bearing plant; a plant classified as being a member of the order Coniferales.
Cotyledonary Mark:	A distinctive depression on the stem of seedlings denoting the location of the seed leaves.
Dry Deposition:	Atmospheric deposition of dry particles, aerosols and gases.
Ectomycorrhizae:	Mycorrhizal association in which the fungi grow over the root surface rather than in root cells (i.e., endomycorrhizae).
Fog:	A type of wet deposition comprised of droplets in the size range of 20 to 100 μm.
Gas Exchange:	Processes involved with the movement of CO_2 and water vapor into and out of leaves of plants (within the context of the present study).
Hoagland's Solution:	A commonly used liquid fertilizer in plant research, patterned after the nutrient content of fertile soil by Hoagland and Arnon in the 1930's.
Injury:	Damage to plant foliage evident as necrosis.
K^+ Leakage:	The loss of K^+ from leaf cells measured as the change in K^+ concentration of the incubation medium over time.
Irradiance:	A measure of light intensity; the flux of radiant energy.
Leaching:	The removal of substances from the leaves.
Macronutrient(s):	The major essential nutrients of plants required in relatively large amounts.
Membrane Permeability:	The degree to which substances pass through a membrane.
Metabolite:	A substance produced as a result of a biochemical process.

GLOSSARY OF SELECTED TERMS
(continued)

Microelement:	The trace elements required in small amounts for plant growth.
Monterey Pine:	*Pinus radiata* d. Don; "Insignis Pine."
Mycorrhizae:	Small roots or root hairs of plants that are infected with certain fungi, and form a mutualistic association.
Old Needles:	Foliage that emerged and developed in the 1986 growing season; also called 1986 needles or primary foliage (within the context of the present study).
Open-Top Field Chamber:	A cylindrical structure comprised of a metal frame and PVC panels routinely used in field studies to examine the effects of air pollutants.
Photosynthate:	Carbohydrate products of photosynthesis.
Photosynthesis:	The capture of light energy and its transformation into chemical energy to produce carbohydrates.
Physiology:	The branch of biology dealing with processes and activities of living organisms, organs, tissues and cells.
Ponderosa Pine:	*Pinus ponderosa* Dougl. ex Laws; "Western Yellow Pine" or "Blackjack Pine."
Seedling:	A young tree less than three feet high.
Shoot:	The aboveground portion of the plant.
Simulant:	A prepared (fog) solution that contains approximately the same chemical constituents as ambient fog (within the context of the present study).
Stomatal Conductance:	A measure of stoma openess based on the water vapor gradient between the atmosphere and the interior of a leaf.
Transpiration:	The evaporation of water vapor from leaves of plants, especially through stomata.
Wet Deposition:	Forms of moisture-laden atmospheric precipitation (e.g., rain, mist, snow, fog).
White Fir:	*Abies concolor* (Gord. & Glend.) Hildebr.; "Silver Fir" or "concolor Fir."
Young Needles:	Foliage that emerged and developed during the 1987 growing season; also called 1987 needles or secondary foliage (within the context of the present study).

SUMMARY

Trees in southern California are exposed to a wide variety of wet and dry deposited air pollutants during the year. Among the major pollutant stresses that can affect trees in mountain sites are acidic fog and photochemical oxidants (primarily O_3). To date, the effects of fog on crops have been more extensively examined than on trees (Bytnerowicz et al., 1986; Granett and Musselman, 1984; Musselman and McCool, 1987; Olszyk et al., 1987; Takemoto et al., 1988b; Temple et al., 1987). The possibility that chemical inputs from fog and cloudwater could contribute to forest decline has led to an increased appreciation of the potential effects of small-particle-size wet deposition (Dollard et al., 1983). Highly acidic fogs have been found at many sites in California (Waldman et al., 1982), with pH levels frequently below 3.0 (Jacob et al., 1985). Injury to leaves and reduced tree growth was demonstrated in tree species exposed to acidic rain (Neufeld et al., 1985) or mist (Skeffington and Roberts, 1985) in controlled studies, but little work has been done to determine if acidic fog can induce similar deleterious effects under field conditions.

Although there is some uncertainty as to the phytotoxicity of acidic fog, the effects of ambient levels of O_3 in the mountain ranges of southern California are well-documented (Miller et al., 1963; Miller et al., 1982). Most studies have focused on O_3 impacts on ponderosa pine, but recent reports have indicated that other species such as Jeffrey pine may also be experiencing similar declines (Peterson et al., 1987). While these studies demonstrate that atmospheric pollution can alter tree growth (over extended time periods of exposure), the metabolic basis for the observed effects remain obscure.

The principal objectives of the present field investigation were:

(1) To determine the metabolic basis of acidic fog induced alterations in the growth of conifer seedlings, by comparing the physiological process rates and metabolite levels of seedlings exposed to fog with different pH values.

(2) To determine if exposures to acidic fog predisposed conifer seedlings to O_3-induced changes, by examining the physiological, biochemical, and growth responses of seedlings to ambient concentrations of ozone following exposure to acidic fog.

The first objective was tested by examining the effects of acidic fog at pH 2.0, 3.0, and 4.0 (16 exposures) on the metabolism and growth of ponderosa pine and white fir seedlings (i.e., first phase of a sequential exposure experiment). The underlying hypothesis in the fog experiment was that H^+ inputs from acidic fog would cause foliar injury and inhibit seedling photosynthesis, leading to reductions in starch accumulation, mycorrhizal colonization of roots and growth. The major findings of the fog exposure study were:

(1) Both tree species (i.e., ponderosa pine and white fir) were able to neutralize acid inputs from pH 3.0 or 4.0 fog via surface buffering processes on leaves, but inputs from pH 2.0 fog exceeded leaf buffering capacities, resulting in foliar injury.

(2) The two tree species intercepted fog equally on a leaf area basis.

(3) Young needles of both species were more susceptible to fog-induced injury than old needles.

(4) White fir was more sensitive to fog-induced injury than ponderosa pine.

(5) Exposure to fog at pH 2.0 increased membrane permeability and K^+ leakage rates in ponderosa pine, but not in white fir.

(6) Foliar pigment levels in young needles did not correlate closely with injury amounts.

(7) Gas exchange rates were extremely low in both species; net photosynthesis in ponderosa pine was consistently reduced by pH 2.0 fog and by pH 3.0 fog on an event basis in white fir, but whole-study average rates were not significantly altered.

(8) Net photosynthetic rates were lowered by pH 2.0 fog in both tree species, but needle starch was reduced only in white fir.

(9) Mycorrhizal infection of both species was not affected by acidic fog treatment.

(10) Growth of both conifers was not altered by exposure to acidic fog.

The second objective was tested in the summer of 1987 (second phase of the sequential exposure experiment). The seedlings from each fog treatment group were divided into two groups, and transferred to open-top field chambers for four months of exposure to clean, charcoal-filtered (CF) air, or polluted, nonfiltered (NF) air to determine the impact of ambient levels of O_3 (seasonal 12-h average concentration of 73 ppb) on their growth and metabolism. In this experiment, the underlying hypothesis was that the reduced vigor of trees pretreated with highly acidic fog in the spring (i.e., pH 2.0), would predispose them to greater adverse effects from gaseous O_3. The major findings of this experiment were:

(1) White fir exposed to ambient levels of O_3 exhibited significantly higher stem and needle dry weights than seedlings exposed to CF air.

(2) Increased stem dry weight in white fir grown in NF air paralleled increases in stem diameter.

(3) Membrane permeability was increased in ponderosa pine grown in NF air compared to CF air, despite the lack of significant O_3 effects on growth.

(4) Membrane permeability was decreased in young needles of white fir grown in NF air compared to CF air, which was coincident with higher organ weights in white fir grown in NF air.

(5) Pretreatment with pH 2.0 fog significantly increased membrane permeability in white fir, but had no significant effect on ponderosa pine.

(6) Root systems of ponderosa pine grown in NF air were infected by mycorrhizal fungi to a significantly greater extent than in CF air.

(7) Exposure to ambient levels of O_3 did not significantly affect injury or growth in ponderosa pine.

(8) The effects of pH 2.0 fog pretreatment on foliar injury in white fir persisted over most of the summer, but O_3-related injury development was not enhanced to any sizeable extent in the current field season.

The results of the present study provide useful information relative to the metabolic basis of acidic fog and ambient O_3 effects on the growth of two coniferous tree species. In general, adverse macroscopic and metabolic effects were detected in seedlings (of both tree species) exposed to multiple applications of pH 2.0 fog. The adverse effects of pH 2.0 fog were largely observed in young needles of both species. Responses of plants exposed to pH 3.0 fog were slightly different from plants treated with pH 4.0 fog or nonfogged plants.

Exposure to O_3 in the summer caused different effects in the two tree species. In ponderosa pine, growth was not significantly affected by O_3, but significant alterations were observed with respect to metabolic responses. While increased membrane permeability was indicative of an adverse O_3 effect, elevated carotenoid levels in young needles, and increased mycorrhizal infection were examples of beneficial compensatory responses (i.e., higher concentrations of accessory pigments to protect against chlorophyll photo-oxidation, and enhanced mineral and water absorption by roots, respectively). Stem, young needle weight and chlorophyll levels of old needles were increased when white fir seedlings grown in NF air were compared to CF grown trees. The NF treatments decreased membrane permeability in young needles. Seedling responses to the two pollutant stresses were highly variable within this single-season study, and additional research is needed to verify the consistency of the observed responses over multi-year periods.

RECOMMENDATIONS

(1) Multiple-season studies are required to fully comprehend the immediate and long-term effects of air pollutants on trees. Single-season studies provide useful baseline data, but fail to consider the influence of environmental conditions the preceding year on tree growth in the current year. Investigations of greater duration are needed, given the long lifespan of trees and the moderate phytotoxicity of ambient air pollution stress.

(2) Plant-to-plant variability must be minimized in order to determine treatment effects on tree growth or fundamental growth processes. Larger sample sizes (i.e., 60 to 80 seedlings per treatment group) should be examined, and longer lead times provided to obtain, properly culture, and acclimate the test seedlings to prolonged durations in open-top chambers.

(3) Changes in membrane permeability induced by acidic fog and ambient O_3 clearly identifies these biochemical parameters as useful bio-indicators of pollution stress in ponderosa pine and white fir. The degree to which membrane permeability is altered by an applied stress may have utility as a rapid, qualitative test for predicting the inherent sensitivity of other tree species in future investigations.

(4) The investigation of air pollution effects on tree physiology should be intensified during the spring and fall when bud break and new foliage begin to develop. Compared to the summer, gas exchange rates and net carbon gains are higher in spring and fall, and pollution exposures would likely have a greater adverse influence.

(5) Gas exchange assessments in subsequent studies should also consider aspects of dark respiration to characterize carbon metabolism from the perspective of net gains or losses.

I. INTRODUCTION

Acidic precipitation is an environmental concern in the United States, Canada and Europe. The exposure of trees to acidic precipitation and photochemical oxidants (primarily O_3) is considered to be a significant contributing factor to forest decline in Europe (Krause, 1983; Schutt and Cowling, 1985). In the United States, the potential for negative impacts of acidic precipitation and O_3 on plants is especially high in the Los Angeles Basin, San Joaquin Valley, and in the mountains adjacent to these areas (Miller et al., 1982). Native conifers which do not shed their leaves each season may be extremely sensitive to exposures to acidic fog in the spring (Waldman et al., 1985), followed by elevated O_3 concentrations in the summer (Miller et al., 1963, 1969).

In recent years, there has been considerable research conducted on the effects of acidic rain on crops and trees (Evans, 1984; Irving, 1985). The consensus among researchers is that acidic rain must be at or below pH 4.0 to injure vegetation (Lindhurst et al., 1982). In contrast to data from the eastern United States, recent evidence suggests that acidic fog and not rain may be of prime interest in California (Heileman, 1973; Roberts, 1982), since fog chemical concentrations tend to be higher, and the frequency of fog events is greater than that of rain events. Acidic fog events at pH levels as low as 2.0 have been reported in the Los Angeles Basin (Waldman et al., 1982; Jacob et al., 1985), and ionic concentrations in these fogs were 10 to 100 times greater than those observed in typical acidic rain events (Waldman et al., 1982; Brewer et al., 1983; Munger et al., 1983). Nitrate-to-sulfate ratios in southern California fogs range from 1:1 to 3:1 (Waldman et al., 1982), which is significantly higher than fog in the eastern United States where the sulfate ion dominates precipitation chemistry. While several studies on the effects of simulated acidic rain and mists on crops and trees have reported alterations in morphological and physiological responses leading to the development of foliar injury (Ferenbaugh, 1976; Evans, 1984; Evans et al., 1977; Hindawi et al., 1980), relatively little is known about the effects of acidic fog on plants.

The combined effect of O_3 and acidic deposition may contribute to increased tree mortality in American and European forests (McLaughlin, 1985; Schutt and Cowling, 1985). Adverse effects of O_3 on plants are well-documented, and at the cellular level, the sites of O_3 action are membranes (Heath, 1975) which control intra- and intercellular fluxes of ions and water. Acidic deposition in the form of fog and rain have been reported to leach essential nutrients from foliage at rates dependent upon precipitation volume, acidity and ion composition (Krause, 1983). Leaching can cause leaves of plants exposed to acidic precipitation to lose K^+, Mg^{2+}, Ca^{2+}, Cl^-, amino acids, proteins, and carbohydrates (Wood and Bormann, 1975; Hindawi et al., 1980; Scherbatskoy and Klein, 1983; Evans et al., 1985). These substances, as well as carbonates, phosphates, and organic acids form the principal components of surface buffering systems of leaves (Keller, 1982; Pylypec and Redmann, 1984). It has been suggested that these buffering substances play an important role in neutralizing acid inputs from rain (Adams and Hutchinson, 1984; Evans et al., 1985). As leaf buffering systems are weakened, H^+ ion concentrations in plant tissues may rise, and foliar injury symptoms can develop as a result of increased cell acidity (Bytnerowicz et al., 1986).

Damage to coniferous trees by O_3 was found in the mountain ranges of southern California more than twenty years ago (Miller et al., 1963; Wert et al., 1970; Miller and Millican, 1971; Miller et al., 1982). While these findings of O_3 impacts on natural forest ecosystems are specific to California, O_3 is also considered to be one of the major environmental factors contributing to forest dieback in western Europe and eastern North America (Schutt and Cowling, 1985; Tomlinson, 1983; McLaughlin, 1985). In controlled studies, ponderosa pine seedlings exposed to 100 ppb for 6 h daily (over the growing season) exhibited reduced root carbohydrate reserves (Tingey et al., 1976). Moreover, in tomato, O_3 treatment has been shown to reduce photosynthate translocation to roots (McCool and Menge, 1983). Thus, the reduction of soluble sugars and starch in tree seedlings under chronic O_3 stress (Miller et al., 1969; Tingey et al., 1976) could limit root growth (Blum and Tingey, 1977), as well as the colonization rates of mycorrhizal fungi (Bevege et al., 1975).

While the impacts of gaseous pollutants and acidic deposition on forest decline are recognized, specific effects on ectomycorrhizae have not been extensively investigated. Beneficial root-inhabiting mycorrhizal fungi are known to enhance growth, nutrient and water uptake, and tolerance to disease in forest trees (Ruehle and Marx, 1979; Bowen, 1973; Sinclair et al., 1982). Brewer and Heagle (1983) demonstrated that the ectomycorrhizae of soybean were sensitive to simulated acidic rain, and similar reductions in infection have been reported for ectomycorrhizae of several forest species (Reich et al., 1986). Ozone exposures have been shown to have detrimental effects on mycorrhizae of citrus and tomato (McCool et al., 1979, 1982), but was found to increase ectomycorrhizal infection of red oak (Reich et al., 1985). While these results suggest that the alteration of root colonization by air pollutants is highly species-specific, air pollutant exposures generally contribute to reductions in tree growth, loss of vigor, and decreased tolerance to subsequent pollutant exposures (McCool et al., 1979, 1982; Brewer and Heagle, 1983; Reich et al., 1986).

Recently, an ARB-sponsored study on the effects of acidic fog and ambient O_3 on the yield, growth, and physiological responses of commercially-important California crops was completed at Riverside (Olszyk et al., 1987). In general, fog treatments more acidic than pH 2.0 and ambient O_3 adversely affected plant responses, but there was no evidence of an interactive effect. Yields of fruit producing crops (e.g., strawberry, green pepper) were more sensitive to fog than measures of vegetative growth (Takemoto et al., 1988a,b). Moreover, net photosynthesis was reduced by 5 to 15% by highly acidic fog applications or ambient O_3. However, since the crops had been exposed to acidic fog and O_3 for only three months, plants with longer growing seasons (i.e., trees) may experience greater yield or growth reductions over their lifespans.

The effects of acidic fog on plants have also been examined by the research groups of Robert C. Musselman and Patrick J. Temple of the SAPRC. Musselman and co-workers have reported that lettuce injured by simulated acidic fogs of pH 2.5 or lower (Granett and Musselman, 1984). Moreover, in controlled environment studies, these workers also found that radish, spinach, celery, bean, tomato, strawberry, azalea, alfalfa, and selected fruit tree seedlings were injured by acidic fogs in the range of pH 2.0 (Musselman and McCool, 1987). Research by Temple and co-workers has included greenhouse studies on the injury responses of giant sequoia and Jeffrey pine seedlings (Temple, 1987), and the yield responses of field-grown alfalfa to acidic fog and ambient O_3 (Temple et al., 1987). Both tree species were sensitive to fog exposures of pH 2.0, and alfalfa yields were reduced by acidic fog and O_3.

Presently, two studies on herbaceous crops found that acidic fog and ambient O_3 did not have an interactive effect on growth or yield responses (Takemoto et al., 1988b; Temple et al., 1987), but it has not been clearly established that exposures to acidic fog predispose plants to the harmful effects of ambient levels of O_3. Leaching of foliar nutrients attributable to fog exposures may increase plant sensitivity to O_3 by altering the inorganic chemical composition of leaf cells, surfaces, and intercellular spaces (Bytnerowicz et al., 1986). In particular, reduced levels of Ca^{2+} could be especially detrimental since Ca^{2+} is known to be important in the regulation of membrane permeability (Mengel and Kirkby, 1982). As a consequence, the disruption of cell membrane integrity by O_3 (Heath and Frederick, 1979; Chimiklis and Heath, 1975) may be enhanced if prior exposure to acidic fog causes significant alterations in the quantities of inorganic elements in leaves.

II. PROJECT OBJECTIVES

The principal objectives of this project were:

(1) To determine the metabolic basis of acidic fog induced alterations in the growth of conifer seedlings, by comparing the physiological process rates and metabolite levels of seedlings exposed to fog with different pH values.

(2) To determine if exposures to acidic fog predisposed conifer seedlings to O_3-induced changes, by examining the physiological, biochemical, and growth responses of seedlings to ambient concentrations of ozone following exposure to acidic fog.

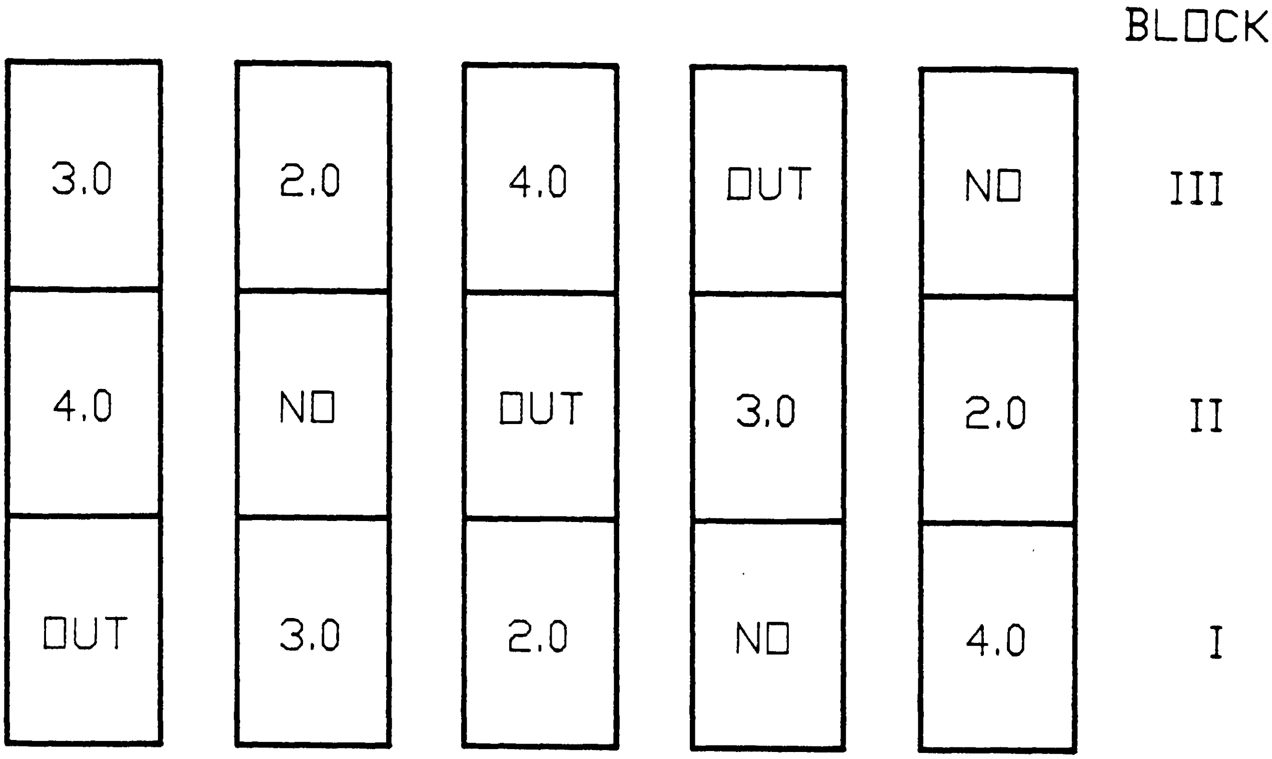

Figure 1. The arrangement of experimental plots for studying effects of acidic fog on ponderosa pine and white fir seedlings. The designated fog treatments were:

(1) Outside -- Plants that are not covered during the scheduled fog applications;

(2) No Fog -- Plants that are covered during the scheduled fog applications, but do not receive fog treatments;

(3) pH 2.0 -- Plants receiving three pH 2.0 fog applications per week;

(4) pH 3.0 -- Plants receiving three pH 3.0 fog applications per week;

(5) pH 4.0 -- Plants receiving three pH 4.0 fog applications per week.

III. METHODS

A. Fog Exposure System

A complete description of the fog exposure system used in the present study is given in a previously submitted final report to the ARB (Contract No. A5-087-32, Olszyk et al., 1987). The fog exposure system consisted of a pressure source to generate fog by impaction; a solution canister to serve as a reservoir for the fog simulants dispensed; and a dispensing unit to distribute the fog over the experimental plots. Fog particles were produced by forcing prepared solutions through the orifice of stainless steel impingement nozzles (Mee Industries, El Monte, CA) at pressures of 690 to 830 kPa (100 to 120 psi). Changes were made in the configuration of the main system pressure lines and in the location of several fog chamber frames to match the arrangement of plots that received fog (Figure 1).

B. Spring Fog Exposure Study

1. Fog Treatments and Measurements

In this study, an investigation of the metabolic basis of conifer seedling growth and injury responses to acidic fog was conducted. This was achieved by comparing the physiological process rates and metabolite levels of seedlings exposed to fog with different pH levels. Four fog treatments were applied to the potted seedlings: three target fog pH levels of 2.0. 3.0, and 4.0 in addition to a no fog treatment. The comparison of responses between plants exposed to the three acidic fog treatments provided a measure of the effects of acid inputs from the multiple fog episodes. Comparison of plant responses in the no fog treatment to those receiving pH 4.0 fog provided a measure of the effects of fog application. Additionally, within each treatment block an outside plot was maintained and monitored to determine if enclosing the plots during fog exposures altered normal plant growth.

The target fog solutions contained a background chemical composition representative of the San Joaquin Valley (Table 1). These analyte levels closely approximate the concentrations of inorganic constituents in fog samples found in the Bakersfield area, which is characterized by a higher $SO_4^{2-}:NO_3^-$ ratio than fogs in other parts of California (Jacob et al., 1984). The reason for this choice is the proximity of the Central Valley

Fog solutions (15.1 L) were placed in the canisters immediately after completing a fog exposure to reduce the amount of time needed to prepare the plots for the next pre-dawn fog exposure. Our previous experiences had shown that fog chemistry was little affected by overnight storage in the canisters (Olszyk et al., 1987). On the morning of a fog event, the plots receiving fog (2.44 m width x 3.05 m length x 1.83 m height) were enclosed with plastic sheets (7 m x 7 m, 4 mil thickness), that were attached to the fog frames by PVC clamps. After all nine plots were enclosed, the canisters were pressurized to 690 kPa (100 psi), and fog application were initiated by opening the plug valve (Series P4T, Nupro Co., Willoughby, OH) on each canister. Fog episodes lasted for approximately 2.5 h. The plastic coverings were removed within 30 min of the completion of a fog event.

Samples of nozzle drip, suspended fog, and moisture on leaves were collected on four occasions (i.e., 24 April, 01 May, 08 May, 15 May). Nozzle drip was sampled by collecting fogwater directly into marked polyethylene bottles (Nalgene Labware, Rochester, NY). Suspended fog was collected using a high volume sampler developed by Michael R. Hoffman and associates at the California Institute of Technology (Jacob et al., 1985). The fog collector is a fan-driven sampler that draws fog-laden air into a rectangular collection port at a rate of 19.3 m^3 min^{-1}. Fog particles that impacted against a series of Teflon threads in the sampler were funneled to a drain for collection into labeled polyethylene bottles. Moisture on leaves was sampled by excising fog-wetted needles and storing them between two sheets of filter paper (Whatman #1, 7.0 cm diameter) in pre-weighed, sealed plastic bags. Within 2 h post-collection, the bags were weighed to determine the amount of water absorbed onto the filter paper discs. The total surface area of the needles was determined by doubling the projected needle leaf area measured with a leaf area meter (LI 3000, Lambda Instruments, Lincoln, NE), and moisture on needles was calculated on an area basis.

2. Environmental Measurements

Environmental conditions during the Spring Fog Exposure Study were monitored at the ARB Citrus Project Site located 125 m southeast of the field site in UCR Field 8C. Irradiance was measured with a quantum sensor (LI 190SB, Lambda Instruments, Lincoln, NE), relative humidity with

Table 1. Ionic Concentrations in the Fog Simulants[a]

I. H^+, NO_3^-, and SO_4^{2-} (μeq L^{-1})

Simulant pH		H^+	NO_3^-	SO_4^{2-}
2.0	7762	3734	10749	
3.0	776	672	1928	
4.0	76	439	1257	

II. Background Inorganic Constituents

Macronutrients (μeq L^{-1})		Microelements (μg L^{-1})	
NH_4^+	1440	Fe^{2+}	404
Ca^{2+}	47	Pb^{2+}	330
Na^+	20	Ni^{2+}	61
K^+	9	Cu^{2+}	34
Mg^{2+}	6	Mn^{2+}	17
Cl^-	47		

[a]Background salts provided base concentrations of 406 and 1161 μeq L^{-1} of NO_3 and SO_4^{2}, respectively, to the fog simulants. NO_3^- and SO_4^{2-} in excess of these levels were provided by adding a concentrated acid mixture (4.2 M HNO_3 and 12.1 M H_2SO_4) during pH adjustment.

Airshed to parts of the Western Sierra Nevada. In the absence of fog or cloudwater data specific to the Sierra Nevada, Bakersfield fog chemistry was selected as the closest surrogate. The different levels of acidity were achieved by adding a nitric and sulfuric acid mixture in an approximate 1 to 1.1 volume ratio.

Fog was applied three times per week, on Monday, Wednesday, and Friday mornings from 0500 to 0700 h PST. Exposures were conducted for six consecutive weeks until a total of 16 fog treatments were applied. Nozzle function was checked at the onset of application, and every 45 min during the exposure to ensure an even application of fog. Routine maintenance of the fog application system was not required in this study because of the infrequency of nozzle clogging that was common in previous investigations (Olszyk et al., 1987).

protect the trees from solar radiation shock, the seedlings were kept in a shaded enclosure until the experimental site was completely prepared. The white fir seedlings were transferred to the field site on 16 March 1987. The seedlings were kept under continuous shade prior to and during the entire experiment. This was essential to tree survival during the summer when ambient air temperatures and irradiance were highest.

From the date of transfer from the lathhouse to the end of the study, the seedlings were fertilized weekly with full-strength North Carolina State University Phytotron nutrient solution, or otherwise supplied with tap water. No herbicide or pesticide treatments were applied at any time during the study. Prior to the initiation of fog exposures, the trees were assigned to one of five treatment groups. Owing to the wide range of tree size classes, the seedlings were separated into four groups based on stem height. Trees from within each stem height group were then randomly assigned to one of the five fog treatment groups to distribute the variability in tree size objectively. In total, twelve seedlings of each species were assigned to each fog plot. The potted trees were transferred to the experimental site over a two day period ending on 03 April 1987, where they remained for seven days prior to the first fog event (10 April 1987). Pots were buried in the soil to a depth of 40 cm to insulate the root systems of the seedlings from high ambient air temperatures. This measure maintained a more normal soil to air temperature gradient necessary to optimize plant growth.

Preliminary assessments of mycorrhizal colonization were performed on 19 March 1987. Because little evidence of natural infection was found, the plants were inoculated with commercially-available ectomycorrhizae spore pellets (05 May 1987). Spores of <u>Pisolithus</u> <u>tinctorius</u> (Pers.) Coker and Couch (International Forest Seed Co., Odenville, AL) were applied to the root systems of the experimental trees. The spores were applied by mixing 2 g of spore pellets with the upper 2 cm of soil in each pot, and watering the plant to aid in spore distribution to the upper root ball.

4. <u>Injury and Growth Responses</u>

Foliar injury assessments were made on three occasions: prior to initiating the fog exposures (03 April), after 11 fog exposures (05-07 May), and after 16 fog exposures had been applied (15-18 May 1987).

a chilled mirror sensor (Dew-10, General Eastern Instruments Corp., Watertown, MA), and air temperature with iron-constantan thermocouples prepared by Environmental Research Laboratory (ERL) technical staff. Data from these sensors were tabulated with a data acquisition and control system (ISAAC, Cyborg Corporation, Newton, MA) interfaced with a micro-computer (Apple IIe, Apple Computer Inc., Cupertino, CA). Mean 12 h values (0800 to 2000 h PST) for irradiance, relative humidity, and air temperature were calculated on a weekly basis.

3. Plant Culture

One-year old and two-year old bare root seedlings of *Pinus ponderosa* Dougl. ex Laws (ponderosa pine) and *Abies concolor* (Gord. & Glend.) Hildebr. (white fir), respectively, were obtained from the California Department of Forestry in February 1986. The Department of Forestry Nursery (Davis, CA) was the source of the ponderosa pine seedlings, and the white fir seedlings were from the Ben Lomond Nursery (Santa Cruz, CA). The seedlings were kept in cold storage (4°C) until transplanted (approximately 14 days). Seedling trees were planted in 3.6 L plastic pots (10 cm x 10 cm x 36 cm tall) containing an Oakley sand, peat, and fir bark mixture (2:1:1 by weight, UCR soil mix #2). After planting, the trees were kept in a lathhouse on the UCR campus, and provided with water and fertilizer (in the form of a modified full-strength Hoagland's solution) through February 1987.

Originally, the proposed study was designed to examine the responses of ponderosa and Monterey pine (*Pinus radiata* D. Don) to acidic fog. With this intent, seedlings of these two pine species were transferred to a sun-exposed, open area adjacent to the field site on 20 February 1987. After several days of allowing the seedlings to acclimate to ambient, outdoor conditions, the Monterey pines developed severe needle injury symptoms. It was estimated that 70% of the Monterey pine seedlings exhibited chlorosis or needle tip burn, and therefore could not be used in the present study. The combined effect of elevated daytime irradiance and air temperature was hypothesized to be the cause of the injury. White fir seedlings, which are also common to the Sierra Nevada and San Bernardino Mountains (Little, 1981) were substituted for Monterey Pine.

While the ponderosa pine seedlings did not sustain extensive foliar injury, measures were taken to reduce the risk of injury development. To stomatal conductance of water vapor represent the average of ten 6 s measurements. Transpiration was calculated from the initial rate of change in humidity.

Plant gas exchange responses were measured on eight occasions during the fog exposure study. Ponderosa pine responses were measured on 15 April, 20 April, 06 May, and 11 May 1987 on the mornings of the 3rd, 5th, 12th, and 14th fog events, respectively. White fir responses were measured on 17 April, 22 April, 08 May, and 13 May 1987 on the mornings of the 4th, 6th, 13th, and 15th fog events, respectively. On each sampling date, measurements were made on branches from four different seedlings per plot, to provide a total of 12 sample measurements for each fog treatment group (i.e., three replicate plots of each treatment). After completing the gas exchange measurements, the number of needles enclosed in the cuvette and the length of the longest needle was recorded to calculate the total needle surface area sampled. All values of plant gas exchange were expressed on a total needle surface area basis.

Equations were generated to estimate total needle surface area from measures of needle number and needle length, in order to quantify needle surface area non-destructively (for calculating the gas exchange parameters). In order to develop a reliable indicator of total needle surface area for white fir, it was necessary to determine the relationship between the length of the longest needle to the mean needle length on 34 mm branch segments (starting at the branch tip). Extra tree seedlings that were not used in the experiment served as the source of material for determining this relationship. Twenty-five branch samples were harvested, and all needles > 10 mm long were counted and measured. Additionally, needle diameter measurements were made using an occular micrometer. Needle volume was measured by monitoring water displacement of needle bundles of known length in 25 mL graduated cylinders. The validity of the derived equations was checked against values obtained using a leaf area meter (LI 3000, Lambda Instruments, Lincoln, NE). For ponderosa pine, the main source of variation was needle width, and a mean needle width measure for area calculations was determined from 100 needle samples from extra trees.

Samples for assessing mycorrhizal establishment were collected eleven days after the 16th fog application (26 May 1987). Whole root systems were washed, and three lateral roots were randomly selected from the upper

Foliar injury was evaluated in terms of the percentage of needles injured (i.e., number of needles exhibiting injury symptoms), the degree of foliar injury (i.e., severity of needle injury), and the total amount of injury on a needle area basis. Percentage of needles injured (PI) and degree of foliar injury (DI) were estimated in 5% increments, and total injury (TI) was determined by multiplication (i.e., PI x DI = TI). All injury data reported hereafter indicate values of TI.

Stem height and stem diameter were measured before exposing the plants to fog (22 March), and after 11 fog exposures (05 May 1987). Measurements were also taken on the plants harvested at the end of the fog exposures (26 May 1987). Stem height was measured from soil level to the top of main trunk. Stem diameter was measured at the height of the pot rim using a caliper (Manostat 5921, KWB, Switzerland).

Dry biomass of five seedlings of each species was measured before the first fog exposure (08 April 1987) to establish mean initial whole-plant and organ dry weight values. The plants were removed from the pots, and surface was soil removed from the lower portion of the stem. The shoot portion of the plant was defined as being those structures growing above the cotyledonary mark. After the soil was washed from the roots, the samples were stored in separate, labeled bags and dried to constant weight in drying ovens (70°C). The shoot portion was later separated into sub-samples identified as young needles (i.e., emerging in 1987), old needles (i.e., foliage present from previous year), or stems. Samples were weighed to the nearest 10 mg with a top-loading electronic balance (Model PE3000, Mettler Instrument Co.). After sixteen fog exposures, four of the twelve seedlings of each species from each fog plot were harvested as described to determine the effects of fog chemistry on seedling growth.

5. Physiological and Biochemical Responses

Measurements of carbon dioxide and water vapor exchange were made using a portable photosynthesis system (LI-COR 6000, Lambda Instruments, Lincoln, NE). The LI-COR 6000 system simultaneously measured irradiance, relative humidity, carbon dioxide concentration, leaf temperature, and air temperature. These data were used to calculate net photosynthesis, stomatal conductance of water vapor, and transpiration. In this study, the 0.25 L leaf cuvette was used, with a total sampling time per seedling of 60 s. For each seedling, mean values of net photosynthesis and foliage samples subjected to chemical and enzymatic digestion (Huber and Israel, 1982). Subsamples of old needle preparations (50 to 100 mg) were extracted with 80% ethanol (v/v) four times in a 80°C water bath until the samples were pigment-free. After the supernatant ethanol was removed, 1 mL of 0.2 M KOH was added to the needle brei, and the mixture was incubated at 100°C for 30 min to facilitate the chemical digestion process. The sample was allowed to cool for 10 min, and the mixture neutralized by adding 0.2 mL of 1 M acetic acid. The mixture was stirred, and placed in a 55°C water bath for 5 min, before adding 1 ml of amyloglucosidase from Rhizopus sp. (400 units mL^{-1}, Sigma Chemical Co., St. Louis, MO) in 50 mM sodium acetate buffer (pH 4.5). Enzymatic digestion was allowed to continue for 90 min, after which the samples were placed in a 100°C water bath for 1 min to stop the enzyme digestion process. The samples were cooled to room temperature, centrifuged, the supernatant adjusted to 10 mL with distilled water, and kept frozen until the time of assay. Aliquots (0.2 to 0.5 mL) were analyzed for glucose content by measuring the production of oxidized o-dianisidine (Glucose Diagnostic Kit, Sigma Chemical Co., St. Louis, MO) at 450 nm with a spectrophotometer. Starch content (as glucose equivalents) on a dry weight basis was calculated.

6. Statistical Analysis

The experimental design for the Spring Fog Exposure Study was a randomized split plot with three replications. Fog chemistry was the main plot factor, and plant species was the subplot factor. Within each treatment block, tree seedling responses to four fog treatments (i.e., pH 2.0, 3.0, and 4.0 simulated fog and no fog treatment) were examined. Treatment blocks were distinguished along a North-South axis; block I was furthest South, and block III furthest North. Additionally, plant responses in ambient plots were monitored to assess the effects of enclosing the plots during fog exposure.

Fog chemistry effects were analyzed using Statistical Analysis Systems procedures for analysis of variance (ANOVA). Differences in injury, growth, physiological, and biochemical responses were analyzed for each tree species, needle age class, and sampling date separately. Because the data for each species, needle age class, and date were analyzed separately, the statistical design differed from the experimental root ball, and kept frozen until the time of assay. Lateral and associated short roots were examined under a dissecting microscope at 60x magnification. The presence of the ectomycorrhizae P. tinctorius was determined by visual inspection.

Membrane permeability was determined by measuring the conductivity of needle extracts (Beckerson and Hofstra, 1980), and K^+ leakage (Elkiey and Ormrod, 1979) of young and old needle samples collected after 16 fog applications. Excised needles were stored on ice and transferred to the ERL for analysis. The needles were cut into sixty 1 cm segments, and placed in 50 mL Erlenmeyer flasks. Needle surface contaminants were removed by rinsing the needle segments three times with distilled water. After the final rinse, 30 mL of distilled water was added to each flask, the flasks sealed with parafilm, and allowed to incubate for 8 h at room temperature (25°C). The contents of the flasks were stirred every hour, and conductivity measured every 2 h (Markson Model 1062 Conductivity Meter, Amber Science Inc., Phoenix, AZ). Measurements taken after 8 h of incubation are presented in this report. The concentration of K^+ in solution was measured after 8 h of incubation with an ion-specific electrode (Model 931900 Potassium Electrode, Orion Research Inc., Boston, MA).

Samples for pigment analysis were collected on two occasions: prior to the first fog application (08 April) and after the 16 fog exposures had been applied (26 May 1987). Foliar chlorophylls and carotenoids were extracted using dimethylsulfoxide (DMSO) (Hiscox and Israelstam, 1979). Needle tissue samples (thirty 1 cm segments) were incubated overnight at 55°C in 10 mL of reagent grade DMSO. Absorbances of diluted aliquots were measured at 665, 649, and 470 nm with a double-beam spectrophotometer (Model DB, Beckman Instruments, Inc., Palo Alto, CA) after 12 to 16 h. The pigment-free needle segments were dried to constant weight at 80°C, and foliar pigment concentrations calculated as total chlorophyll and total carotenoids on a dry weight basis (Lichtenthaler and Wellburn, 1983).

Samples for starch analysis were obtained from the trees harvested for determining the initial and post-fog organ weight responses. Dried needles were ground with a Wiley mill to pass a 40 mesh screen. Starch contents of old needles were determined by assaying the glucose content of

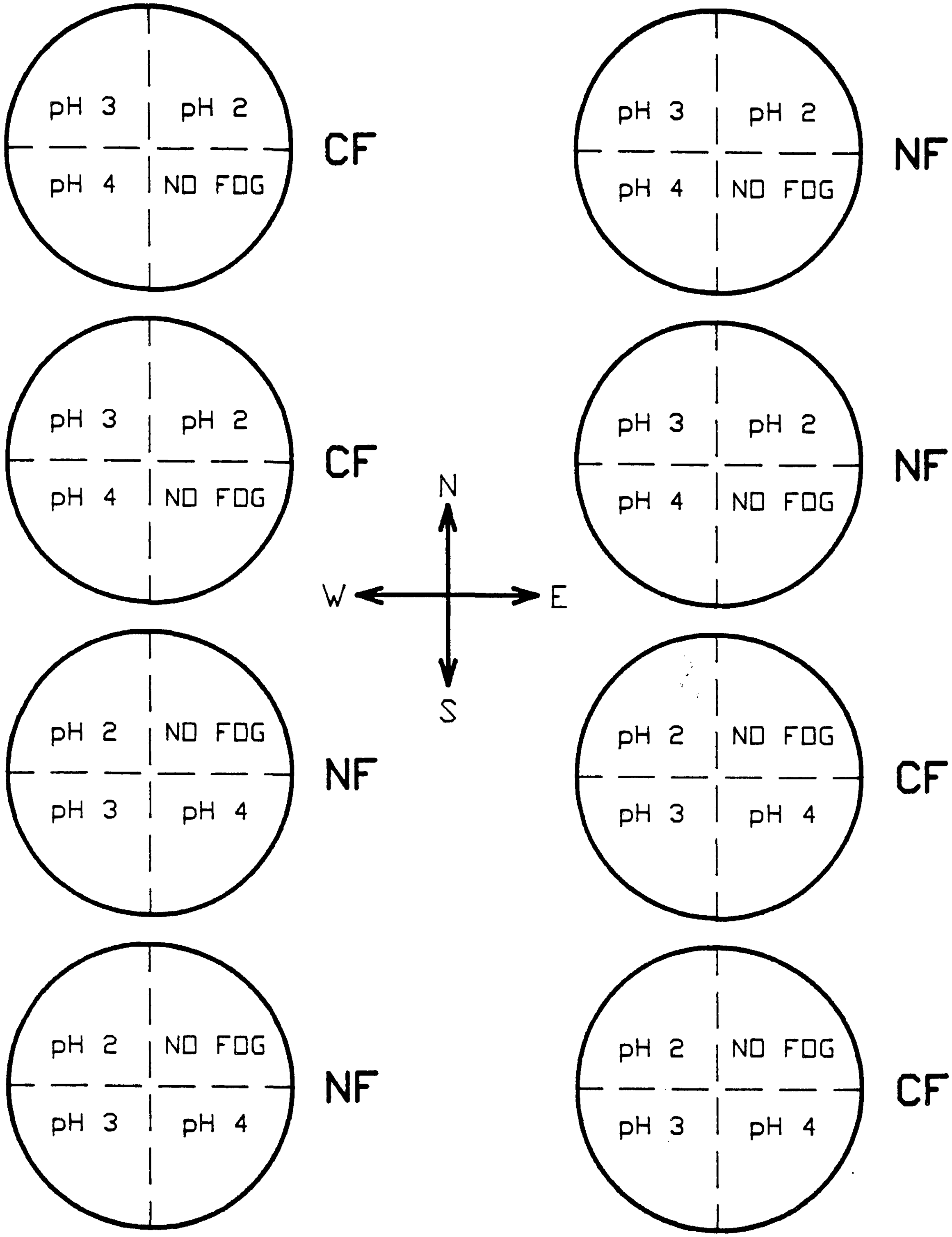

Figure 2. Plot diagram for the ozone exposures. The designation No Fog indicates the sector of the plot in which trees that were not previously exposed to fog are located. The labels pH 4, pH 3, or pH 2 indicate the pH of the fog previously applied to trees in that sector. Replicate treatment blocks are arranged as four groups of two plots, oriented from south to north.

design. In the statistical design used, fog chemistry was the main treatment variable. Differences in plant response due to plot containment (i.e., outside vs. no fog) were examined with t-tests (Steel and Torrie, 1960).

C. Summer Ozone Exposure Study

1. Oxidant Treatments and Measurements

Following the Spring Fog Exposure Study, the seedlings were transferred to open-top field chambers and exposed to charcoal-filtered (CF) or nonfiltered (NF) air (Figure 2). Within each open-top chamber, twelve seedlings of each species were assigned (i.e., three from each fog pretreatment group-no fog, pH 2.0, 3.0 and 4.0). The objective was to determine if the spring acidic fog exposures predisposed the seedlings the phytotoxic effects of O_3. The air quality treatments were applied from 22 May to 14 September 1987. During this period, the chamber blowers operated from 0800 to 2000 h PST, and air quality was sampled at the center of the chamber at a height of 0.3 m. The blowers were shut off between the hours of 2000 to 0800 h PST. Ozone concentrations were monitored continuously with an O_3 analyzer (Model 1003-AH, Dasibi Environmental Corp., Glendale, CA). Monthly calibrations were performed using a transfer standard maintained by the ARB.

2. Environmental Monitoring

Monitoring of environmental conditions was conducted as previously described in the Spring Fog Exposure Study.

3. Plant Culture

The plants transferred to the open-top field chambers were maintained in the same manner as in the Spring Fog Exposure Study insofar as fertilizer and water treatments. Irradiance levels in the chambers were reduced by attaching shadecloth to the chamber frame at height of 1.2 m. No herbicide or pesticide treatments were applied during the Summer Ozone Exposure Study.

4. Injury and Growth Responses

Needle injury and stem growth were measured monthly. Needle injury was measured on three occasions: 22 June, 22 July, and 14 August 1987. Stem height and diameter were measured on 17 June, 15 July, 10 August, and 08 September 1987. The final whole-tree harvest was conducted

6. Statistical Analysis

The experimental design for the Summer Ozone Exposure Study was a randomized split-plot with four replications. Air quality and fog pretreatment were the main plot factors, and tree species was the subplot factor. Plants maintained in the three outside plots in the Spring Fog Exposure Study were randomly re-assigned to four outside plots, corresponding to four air quality treatment blocks.

Data were analyzed using ANOVA, for each tree species, needle age class, and sampling date separately. Because statistical analyses were done for each species, needle age class, and date separately, the statistical design differed from the experimental design. In the statistical design, air quality and fog pretreatment were the main treatment variables, and (air quality x fog pretreatment) was the interaction variable. Plant-to-plant variability was determined separately as a sampling error term.

on 14 September 1987. The aboveground portions were harvested into labeled bags and placed in drying ovens immediately. Washing of the root systems was completed over a three day period ending on 17 September 1987.

5. Physiological and Biochemical Responses

Gas exchange measurements with the LI-COR 6000 photosynthesis system were performed eight times for each species during the 115 day Summer Ozone Exposure Study. Ponderosa pine responses were measured on 02 June, 09 June, 16 June, 07 July, 21 July, 28 July, 11 August, and 25 August. White fir responses were monitored on 03 June, 11 June, 18 June, 09 July, 23 July, 30 July, 13 August, and 26 August 1987. On each sampling date, the responses of eight plants per chamber were measured; two from each fog pretreatment group (i.e., pH 2.0, 3.0, 4.0, or no fog).

In the Summer Ozone Exposure Study procedures to determine the extent of mycorrhizal colonization were modified. Two core samples (2.5 cm diameter) were taken to a depth of 15 cm from opposite sides of the main stem. Cores taken to this depth allowed for a thorough sampling of the upper root mass where mycorrhizal infection is greatest. The soil cores were screened through a 2 mm wire mesh to separate the soil from the roots. Four lateral roots from each soil core (i.e., a total of eight lateral roots per tree), were randomly selected and examined for mycorrhizal infection as previously described. The total length of each lateral root, number of short roots, and number of infected short roots were determined for each lateral root selected. Percent of mycorrhizal infection, number of short roots cm^{-1} of lateral root, and number of infected short roots cm^{-1} of lateral root were calculated.

Samples for assaying membrane permeability were collected and immediately frozen in liquid nitrogen. This method of sample collection was employed owing to the two-fold increase in the number of treatment groups, which did not allow for all samples to be analyzed immediately.

Foliage for pigment analysis were collected monthly on 18 June, 14 July, 12 August, and 09 September 1987, and were kept frozen (-20°C) until the time of assay. The method of pigment extraction was the same as described in the Spring Fog Exposure Study.

Samples for starch analysis were prepared after the final harvest weight measurements were completed. Subsamples from four trees per air quality/acidic fog pretreatment regime were randomly selected for assay. Starch content of old needles was assayed as previously described.

Table 2. Calculated Total H^+, NO_3^- and SO_4^{2-} Deposited on a Single Event and Whole Study Basis[a]

Simulant pH	Deposition ($\mu eq\ m^{-2}$) H^+	NO_3^-	SO_4^{2-}
Single Event			
2.0	1210	582	1675
3.0	121	105	301
4.0	12	68	196
Whole Study			
2.0	19358	9312	26808
3.0	1935	1676	4808
4.0	190	1095	3135

[a]Deposition to tree seedlings adjusted for nozzle drip, evaporation, and deposition to chamber walls.

Table 3. pH of Nozzle Drip, Suspended Fog, and Leaf Moisture Collections[a]

Fog Treatment	Nozzle Drip	Suspended Fog	Leaf Moisture: Ponderosa Pine	Leaf Moisture: White Fir[b]
pH 2.0	2.10 (0.08)	2.12 (0.06)	2.32 (0.64)	1.78 (0.13)
pH 3.0	3.09 (0.06)	3.17 (0.09)	8.54 (3.70)	6.90 (3.54)
pH 4.0	4.01 (0.91)	4.63 (0.91)	5.47 (4.02)	5.92 (3.84)
No Fog			6.68 (5.65)	11.04 (0.52)

[a]Mean (± one standard deviation) for four sampling dates. The pH values for ponderosa pine and white fir leaf moisture collections were calculated from data on moisture weight and pH of the distilled water used to prepare the leaf wash samples.
[b]pH 2.0 and 4.0 collections are based on three sampling dates.

IV. RESULTS AND DISCUSSION

Because of the volume of collected data, only the results which are relevant for discussion of the major findings (in most cases statistically significant changes or persistent trends) are included in the body of the report. The remaining data in the form of graphs and tables are presented in appendices to the report.

A. Spring Fog Exposure Study

1. Fog Chemistry

The Spring Fog Exposure Study was initiated on 10 April 1987, and continued until 15 May 1987. The seedlings were exposed to fog three times per week over a six week period, in which 16 fog exposures were applied. Nozzle drip samples were collected from each fog plot for pH determination (Appendix B-1). Fog pH values varied slightly on an event basis, and the measured pH levels closely matched the target values initially selected. The total loading of H^+, NO_3^-, and SO_4^{2-} on a single-event and whole-study basis was calculated, adjusting for evaporation, nozzle drip, and fog deposition to the chamber walls (Table 2).

During four of the fog exposures (i.e., 24 April, 01 May, 08 May, 15 May), samples of suspended fog and leaf moisture were collected in addition to nozzle drip (Table 3). While the pH of the suspended fog and nozzle drip samples were similar to each other, they were markedly different from the pH of the leaf moisture samples. Both tree species appeared to effectively buffer the acidic inputs from fog at pH 3.0 or 4.0, but were not able to neutralize pH 2.0 fog to any appreciable extent. Dry deposited materials on leaves of herbaceous crops have been found to be important to surface acid-buffering capacities (Olszyk et al. 1987), and may also be important in coniferous trees. The ability to neutralize precipitation acidity by leaf surface processes does, however, appear to be effective over the pH range of 3.0 to 4.0, but may be of little significance with respect to highly acidic fog exposures (i.e., pH 2.0).

The ability to intercept fog on a leaf area basis was not markedly different between the two tree species (Table 4). Fog particles that were deposited to, or impacted against the needles of the seedlings tended to coalesce into droplets at needle tips. It is likely that the data in

Table 5. Ambient Ozone Concentrations During the Spring Fog Exposure and Summer Ozone Exposure Studies[a]

Week	Ozone ($nL\ L^{-1}$)	Maximum 1 h Avg. ($nL\ L^{-1}$)
Spring Fog Exposure Study		
04 April	55 ± 23	118
11	70 ± 38	156
18	57 ± 26	150
25	63 ± 31	144
02 May	79 ± 33	166
09	80 ± 36	184
16	54 ± 19	96
Mean	65 ± 11	145 ± 30
Summer Ozone Exposure Study		
23 May	58 ± 16	102
30	97 ± 38	205
06 June	82 ± 31	140
13	73 ± 31	159
20	100 ± 39	186
27	90 ± 36	162
04 July	72 ± 27	119
11	74 ± 39	167
18	57 ± 26	119
25	116 ± 38	212
01 August	94 ± 43	267
08	72 ± 35	148
15	84 ± 37	162
22	94 ± 45	223
29	81 ± 43	219
05 September	76 ± 36	175
12	66 ± 36	184
19	71 ± 45	207
26	89 ± 55	246
Mean	81 ± 15	179 ± 44

[a]Mean (± one standard deviation) for 12 h average ozone concentration (0800 to 2000 h PST), and maximum 1 h average ozone concentration for the week beginning on the date provided at the ARB Citrus Project Site. To calculate ozone concentration in ppm, divide table values by 1000.

Table 4. Weight of Moisture on Coniferous Tree Seedling Needles after a Fog Exposure Episode[a]

Tree Species	Leaf Moisture ($g\ m^{-2}$)	
	Fog Exposed	No Fog
Ponderosa Pine	88 ± 27	11 ± 8
White Fir	102 ± 44	13 ± 4

[a]Each value represents the average of eight sample collections (± one standard deviation). Leaf moisture expressed on a total needle surface area basis.

Table 4 underestimate the actual leaf moisture values in that a large number of droplets were lost prior to collection (during the needle excision process). Nevertheless, the difference in sensitivity to fog between the young needles of white fir and ponderosa pine does not appear to be a function of moisture collection.

2. Air Quality

During the six week Spring Fog Exposure Study, weekly 12 h average O_3 concentrations ranged from 54 to 80 nL L^{-1} (ppb), and 1 h maxima from 96 to 184 nL L^{-1} (Table 5). The overall 12 h average O_3 concentration was 65 nL L^{-1}, which has not been found to cause significant deleterious impacts to other coniferous tree species (Hogsett et al., 1985; Reich et al., 1987). Ambient springtime 12 h average O_3 levels were 20% lower than levels in the Summer Ozone Exposure Study.

3. Environmental Conditions

Measures of ambient environmental conditions during the early spring in Riverside are listed in Appendix B-2. Weekly 12 h average air temperatures ranged from 18.5 to 31.8°C, irradiance from 569 to 984 µmol $m^{-2}\ s^{-1}$, and relative humidity from 17 to 57%. The shading of the open plots reduced air temperatures by 1 to 3°C, and irradiance by up to 50%, depending on the time of day (experimental observations).

Table 6. Effects of Acidic Fog on the Growth of Ponderosa Pine[a]

Fog Treatment	Dry Weight (g) Root	Stem	Needle	Shoot
I. Prior to the First Fog Application				
	5.1 (4.9)	2.9 (1.7)	3.1 (1.4)	6.0 (2.9)
II. After 16 Fog Applications				
pH 2.0	7.25 (5.37)	5.37 (2.31)	4.85 (2.05)	10.22 (4.24)
pH 3.0	7.72 (6.73)	5.10 (2.43)	4.91 (2.78)	10.01 (5.16)
pH 4.0	5.48 (4.64)	4.60 (2.56)	4.08 (1.88)	8.69 (4.27)
No Fog	7.46 (5.89)	5.67 (2.68)	5.24 (2.41)	10.91 (5.04)
ANOVA[b]	NS	NS	NS	NS

[a]Mean (± one standard deviation). Values represent the average response of five or twelve seedlings for the pre-fog and post-fog analyses, respectively.
[b]Results of the ANOVA for the effects of fog chemistry on the post-fog growth values; NS = not significant at $p<0.05$.

Table 7. Effects of Acidic Fog on the Whole-Study Average Gas Exchange Rates of Ponderosa Pine[a]

Fog Treatment	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)	Stomatal Conductance ($cm\ s^{-1}$)	Net Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)
pH 2.0	25.5 (12.8)	0.066 (0.04)	0.074 (0.04)
pH 3.0	29.3 (10.0)	0.079 (0.04)	0.089 (0.05)
pH 4.0	32.7 (12.1)	0.095 (0.04)	0.104 (0.05)
No Fog	30.2 (12.9)	0.081 (0.04)	0.094 (0.03)
ANOVA[b]	NS	NS	NS

[a]Mean (± one standard deviation). Values represent the average response of 48 seedlings (i.e., 12 measurements on four sampling dates) during the fog exposure study.
[b]Results of the ANOVA; NS = not significant at $p < 0.05$.

4. Effects of Fog Chemistry on Ponderosa Pine

a. Needle Injury Responses

No significant fog chemistry effects on foliar injury to old or young needles were observed (Appendix B-3). Histograms are used to illustrate the distribution of seedlings with different amounts of injury over time (Appendices A-1 to A-4). A vertical broken line was used to demark 10% foliar injury on the histograms as a point of reference. Plants exhibiting injury amounts > 10% were considered to be severely injured. Seedlings with <10% injury were considered to exhibit low or moderate amounts of injury.

(1) Old Needles

Foliar injury assessments made before the first fog application showed that injury amounts were low (<3%) across the fog treatments. Injury amount tended to increase with the number of fog applications. On 7 May, the highest number of trees with >10% injury occurred in the pH 2.0 and 3.0 groups (Appendix A-2). On 18 May, the highest number of trees with >10% injury occurred in the pH 2.0 group (Appendix A-3). None of the trees exposed to pH 4.0 fog showed >10% injury (Appendices A-1 to A-3, B-3).

(2) Young Needles

No significant effects of fog chemistry on injury to young needles were observed (Appendices A-4, B-3).

b. Growth Responses

Mean initial (i.e., pre-fog exposure) and post-fog measures of seedling dry weight are listed in Table 6. Compared to the organ dry weights of seedlings analyzed prior to the first fog application, shoot and root dry weight increased by 67 and 37%, respectively, after 16 fog exposures. While seedling growth was relatively rapid during the six week fog application period, no significant impacts of fog chemistry were observed in any measure of plant dry weight. Seedlings exposed to pH 4.0 fog tended to exhibit lower organ dry weight responses than seedlings in other treatment groups. Mean stem height and diameter measures were increased by 16 and 10%, respectively, by the end of the fog exposures, however, no significant fog chemistry effects were noted (Appendix B-4).

Table 8. Effects of Acidic Fog on Needle Segment Extract Conductivity and K^+ Leakage in Ponderosa Pine[a]

Fog Treatment	Needle Age Class: Young	Needle Age Class: Old
Conductivity (μmhos cm^{-1} g^{-1} dry weight)		
pH 2.0	139 ± 31 a	96 ± 20
pH 3.0	75 ± 9 b	74 ± 25
pH 4.0	91 ± 21 b	86 ± 16
No Fog	81 ± 19 b	73 ± 4
ANOVA[b]	*	NS
L.S.D.[c]	38	---
K^+ Leakage (μg K^+ g^{-1} dry weight)		
pH 2.0	1237 ± 350 a	525 ± 129 a
pH 3.0	804 ± 167 b	452 ± 63 a
pH 4.0	892 ± 152 b	449 ± 94 a
No Fog	751 ± 161 b	301 ± 42 b
ANOVA[b]	*	*
L.S.D.[c]	325	122

[a]Values represent the average conductivity or K^+ leakage response of five seedlings for each fog treatment group (± one standard deviation). Means followed by dissimilar letter designations for each needle age class separately, indicate statistically significant differences at $p < 0.05$ using Duncan's New Multiple Range Test.

[b]Results of the ANOVA; NS = not significant at $p < 0.05$, * = significant at $p < 0.05$.

[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).

c. Physiological Responses

(1) Old Needles

Average values for transpiration (Ts), stomatal conductance of water vapor (Cs), and net photosynthesis (Pn) on a sampling event-basis are listed in Appendix B-5. Gas exchange rates were measured only in old needles (the young needles did not emerge until mid-April). Although gas exchange rates by pine needles varied from one sampling date to the next, plants exposed to pH 2.0 fog displayed the lowest rates on each sampling date compared to plants in the other fog treatment groups. While this was a consistently observed phenomenon, whole-study average process rates were not significantly inhibited (Table 7).

(2) Young Needles

The young needles had not elongated to the point where the measurement of gas exchange rates was possible during the Spring Fog Exposure Study.

(3) Roots

Mycorrhizal infection was evident on < 5% of the seedlings sampled at the end of the fog exposure study (i.e., in two of the 60 seedlings sampled). The low levels of infection may be attributed to the short duration between tree inoculation and root assay. Given the low amount of infection, there was no evidence of significant treatment effects on mycorrhizal establishment in ponderosa pine.

d. Biochemical Responses

(1) Old Needles

The conductivity of needle segment extracts and amount of K^+ leakage was highest in the pH 2.0 fog-treated plants, but the differences among fog treatments were not statistically significant (Table 8). Pre-fog chlorophyll concentrations were on average 31% higher than post-fog levels (Table 9). The only significant fog chemistry effect was observed in plants exposed to pH 2.0 fog, where chlorophyll levels were 28 or 37% higher than unfogged or pH 3.0 fog-exposed seedlings, respectively. Carotenoid levels in ponderosa pine foliage before the first fog application were markedly higher than post-fog concentrations (Table 9). On average, levels were 45% lower in old needles of ponderosa pine by the end of the fog exposures. Seedlings exposed to pH 2.0 fog had the highest carotenoid contents, but levels were significantly higher relative to nonfogged plants only. Exposure to fog induced no significant alterations in ponderosa pine starch concentrations. Needle starch levels were increased by an average of 4% by the end of the fog exposures (Table 9).

(2) Young Needles

Exposure to pH 2.0 fog resulted in significantly higher needle extract conductivity and K^+ leakage responses in young pine needles (Table 9). Conductivity was 53% higher, and K^+ leakage was 39% higher than in plants exposed to pH 4.0 fog. After 16 exposures to fog, there was no evidence of significant fog chemistry impacts on needle chlorophyll responses. Among fog treatment groups, carotenoid levels varied by only 7%, and the effects of fog chemistry were not significant.

e. Effects of Fog Application

The effects of fog application were assessed by comparing the responses of plants that were not fogged and plants that received applications of pH 4.0 fog. In ponderosa pine, no significant fog chemistry effects were observed insofar as injury or growth responses (Table 6, Appendix B-4). Relative to biochemical responses, the only significant effect was found in old pine needle K^+ leakage, where plants exposed to pH 4.0 fog exhibited levels that were 49% higher than unfogged plants (Table 8). These data clearly demonstrate that the application of pH 4.0 fog did not cause appreciable alterations in injury, growth, physiological, or biochemical responses in ponderosa pine seedlings. Since the total loading of inorganic elements from pH 4.0 fog over the entire study was low (Table 2), marked changes in foliar inorganic chemistry are not likely to occur. The greatest impact of fog application may be in providing moisture directly to foliage (Tukey, 1980).

f. Effects of Fog Enclosures

The responses of plants in outside and no fog plots were compared to determine if periodically enclosing the plants during fog episodes altered plant injury, growth, or biochemical responses. In ponderosa pine, there was little difference in the value of responses between plants in outside or no fog plots (Appendices B-6, B-7), and none of the differences were found to be statistically significant. Since none of the twenty-five parameters monitored differed significantly between plants in these two treatments, the practice of including both types of treatments in future fog studies does not appear to be necessary.

Table 9. Effects of Acidic Fog on the Foliar Chlorophyll, Carotenoid, and Starch Concentrations of Ponderosa Pine[a]

Fog Treatment	Needle Age Class	
	Young	Old
Chlorophyll (mg g^{-1} dry weight)		
pH 2.0	2.56 ± 0.59	3.46 ± 0.40 a
pH 3.0	2.49 ± 0.37	2.53 ± 0.45 b
pH 4.0	2.64 ± 0.51	3.02 ± 0.63 ab
No Fog	2.94 ± 0.66	2.71 ± 0.43 b
ANOVA[b]	NS	*
L.S.D.[c]	---	0.59
Carotenoids (mg g^{-1} dry weight)		
pH 2.0	0.42 ± 0.10	0.56 ± 0.04 a
pH 3.0	0.41 ± 0.06	0.47 ± 0.07 ab
pH 4.0	0.43 ± 0.08	0.45 ± 0.04 ab
No Fog	0.44 ± 0.09	0.37 ± 0.16 b
ANOVA	NS	*
L.S.D.	---	0.11
Starch (mg g^{-1} dry weight)		
pH 2.0	-----	8.57 ± 1.94
pH 3.0	-----	10.72 ± 3.41
pH 4.0	-----	8.76 ± 2.45
No Fog	-----	10.96 ± 4.14
ANOVA	-----	NS

[a]Values represent the average response of six, six, or four seedlings from each fog treatment group (± one standard deviation) for chlorophyll, carotenoid, and starch responses, respectively. Means followed by dissimilar letter designations indicate statistically significant differences at $p < 0.05$ using Duncan's New Multiple Range Test. Mean chlorophyll, carotenoid, and starch concentrations prior to the first fog exposure were 4.22 ± 0.64, 0.84 ± 0.10, and 9.35 ± 5.76 mg g^{-1} dry weight, respectively, based on a random sample of five seedling trees.

[b]Results of the ANOVA; NS = not significant at $p < 0.05$, * = significant at $p < 0.05$.

[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).

Table 10. Effects of Acidic Fog on White Fir Foliar Injury[a]

Fog Treatment	Needle Age Class: Young	Needle Age Class: Old
03 April 1987 (Pre-Exposure)		
pH 2.0	---	0.65 ± 1.96
pH 3.0	---	0.41 ± 1.27
pH 4.0	---	0.80 ± 2.46
No Fog	---	0.40 ± 1.48
ANOVA[b]	---	NS
05 May 1987 (After 11 Fog Exposures)		
pH 2.0	---	3.31 ± 3.32 a
pH 3.0	---	1.10 ± 1.81 b
pH 4.0	---	1.11 ± 2.89 b
No Fog	---	1.49 ± 2.71 b
ANOVA	---	*
L.S.D.[c]	---	1.76
15 May 1987 (After 16 Fog Exposures)		
pH 2.0	8.71 ± 9.25 a	8.38 ± 7.73 a
pH 3.0	0.39 ± 1.44 b	2.52 ± 5.87 b
pH 4.0	0.14 ± 0.40 b	2.49 ± 4.36 b
No Fog	0.33 ± 1.53 b	1.43 ± 3.22 b
ANOVA	*	*
L.S.D.	2.02	2.32

[a]Foliar injury expressed as the percentage of the total needle surface area exhibiting necrosis (± one standard deviation). Values listed represent the average response of 36 tree seedlings for each fog treatment group. Treatments followed by dissimilar letter designations for each needle class separately, indicate statistically significant differences at a level of $p < 0.05$ using Duncan's New Multiple Range Test.

[b]Results of the ANOVA; NS = not significant at $p < 0.05$, * = significant at $p < 0.05$.

[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).

g. Assessment of Ponderosa Pine Responses to Acidic Fog

The foliage of ponderosa pine was relatively tolerant of acid inputs from pH 2.0 fog. Although visible injury was observed in both old and young needle age classes, the amounts were not appreciable, and were not evident as alterations in foliar pigment levels. However, young needle conductivity and K^+ leakage responses were increased, indicating that the integrity of cellular membrane may have been affected. Trends toward reductions in gas exchange process rates, stem height, and starch content in pH 2.0 fog-exposed plants may be related to the development of foliar necrosis. Young needles appear to be more sensitive to highly acidic fog than old needles, and the lack of significant fog application or fog enclosure effects suggest that the observed impacts on pine were attributable to the fog chemistry treatments. Moreover, no apparent deleterious impacts were observed with respect to the application of pH 3.0 or 4.0 fog. Overall, the absence of significant injury or growth effects paralleled the relative lack of significant physiological and biochemical effects.

5. Effects of Fog Chemistry on White Fir

a. Needle Injury

Unlike the ponderosa pine seedlings, the white fir seedlings exhibited statistically significant differences in injury due to fog chemistry (Table 10, Figures 3 to 5).

(1) Old Needles

Average foliar injury before the fog exposures was very low (<1%) in all the treatment groups (Appendix A-5). On 05 May, the number of seedlings that exhibited >10% injury was highest in the pH 2.0 fog group (Figure 3). The same trend was evident on 15 May after five additional fog applications (Figure 4). The average amount of injury was significantly higher in the pH 2.0 fog group than in any other fog treatment group (Table 10).

(2) Young Needles

Young needles showed a high sensitivity to pH 2.0 fog. The average amount of injury and number of trees with >10% injury was highest in the pH 2.0 fog treatment group (Table 10, Figure 5).

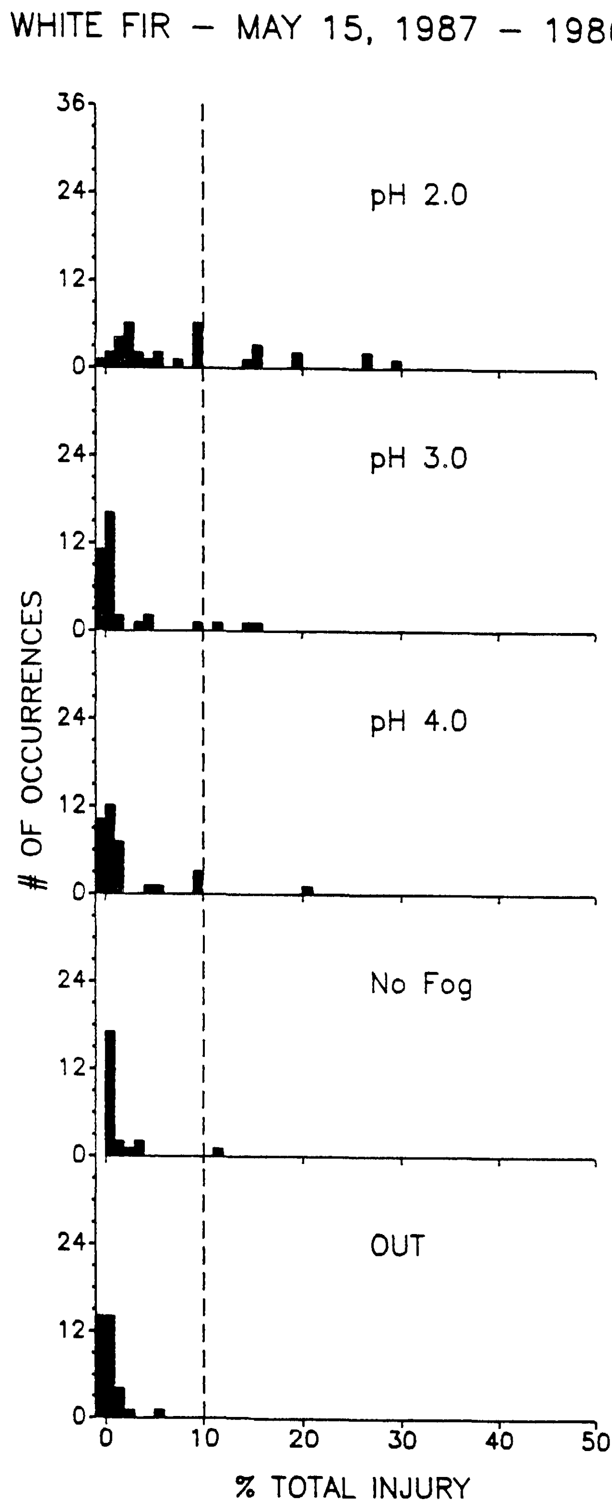

Figure 4. Effects of fog chemistry on distribution of injury to 1986 needles of white fir on May 15, 1987 (see Figure 3 for explanation).

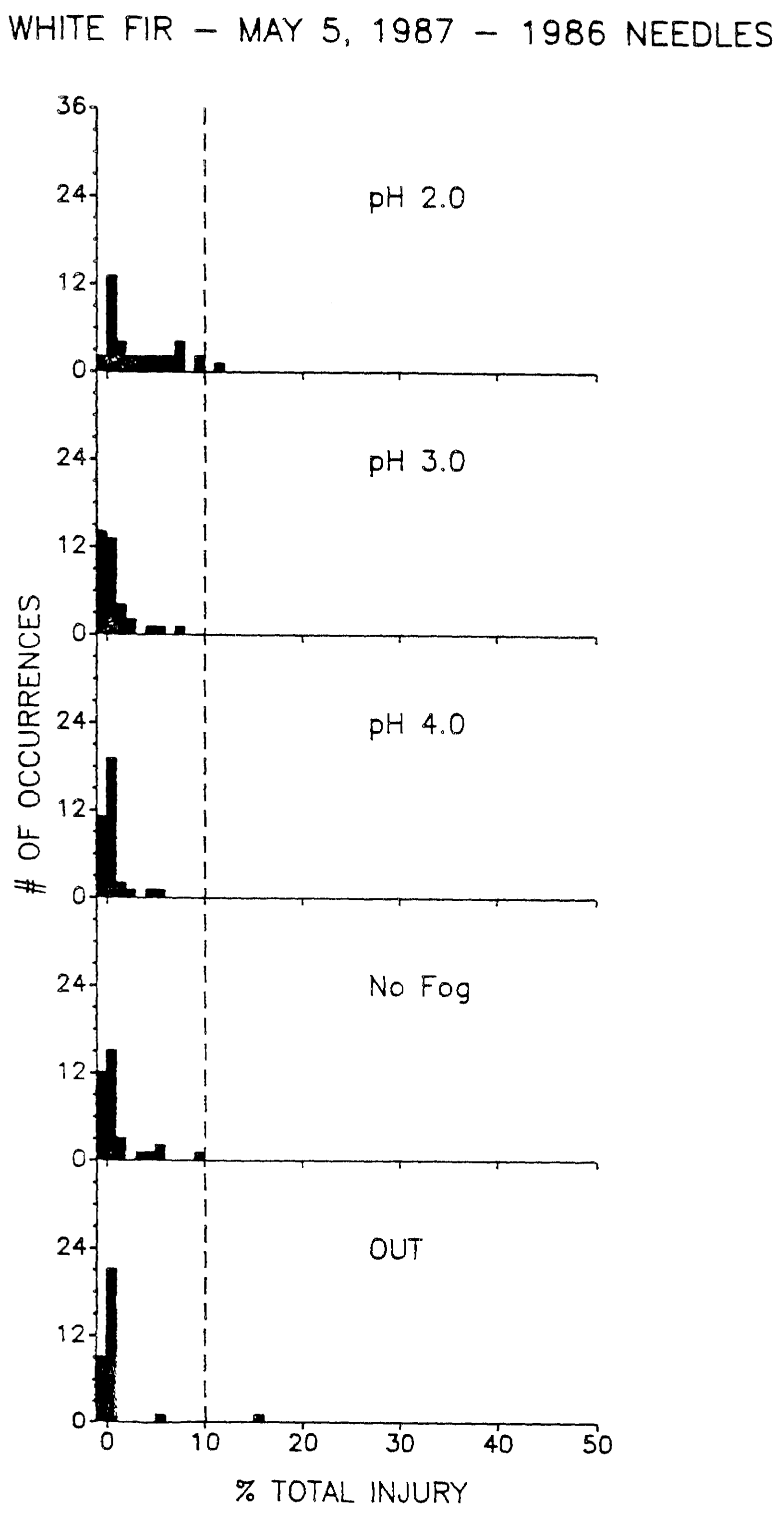

Figure 3. Effects of fog chemistry on distribution of injury to 1986 needles of white fir on May 5, 1987. The broken vertical line at 10% total foliar injury was arbitrarily selected, and separates trees considered severely injured (>10% total injury) from less injured trees.

b. Growth Responses

Although no statistically significant fog chemistry effects were observed, seedlings exposed to pH 2.0 fog tended to exhibit lower organ dry weights than plants exposed to the other fog treatments (Table 11). No significant impacts of fog chemistry on stem height or diameter were noted (Appendix B-8).

c. Physiological Responses

(1) Old Needles

Whole-study average gas exchange process rates were not significantly affected by acidic fog treatment (Table 12). Mean values for Ts, Cs, and Pn for each sampling date are provided (Appendix B-9). Plants exposed to pH 3.0 fog exhibited the lowest Ts and Cs values on all four sampling dates, but Pn was comparable to pH 2.0 plants.

(2) Young Needles

Owing to the lack of development, young needle gas exchange rates were not measured in this study.

(3) Roots

Similar to ponderosa pine, mycorrhizal colonization rates were extremely low, and infected short roots were found on fewer than 5% of the trees sampled.

d. Biochemical Responses

(1) Old Needles

The conductivity of needle extracts or K^+ leakage responses were not significantly altered by fog chemistry (Table 13). Moreover, differences among fog treatment groups for chlorophyll and carotenoid concentrations (Table 14) were not significant. Exposure to pH 2.0 fog significantly reduced starch levels by 36 to 42% compared to values found in the other fog treatment groups (Table 14).

(2) Young Needles

No significant fog chemistry effects were observed with respect to needle extract conductivity, K^+ leakage, chlorophyll or carotenoid responses (Tables 13, 14).

e. Effects of Fog Application

The application of pH 4.0 fog did not induce any significant differences in injury, growth, stem dimension, physiological, or biochemical responses compared to plants that received no fog application.

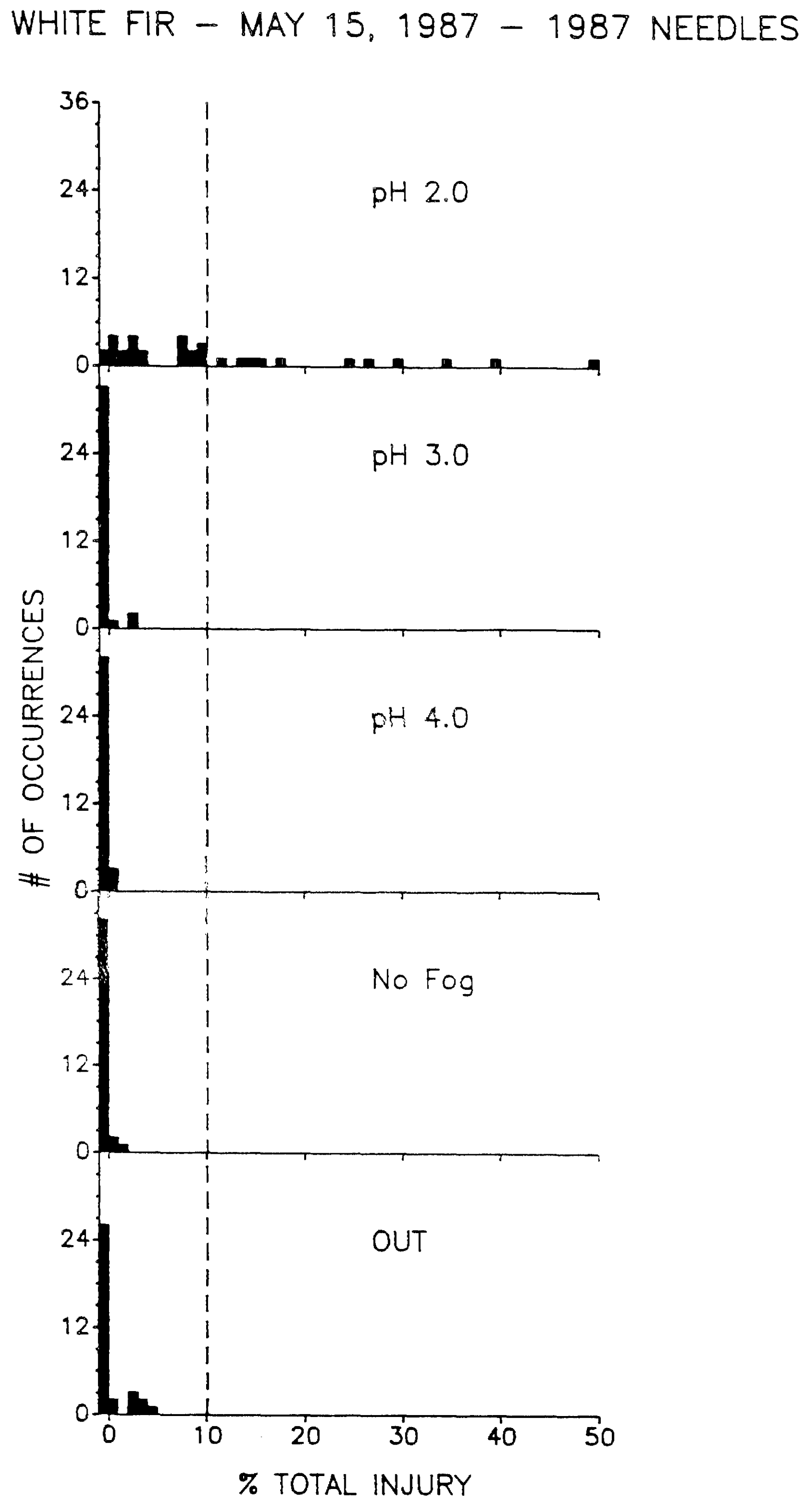

Figure 5. Effects of fog chemistry on distribution of injury to 1987 needles of white fir on May 15, 1987 (see Figure 3 for explanation).

Table 13. Effects of Acidic Fog on Needle Segment Extract Conductivity and K^+ Leakage in White Fir[a]

Fog Treatment	Needle Age Class: Young	Needle Age Class: Old
Conductivity (μmhos cm^{-1} g^{-1} dry weight)		
pH 2.0	529 ± 196	95 ± 13
pH 3.0	689 ± 155	94 ± 15
pH 4.0	417 ± 177	97 ± 30
No Fog	591 ± 191	95 ± 15
ANOVA[b]	NS	NS
K^+ Leakage (μg K^+ g^{-1} dry weight)		
pH 2.0	1806 ± 473	374 ± 42
pH 3.0	1948 ± 554	429 ± 131
pH 4.0	1356 ± 643	514 ± 191
No Fog	1973 ± 575	409 ± 129
ANOVA	NS	NS

[a]Values represent the average conductivity response of five seedlings for each fog treatment group (± one standard deviation).
[b]Results of the ANOVA; NS = not significant at $p < 0.05$.

Table 11. Effects of Acidic Fog on the Growth of White Fir[a]

Fog Treatment	Dry Weight (g) Root	Stem	Needle	Shoot
I. Prior to the First Fog Application				
	10.1 (5.6)	2.9 (1.7)	3.2 (1.4)	6.1 (4.9)
II. After 16 Fog Applications				
pH 2.0	16.60 (8.10)	6.64 (3.39)	7.58 (3.48)	14.22 (6.51)
pH 3.0	18.42 (6.74)	7.20 (2.99)	7.77 (1.59)	14.97 (4.24)
pH 4.0	18.86 (9.32)	7.02 (3.46)	8.25 (4.60)	15.26 (7.98)
No Fog	18.86 (9.43)	6.94 (3.47)	8.62 (3.94)	15.55 (7.28)
ANOVA[b]	NS	NS	NS	NS

[a]Mean ± one standard deviation. Values represent the average response of five or twelve seedlings for the pre-fog and post-fog analyses, respectively.
[b]Results of the ANOVA for the effects of fog chemistry on post-fog growth values; NS = not significant at $p < 0.05$.

Table 12. Effects of Acidic Fog on the Whole-Study Average Gas Exchange Rates of White Fir[a]

Fog Treatment	Transpiration ($mg\ H_2O\ m^{-2}\ s^{-1}$)	Stomatal Conductance ($cm\ s^{-1}$)	Net Photosynthesis ($mg\ CO_2\ m^{-2}\ s^{-1}$)
White Fir			
pH 2.0	32.2 (17.8)	0.092 (0.06)	0.074 (0.05)
pH 3.0	27.8 (14.7)	0.078 (0.05)	0.075 (0.04)
pH 4.0	31.4 (15.3)	0.099 (0.06)	0.096 (0.05)
No Fog	27.7 (13.5)	0.078 (0.05)	0.084 (0.05)
ANOVA[b]	NS	NS	NS

[a]Mean (± one standard deviation). Values represent the average response of 48 seedlings (i.e., 12 measurements on four sampling dates) during the fog exposure study.
[b]Results of the ANOVA; NS = not significant at $p < 0.05$.

f. Effects of Fog Enclosures

No significant treatment effects were observed in any of the twenty-five parameters monitored (Appendix B-10, B-11).

g. Assessment of White Fir Responses to Acidic Fog

Similar to ponderosa pine, no significant impacts on white fir were observed with respect to the application of pH 3.0 or 4.0 fog, or from the fog enclosures. In contrast, foliar injury caused by the application of pH 2.0 fog was severe, and more pronounced in young needles than old needles. Despite significant amounts of injury to both needle age classes of foliage, fog at pH 2.0 did not induce deleterious impacts on membrane permeability or foliar pigment responses. Although sizeable reductions in net photosynthesis were observed in plants exposed to pH 2.0 and 3.0 fog compared to pH 4.0 fog-exposed plants, needle starch levels were significantly reduced only in pH 2.0 fog-treated plants. Growth was not significantly affected during this experimental period, but the obvious impact of pH 2.0 fog insofar as inducing visible injury in the current growing season could potentially have longer-term effects. For example, old needle starch levels were reduced, which could limit needle development in the next flush period. Nonetheless, the lack of significant effects on growth was largely reflected by the paucity of significant impacts on physiological and biochemical responses.

B. Summer Ozone Exposure Study

1. Air Quality

Ambient air quality data (i.e., weekly 12 h average O_3 concentrations and maximum 1 h levels) were measured at the ARB Citrus Project Site (Table 5). These data closely correlated with ambient data from the ARB Humidity Project Site, and are provided in their place for comparative purposes with respect to the Spring Fog Exposure Study. Monthly 12 h average O_3 levels and 1 h maxima in the NF and CF open-top field chambers used in the Summer Ozone Exposure Study are provided in Table 15. During this 115 day study period, air quality data were not collected on five days due to mechanical problems (i.e., 4% down time). The four month average O_3 concentration in NF chambers was 4.1 times greater than in CF chambers.

Table 14. Effects of Acidic Fog on the Foliar Chlorophyll, Carotenoid, and Starch Concentrations of White Fir[a]

Fog Treatment	Needle Age Class: Young	Needle Age Class: Old
Chlorophyll (mg g^{-1} dry weight)		
pH 2.0	2.38 ± 0.67	4.31 ± 0.82
pH 3.0	3.04 ± 0.30	4.74 ± 0.45
pH 4.0	2.85 ± 0.25	4.63 ± 0.47
No Fog	2.45 ± 0.51	4.78 ± 0.62
ANOVA[b]	NS	NS
Carotenoids (mg g^{-1} dry weight)		
pH 2.0	0.55 ± 0.08	0.66 ± 0.08
pH 3.0	0.63 ± 0.07	0.66 ± 0.09
pH 4.0	0.59 ± 0.13	0.62 ± 0.09
No Fog	0.58 ± 0.05	0.70 ± 0.09
ANOVA	NS	NS
Starch (mg g^{-1} dry weight)		
pH 2.0	---	14.63 ± 2.51 b
pH 3.0	---	24.88 ± 4.93 a
pH 4.0	---	22.81 ± 2.27 a
No Fog	---	25.39 ± 4.30 a
ANOVA	---	***
L.S.D.[c]	---	5.67

[a]Values represent the average response of six, six, or four seedlings from each fog treatment group (± one standard deviation) for chlorophyll, carotenoid, and starch responses, respectively. Means followed by dissimilar letter designations indicate statistically significant differences at $p < 0.05$ using Duncan's New Multiple Range Test. Mean chlorophyll, carotenoid, and starch concentrations prior to the first fog exposure were 4.00 ± 0.56, 0.85 ± 0.11, and 21.11 ± 3.85 mg g^{-1} dry weight, respectively, based on a random sample of five seedling trees.

[b]Results of the ANOVA; NS = not significant at $p < 0.05$, *** = significant at $p < 0.001$.

[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).

illustrating injury development were prepared (Appendices A-6 to A-11). No definitive trends in injury development between the CF and NF treatments were observed (Appendices A-6 to A-8).

(2) Young Needles

No apparent effects of air quality on young needle injury were evident in June, July or August (Appendices A-9 to A-11).

b. Growth Responses

While there were no significant air quality impacts on ponderosa pine growth responses, organ dry weights were slightly higher (i.e., 6 to 12%) in seedlings grown in NF air (Table 16). Moreover, air quality had no significant effect on stem dimension in all four months of sampling (Appendices B-13, B-14).

c. Physiological Responses

(1) Old Needles

Gas exchange responses in old needles were not monitored during the summer.

(2) Young Needles

Air quality impacts on whole-study average gas exchange process rates were not significant (Table 17). Relative to the spring (Table 7), process rates were 20 to 46% lower despite the fact that measurements were made on the more active needle age class.

(3) Roots

By the end of the summer, % mycorrhizal infection and the number of short roots cm^{-1} of lateral root were significantly increased in ponderosa pine exposed to NF air relative to plants in CF air (Table 18). Although the number of infected roots cm^{-1} of lateral roots was 49% greater in NF air plants than in CF air plants, the difference was not statistically significant.

d. Biochemical Responses

(1) Old Needles

Plants exposed to NF air exhibited significantly higher needle extract conductivity and K^+ leakage than plants grown in CF air (Tables 19 and 20, respectively). In NF air seedlings, conductivity was 26% higher, and K^+ leakage was 31% greater than CF air plants. No significant air quality impacts on foliar chlorophyll (Appendices B-15 to B-17, Table 21), carotenoid (Appendices B-18 to B-20), or starch (Appendix B-21) levels were observed during the four months of monitoring.

Table 15. Ozone Air Quality in the Summer Ozone Exposure Study[a]

Parameter	Air Quality Treatment[b] NF	CF
12 h Avg. O_3 Concentration		
June	70 ± 33	11 ± 11
July	75 ± 38	16 ± 15
August	83 ± 42	21 ± 13
September	64 ± 41	22 ± 23
Whole Study	73 ± 39	18 ± 16
Maximum 1 h Avg. Concentration		
June	178	72
July	202	84
August	235	70
September	195	61

[a]Ozone concentrations expressed in nL L^{-1}; 12 h Avg. concentrations measured over the period of 0800 to 2000 h PST (± one standard deviation) at the ARB Humidity Project Site.
[b]NF = nonfiltered air; CF = charcoal filtered air.

2. Environmental Conditions

Measures of ambient environmental conditions during the Summer Ozone Exposure Study are listed in Appendix B-12. Air temperatures ranged from 21.1 to 33.3°C, irradiance from 653 to 1084 µmol m^{-2} s^{-1}, and relative humidity from 32 to 55%.

3. Effects of Ozone on Ponderosa Pine

a. Needle Injury

(1) Old Needles

The summer O_3 exposures did not cause substantive changes in injury amount to old needles. Unlike the data from the spring acidic fog experiment, the data were highly skewed, and the distribution of injury amounts was bimodal. Because of these distribution anomalies, statistical analyses were deemed inappropriate. Instead, histograms

Table 17. Statistical Analysis of the Effects of Ozone on the Gas Exchange Rates of Ponderosa Pine Pretreated with Acidic Fog[a]

Treatment Variable	Transpiration (mg H_2O m^{-2} s^{-1})	Stomatal Conductance (cm s^{-1})	Net Photosynthesis (mg CO_2 m^{-2} s^{-1})
Air Quality[b] (AQ)			
CF	15.6 (7.4)	0.061 (0.04)	0.053 (0.04)
NF	16.5 (9.0)	0.067 (0.05)	0.057 (0.04)
Fog Pretreatment[c] (FP)			
pH 2.0	16.3 (9.0)	0.063 (0.04)	0.054 (0.03)
pH 3.0	16.0 (8.0)	0.067 (0.05)	0.054 (0.03)
pH 4.0	17.0 (8.5)	0.066 (0.04)	0.057 (0.04)
No Fog	14.8 (7.4)	0.060 (0.04)	0.054 (0.04)
AQ x FP			
CF/pH 2.0	15.3 (7.9)	0.056 (0.04)	0.051 (0.03)
NF/pH 2.0	17.3 (10.0)	0.070 (0.05)	0.058 (0.04)
CF/pH 3.0	14.7 (7.1)	0.061 (0.05)	0.055 (0.04)
NF/pH 3.0	17.4 (8.7)	0.074 (0.05)	0.053 (0.03)
CF/pH 4.0	16.7 (8.1)	0.064 (0.03)	0.051 (0.04)
NF/pH 4.0	17.4 (8.9)	0.068 (0.04)	0.063 (0.05)
CF/No Fog	15.8 (6.5)	0.065 (0.04)	0.056 (0.04)
NF/No Fog	13.8 (8.0)	0.056 (0.05)	0.053 (0.03)
ANOVA Effect[d]			
AQ	NS	NS	NS
FP	NS	NS	NS
AQ x FP	NS	NS	NS
C.V. (%)[e]	42.0	48.8	47.1

[a]Mean (± one standard deviation). Sample sizes of 256, 128, and 64 were used to examine the AQ, FP, and AQ x FP effects, respectively.
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]Fog treatment applied in the Spring Fog Exposure Study.
[d]Results of the ANOVA; NS = not significant at $p<0.05$.
[e]C.V. = coefficient of variation.

Table 16. Statistical Analysis of the Effects of Ozone on the Growth of Ponderosa Pine Pretreated with Acidic Fog[a]

Treatment Variable	Dry Weight (g)		
	Root	Stem	Needles
<u>Air Quality[b] (AQ)</u>			
CF	13.09 (5.73)	9.38 (3.53)	11.65 (4.79)
NF	14.72 (6.55)	9.97 (3.72)	12.61 (5.46)
<u>Fog Pretreatment[c] (FP)</u>			
pH 2.0	14.23 (5.70)	10.44 (3.94)	13.31 (5.16)
pH 3.0	14.01 (5.67)	9.83 (3.21)	12.50 (4.79)
pH 4.0	13.19 (6.32)	9.18 (3.79)	10.20 (4.46)
No Fog	14.18 (7.23)	9.24 (3.60)	12.52 (5.80)
<u>AQ x FP</u>			
CF/pH 2.0	13.50 (4.95)	10.43 (3.85)	13.28 (4.55)
NF/pH 2.0	14.97 (6.51)	10.45 (4.20)	13.34 (5.89)
CF/pH 3.0	14.23 (5.89)	9.51 (3.60)	12.44 (5.14)
NF/pH 3.0	13.78 (5.69)	10.16 (2.88)	12.56 (4.64)
CF/pH 4.0	12.00 (5.90)	8.94 (3.46)	9.50 (4.13)
NF/pH 4.0	14.38 (6.75)	9.43 (4.22)	10.90 (4.84)
CF/No Fog	12.62 (6.58)	8.62 (3.40)	11.41 (5.00)
NF/No Fog	15.74 (7.79)	9.86 (3.83)	13.64 (6.52)
<u>ANOVA Effect[d]</u>			
AQ	NS	NS	NS
FP	NS	NS	NS
AQ x FP	NS	NS	NS
C.V. (%)[e]	43.4	37.6	41.6

[a]Mean (± one standard deviation). Sample sizes of 48, 24, and 12 were used to examine the AQ, FP, and AQ x FP effects, respectively.
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]Fog treatment applied in the Spring Fog Exposure Study.
[d]Results of the ANOVA; NS = not significant at $p<0.05$.
[e]C.V. = coefficient of variation.

Table 19. Statistical Analysis of the Effects of Ozone on the Conductivity of Needle Segment Extracts of Ponderosa Pine Pretreated with Acidic Fog[a]

Treatment Variable	Needle Age Class: Young	Needle Age Class: Old
Air Quality[b] (AQ)		
CF	1233 ± 146	1019 ± 142
NF	1333 ± 175	1285 ± 251
L.S.D.[c]	81	118
Fog Pretreatment[d] (FP)		
pH 2.0	1209 ± 132	1177 ± 200
pH 3.0	1314 ± 218	1080 ± 172
pH 4.0	1251 ± 185	1101 ± 192
No Fog	1356 ± 83	1252 ± 356
AQ/FP		
CF/pH 2.0	1258 ± 114 xyz	1067 ± 173 yz
NF/pH 2.0	1160 ± 143 yz	1286 ± 173 xy
CF/pH 3.0	1130 ± 71 z	1072 ± 186 yz
NF/pH 3.0	1499 ± 130 w	1087 ± 178 xyz
CF/pH 4.0	1162 ± 178 yz	938 ± 82 z
NF/pH 4.0	1339 ± 161 wxy	1263 ± 102 xy
CF/No Fog	1380 ± 59 wx	999 ± 99 z
NF/No Fog	1332 ± 103 wxy	1504 ± 341 x
L.S.D.	162	236
ANOVA Effect[e]		
AQ	*	***
FP	NS	NS
AQ x FP	***	*
C.V. (%)[f]	9.8	15.9

[a]Mean ± one standard deviation in µmhos cm^{-1} g^{-1} dry weight. Sample sizes of 20, 10, and 5 were used to examine the AQ, FP, and AQ x FP effects, respectively. Dissimilar letter designations for each needle class separately, indicate significant differences at $p<0.05$ (Duncan's New Multiple Range Test).
[b]CF = charcoal filtered air; NF = nonfiltered air.
[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).
[d]Fog treatment applied in the Spring Fog Exposure Study.
[e]Results of the ANOVA; NS = not significant at $p<0.05$, * = significant at $p<0.05$, *** = significant at $p<0.001$.
[f]C.V. = coefficient of variation.

Table 18. Statistical Analysis of the Effects of Ozone on Mycorrhizal Colonization of Ponderosa Pine Pretreated with Acidic Fog[a]

Treatment Variable	% Infection	No. of Short Roots cm^{-1}	No. of Infected Roots cm^{-1}
Air Quality[b] (AQ)			
CF	27.43 (7.09)	3.28 (0.90)	1.46 (0.50)
NF	37.14 (13.79)	4.15 (1.15)	2.17 (0.79)
L.S.D.[c]	7.67	0.51	---
Fog Pretreatment[d] (FP)			
pH 2.0	35.95 (12.40)	3.59 (1.20)	1.82 (0.93)
pH 3.0	32.61 (10.74)	4.22 (1.20)	1.82 (0.70)
pH 4.0	30.25 (11.71)	3.55 (1.07)	1.81 (0.73)
No Fog	30.34 (13.85)	3.48 (1.05)	1.80 (0.76)
AQ x FP			
CF/pH 2.0	26.53 (3.33)	2.96 (0.36)	1.24 (0.32)
NF/pH 2.0	45.38 (10.54)	4.22 (1.38)	2.40 (1.01)
CF/pH 3.0	30.18 (5.96)	4.10 (1.18)	1.74 (0.35)
NF/pH 3.0	35.05 (14.76)	4.35 (1.38)	1.91 (1.00)
CF/pH 4.0	29.48 (4.07)	3.18 (0.95)	1.68 (0.79)
NF/pH 4.0	31.03 (17.38)	3.93 (1.17)	1.95 (0.76)
CF/No Fog	23.55 (12.31)	2.87 (0.58)	1.19 (0.26)
NF/No Fog	37.13 (13.16)	4.09 (1.12)	2.42 (0.50)
ANOVA Effect[e]			
AQ	*	*	NS
FP	NS	NS	NS
AQ x FP	NS	NS	NS
C.V. (%)[f]	34.1	27.1	31.7

[a]Mean (± one standard deviation) for % infection, no. of short roots cm^{-1} of lateral root, and no. of infected roots cm^{-1} of lateral root. Sample sizes of 48, 24, and 12 were used to examine the AQ, FP, and AQ x FP effects, respectively.
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).
[d]Fog treatment applied in the Spring Fog Exposure Study.
[e]Results of the ANOVA; NS = not significant at $p<0.05$, * = significant at $p<0.05$.
[f]C.V. = coefficient of variation.

Table 21. Statistical Analysis of the Effects of Ozone on the Chlorophyll Content of Ponderosa Pine Needles Pretreated with Acidic Fog[a]: September 1987

Treatment Variable	Needle Age Class	
	Young	Old
Air Quality[b] (AQ)		
CF	2.57 ± 0.94	3.57 ± 0.94
NF	2.85 ± 0.94	3.55 ± 0.72
Fog Pretreatment[c] (FP)		
pH 2.0	2.78 ± 1.07	3.48 ± 0.68
pH 3.0	2.86 ± 0.89	3.49 ± 0.93
pH 4.0	2.47 ± 0.91	3.73 ± 0.97
No Fog	2.75 ± 0.70	3.53 ± 0.75
AQ x FP		
CF/pH 2.0	2.55 ± 1.05	3.44 ± 0.88
NF/pH 2.0	3.00 ± 1.10	3.52 ± 0.44
CF/pH 3.0	2.76 ± 1.13	3.74 ± 1.01
NF/pH 3.0	2.95 ± 0.65	3.23 ± 0.83
CF/pH 4.0	2.27 ± 0.80	3.59 ± 0.99
NF/pH 4.0	2.66 ± 1.02	3.87 ± 1.01
CF/No Fog	2.71 ± 0.84	3.51 ± 1.02
NF/No Fog	2.79 ± 0.58	3.56 ± 0.40
ANOVA Effect[d]		
AQ	NS	NS
FP	NS	NS
AQ x FP	NS	NS
C.V. (%)[e]	23.7	23.4

[a]Mean ± one standard deviation in mg g^{-1} dry weight. Sample sizes of 32, 16, and 8 were used to examine the AQ, FP, and AQ x FP effects, respectively.
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]Fog treatment applied in the Spring Fog Exposure Study.
[d]Results of the ANOVA; NS = not significant at $p<0.05$.
[e]C.V. = coefficient of variation.

Table 20. Statistical Analysis of the Effects of Ozone on K^+ Leakage from Needle Segments of Ponderosa Pine Pretreated with Acidic Fog[a]

Treatment Variable	Needle Age Class: Young	Needle Age Class: Old
Air Quality[b] (AQ)		
CF	5654 ± 1500	3850 ± 907
NF	7072 ± 1960	5028 ± 1310
L.S.D.[c]	1059	699
Fog Pretreatment[d] (FP)		
pH 2.0	5649 ± 952	4371 ± 1270
pH 3.0	6177 ± 1780	3993 ± 975
pH 4.0	6932 ± 1970	4434 ± 142
No Fog	6692 ± 2470	4959 ± 134
AQ/FP		
CF/pH 2.0	5910 ± 1130	3926 ± 1310
NF/pH 2.0	5388 ± 769	4815 ± 1190
CF/pH 3.0	5093 ± 1140	3971 ± 1050
NF/pH 3.0	7262 ± 1490	4015 ± 1020
CF/pH 4.0	6535 ± 972	3456 ± 693
NF/pH 4.0	7330 ± 2720	5412 ± 1290
CF/No Fog	5078 ± 2170	4050 ± 574
NF/No Fog	8307 ± 1590	5869 ± 1290
ANOVA Effect[e]		
AQ	*	*
FP	NS	NS
AQ x FP	NS	NS
C.V. (%)	25.8	24.4

[a]Mean ± one standard deviation in μg K^+ g^{-1} dry weight. Sample sizes of 20, 10, and 5 were used to examine of the AQ, FP, and AQ x FP effects, respectively.
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).
[d]Fog treatment applied in the Spring Fog Exposure Study.
[e]Results of the ANOVA; NS = not significant at $p<0.05$, * = significant at $p<0.05$.
[f]C.V. = coefficient of variation.

(2) Young Needles

Exposure to NF air caused a statistically significant 8% increase in conductivity, and 25% increase in K^+ leakage in young needles (Tables 19 and 20, respectively). There were no significant air quality effects on needle chlorophyll content (Appendices B-15 to B-17, Table 21). The only significant air quality effect on foliar carotenoid levels was observed in September (Table 22), where plants exposed to NF air displayed 25% higher levels than CF air plants.

e. Effects of Chambers on Ponderosa Pine

The responses of NF/No Fog (i.e., exposed to NF air in open-top field chambers, and received no fog pretreatment) and outside plants were compared to examine the effects of field chambers on modifying plant growth and biochemical responses (i.e., chamber effects). In ponderosa pine, none of the growth responses differed significantly. Biochemically, significant differences were observed with respect to old needle conductivity and K^+ leakage responses (Appendix B-23). Moreover, young needle K^+ leakage, and carotenoid level in August were also found to be significantly affected. Plants in outside plots displayed lower responses than plants in NF/No Fog plots for old needle conductivity and K^+ leakage (Appendix B-23), and young needle K^+ leakage, and carotenoid level in August.

f. Assessment of Ponderosa Pine Responses to Ozone

Four months of exposure to ambient levels of O_3 did not adversely affect the growth of ponderosa pine. Moreover, there was no evidence of appreciable amounts of O_3-induced injury development or severe physiological or biochemical disruption. However, consistent with the potential for O_3 to increase membrane leakiness (Beckerson and Hofstra, 1980), needle extract conductivity and K^+ leakage increased in both needle age classes. On the other hand, greater mycorrhizal colonization occurred in plants grown in NF air compared to plants in CF air. This response been reported elsewhere in other plant species (Reich et al., 1985; Reich et al., 1986). Despite the occurrence of significant chamber effects on membrane permeability and foliar pigment responses, the lack of consistency in plant responses to O_3 make it unlikely that chamber effects altered pine responses to O_3.

Table 22. Statistical Analysis of the Effects of Ozone on the Carotenoid Content of Ponderosa Pine Needles Pretreated with Acidic Fog[a]: September 1987

Treatment Variable	Needle Age Class	
	Young	Old
Air Quality[b] (AQ)		
CF	0.24 ± 0.08	0.38 ± 0.12
NF	0.30 ± 0.09	0.43 ± 0.12
L.S.D.[c]	0.05	---
Fog Pretreatment[d] (FP)		
pH 2.0	0.26 ± 0.13	0.39 ± 0.15
pH 3.0	0.26 ± 0.11	0.41 ± 0.14
pH 4.0	0.26 ± 0.05	0.44 ± 0.09
No Fog	0.29 ± 0.06	0.39 ± 0.10
AQ x FP		
CF/pH 2.0	0.21 ± 0.04	0.34 ± 0.12
NF/pH 2.0	0.31 ± 0.17	0.43 ± 0.17
CF/pH 3.0	0.22 ± 0.12	0.38 ± 0.15
NF/pH 3.0	0.31 ± 0.07	0.44 ± 0.13
CF/pH 4.0	0.25 ± 0.06	0.41 ± 0.10
NF/pH 4.0	0.27 ± 0.04	0.46 ± 0.08
CF/No Fog	0.28 ± 0.06	0.38 ± 0.13
NF/No Fog	0.30 ± 0.07	0.39 ± 0.06
ANOVA Effect[d]		
AQ	*	NS
FP	NS	NS
AQ x FP	NS	NS
C.V. (%)[e]	29.6	24.1

[a]Mean ± one standard deviation in mg g^{-1} dry weight. Sample sizes of 32, 16, and 8 were used to examine the AQ, FP, and AQ x FP effects, respectively. Dissimilar letter designations indicate significant differences at $p<0.05$ (Duncan's New Multiple Range Test).
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).
[d]Fog treatment applied in the Spring Fog Exposure Study.
[e]Results of the ANOVA; NS = not significant at $p<0.05$, * = significant at $p<0.05$.
[f]C.V. = coefficient of variation.

4. Effects of Fog Pretreatment on Ponderosa Pine

a. Needle Injury

(1) Old Needles

More seedlings from the pH 2.0 fog pretreatment group exhibited >10% injury than from any other fog pretreatment group (Appendices A-6 to A-8).

(2) Young Needles

In June, none of the seedlings exhibited >10% injury (Appendix A-9). By July, some of the trees pretreated with pH 2.0 fog in both the CF and NF plots began to show >10% injury (Appendix A-10). In August, a few trees from the pH 3.0 fog pretreatment group displayed >10% injury as well (Appendix A-11).

b. Growth Responses

Pretreatment with fog did not induce any significant alterations in pine growth responses (Table 16). However, plants previously exposed to pH 4.0 fog tended to have lower organ dry weight responses than plants in the other fog pretreatment groups. Additionally, fog pretreatment did not induce significant alterations in stem dimension (Appendices B-13, B-14).

c. Physiological Responses

(1) Old Needles

Gas exchange process rates in old needles were not measured in the summer (owing to the greater development of the young foliage and the higher physiological activity of the young needles).

(2) Young Needles

Whole-study average gas exchange rates were not significantly affected by fog pretreatment (Table 17). Process rates tended to be slightly higher in pH 4.0 fog pretreated plants than in other fog pretreatment groups.

(3) Roots

Fog pretreatment had no significant effect on mycorrhizal colonization in pine (Table 18).

d. Biochemical Responses

(1) Old Needles

Fog pretreatment did not cause significant impacts on extract conductivity (Table 19), K^+ leakage (Table 20), chlorophyll (Appendices B-15, B-16, Table 21), carotenoid (Appendices B-18 to B-20, Table 22) or starch (Appendix B-21) responses. Foliar chlorophyll levels were significantly depressed in plants that received no fog pretreatment relative to plants in the other fog treatment groups during the month of August (Appendix B-17). In the other months, fog pretreatment had no significant impact on foliar chlorophyll responses.

(2) Young Needles

Prior exposure to acidic fog had no significant effect on extract conductivity (Table 19), K^+ leakage (Table 20), foliar chlorophyll (Appendices B-15 to B-17, Table 21) or foliar carotenoid (Appendices B-18 to B-20; Table 22) responses.

e. Assessment of Ponderosa Pine Responses to Fog Pretreatment

With one exception (i.e., old needle chlorophyll responses in August), there was no evidence of persistent fog chemistry effects on ponderosa pine. No significant injury, growth, physiological, or biochemical responses were observed, indicating that the impacts of acidic fog were not prolonged (i.e., within the context of the current growing season).

5. Interactive Effects of Ozone and Fog Pretreatment on Ponderosa Pine

There were no significant [air quality x fog pretreatment] interactive effects on growth (Table 16), stem dimension (Appendices B-13, B-14), gas exchange (Table 17), mycorrhizal colonization (Table 18), K^+ leakage (Table 20), or foliar chlorophyll (Appendices B-15 to B-17; Table 21) responses. Additionally, no significant impacts were observed for old needle carotenoid (Appendices B-18 to B-20; Table 22) and starch (Appendix B-21) responses, or young needle carotenoid responses in selected months (Appendices B-18, B-19; Table 22).

a. Old Needles

In plants that received either pH 4.0 or no fog pretreatment, needle extract conductivity was 34 or 51% higher in NF air than in CF air, respectively (significant [AQ x FP] effect, Table 19).

b. Young Needles

Similar to the air quality impacts on old needles, seedlings grown in NF air that had been previously pretreated with pH 3.0 fog exhibited significantly higher (i.e., 33%) needle extract conductivity (Table 19) than seedlings in CF air. In August, fog pretreated plants exhibited higher carotenoid levels when grown in CF air than in NF air, but the opposite response was found for unfogged plants (Appendix B-20).

c. Assessment of Ponderosa Pine Responses to Ozone and Fog Pretreatment in Combination

The effects of O_3 and fog pretreatment in combination were not significant for essentially all the parameters monitored. Only two significant effects were observed, which have no obvious functional interrelationship. No consistent trend in plant response to the combined stress treatment was observed. There does not appear to be a clearly distinguishable interactive effect of O_3 and fog pretreatment on ponderosa pine seedlings.

6. Effects of Ozone on White Fir

a. Needle Injury

(1) Old Needles

Because the injury data were highly skewed, no statistical analyses of the data were performed. Instead, histograms of the distribution of injury amounts were prepared (Appendices A-12 to A-17). No clear trends in injury development due to air quality were evident (Appendices A-12 to A-14).

(2) Young Needles

Most of the trees showed very little injury during the Summer Ozone Exposure Study (Appendices A-15 to A-17).

b. Growth Responses

Seedlings grown in NF air had significantly higher stem and needle dry weights (i.e., 24 and 23%, respectively) than plants grown in CF air (Table 23). Root dry weight was also greater in NF air grown plants than in CF air plants (i.e., 10% greater) but the difference was not statistically significant. Although stem height was not affected by air quality (Appendix B-24), plants exposed to NF air had larger stem diameters than plants grown in CF air during the months of June, July, and August (Table 24).

Table 23. Statistical Analysis of the Effects of Ozone on the Growth of White Fir Pretreated with Acidic Fog[a]

Treatment Variable	Dry Weight (g)		
	Root	Stem	Needles
Air Quality[b] (AQ)			
CF	22.04 (10.70)	9.97 (4.37)	8.22 (4.43)
NF	24.33 (8.68)	12.32 (4.69)	10.07 (4.37)
L.S.D.[c]	---	1.18	1.31
Fog Pretreatment[d] (FP)			
pH 2.0	21.04 (8.69)	10.34 (3.86)	8.74 (5.27)
pH 3.0	24.62 (10.20)	12.06 (4.68)	10.18 (4.60)
pH 4.0	23.19 (11.60)	10.07 (4.02)	8.26 (3.54)
No Fog	23.89 (8.50)	12.11 (5.75)	9.42 (4.37)
AQ x FP			
CF/pH 2.0	19.44 (8.66)	9.71 (3.80)	7.45 (4.91)
NF/pH 2.0	22.64 (8.80)	10.96 (3.99)	10.02 (5.50)
CF/pH 3.0	23.77 (11.60)	10.93 (5.77)	9.98 (5.30)
NF/pH 3.0	25.47 (8.87)	13.20 (3.11)	10.37 (4.02)
CF/pH 4.0	22.79 (14.50)	8.73 (3.83)	7.25 (3.69)
NF/pH 4.0	23.59 (8.39)	11.42 (3.89)	9.26 (3.22)
CF/No Fog	22.15 (7.54)	10.52 (4.00)	8.21 (3.61)
NF/No Fog	25.63 (9.39)	13.70 (6.89)	10.63 (4.88)
ANOVA Effect[e]			
AQ	NS	***	**
FP	NS	NS	NS
AQ x FP	NS	NS	NS
C.V. (%)[f]	44.3	44.4	50.6

[a]Mean (± one standard deviation). Sample sizes of 48, 24, and 12 were used to examine the AQ, FP, and AQ x FP effects, respectively.
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).
[d]Fog treatment applied in the Spring Fog Exposure Study.
[e]Results of the ANOVA; NS = not significant at $p<0.05$, ** = significant at $p<0.01$, *** = significant at $p<0.001$.
[f]C.V. = coefficient of variation.

Table 24. Statistical Analysis of the Effects of Ozone on Stem Diameter of White Fir Pretreated with Acidic Fog[a]

Treatment Variable	Month of Sampling			
	June	July	August	September
Air Quality[b] (AQ)				
CF	8.7 (1.5)	9.4 (1.6)	9.7 (1.7)	9.9 (1.5)
NF	9.7 (1.6)	10.2 (1.6)	10.6 (1.5)	10.5 (1.4)
L.S.D.[c]	0.7	0.6	0.7	---
Fog Pretreatment[d] (FP)				
pH 2.0	9.1 (1.6)	9.7 (1.8)	10.1 (1.8)	10.1 (1.3)
pH 3.0	9.5 (1.6)	9.9 (1.5)	10.4 (1.6)	10.5 (1.6)
pH 4.0	9.0 (1.8)	9.5 (1.5)	9.9 (1.5)	9.8 (1.3)
No Fog	9.2 (1.7)	10.1 (1.8)	10.3 (1.8)	10.4 (1.7)
AQ x FP				
CF/pH 2.0	8.7 (1.3)	9.3 (1.7)	9.7 (1.9)	10.0 (1.3)
NF/pH 2.0	9.6 (1.9)	10.1 (1.8)	10.5 (1.6)	10.3 (1.4)
CF/pH 3.0	9.1 (1.9)	9.9 (1.7)	10.1 (1.8)	10.3 (1.9)
NF/pH 3.0	9.8 (1.3)	9.9 (1.4)	10.8 (1.3)	10.6 (1.3)
CF/pH 4.0	8.4 (1.3)	8.9 (1.5)	9.3 (1.4)	9.3 (1.3)
NF/pH 4.0	9.6 (1.6)	10.1 (1.3)	10.5 (1.4)	10.4 (1.0)
CF/No Fog	8.7 (1.4)	9.6 (1.5)	9.9 (1.7)	10.0 (1.5)
NF/No Fog	9.7 (1.8)	10.6 (1.9)	10.7 (2.0)	10.7 (2.0)
ANOVA Effect[e]				
AQ	*	*	*	NS
FP	NS	NS	NS	NS
AQ x FP	NS	NS	NS	NS
C.V. (%)[f]	17.5	16.6	16.8	15.1

[a]Mean (± one standard deviation) in mm. Sample sizes of 48, 24, and 12 were used to examine the AQ, FP, and AQ x FP effects, respectively.
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).
[d]Fog treatment applied in the Spring Fog Exposure Study.
[e]Results of the ANOVA; NS = not significant at $p<0.05$, * = significant at $p<0.05$.
[f]C.V. = coefficient of variation.

c. Physiological Responses

(1) Old Needles

Gas exchange process rates were not measured in old needles during the Summer Ozone Exposure Study.

(2) Young Needles

Air quality had no significant impacts on whole-study average gas exchange responses in the summer (Table 25).

(3) Roots

Air quality had no significant impact on mycorrhizal colonization (Appendix B-25).

d. Biochemical Responses

(1) Old Needles

Air quality impacts on fir needle extract conductivity (Table 26), K^+ leakage (Table 27), foliar chlorophyll (Appendices B-26, B-27; Tables 28, 29), foliar carotenoid (Appendices B-28 to B-30; Table 30), and starch (Appendix B-31) responses were not significant.

(2) Young Needles

Needle extract conductivities of plants grown in NF air were significantly lower (i.e., 13%) than plants grown in CF air (Table 26). Similarly, K^+ leakage responses were decreased significantly (by 17%) in NF air plants compared with CF air plants (Table 27). There were no significant air quality effects on young needle chlorophyll or carotenoid contents in any of the four months of O_3 exposures (Appendices B-26 through B-30).

e. Effects of Chambers on White Fir

In white fir, there were no significant chamber effects with respect to growth responses (Appendix B-32). However, ten of twenty-one biochemical responses were found to differ significantly (Appendix B-33). In old needles, conductivity of needle extracts were higher in NF/No Fog plants than in outside plants, and chlorophyll levels were lower in July, August, and September. Total carotenoid responses in old needles were higher in NF/No Fog plants in August, but lower than outside plants in September. In young needles, conductivity of needle extract and K^+ leakage responses were higher in NF/No Fog plants than in outside plants (Appendix B-33). On the other hand, chlorophyll responses were not consistent; in June, levels were higher in NF/No Fog plants, but in August, outside plants displayed higher levels.

Table 25. Statistical Analysis of the Effects of Ozone on the Gas Exchange Rates of White Fir Pretreated with Acidic Fog[a]

Treatment Variable	Transpiration (mg H_2O m^{-2} s^{-1})	Stomatal Conductance (cm s^{-1})	Net Photosynthesis (mg CO_2 m^{-2} s^{-1})
Air Quality[b] (AQ)			
CF	19.4 (10.4)	0.066 (0.04)	0.076 (0.04)
NF	18.4 (10.6)	0.066 (0.04)	0.076 (0.04)
Fog Pretreatment[c] (FP)			
pH 2.0	20.6 (9.4)	0.072 (0.04)	0.083 (0.04)
pH 3.0	18.5 (11.7)	0.063 (0.04)	0.073 (0.04)
pH 4.0	17.8 (9.7)	0.061 (0.04)	0.073 (0.04)
No Fog	18.6 (11.0)	0.068 (0.05)	0.075 (0.04)
AQ x FP			
CF/pH 2.0	19.4 (8.7)	0.068 (0.04)	0.075 (0.03)
NF/pH 2.0	21.8 (10.0)	0.076 (0.04)	0.090 (0.04)
CF/pH 3.0	20.9 (12.5)	0.068 (0.04)	0.078 (0.04)
NF/pH 3.0	16.1 (10.4)	0.057 (0.04)	0.069 (0.04)
CF/pH 4.0	17.4 (8.9)	0.059 (0.03)	0.069 (0.04)
NF/pH 4.0	18.2 (10.5)	0.063 (0.04)	0.069 (0.04)
CF/No Fog	19.7 (11.1)	0.067 (0.04)	0.080 (0.04)
NF/No Fog	17.6 (10.8)	0.068 (0.05)	0.069 (0.04)
ANOVA Effect[d]			
AQ	NS	NS	NS
FP	NS	NS	NS
AQ x FP	NS	NS	NS
C.V. (%)[e]	38.9	44.0	43.8

[a]Mean (± one standard deviation). Sample sizes of 256, 128, and 64 were used to examine the AQ, FP, and AQ x FP effects, respectively.
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]Fog treatment applied in the Spring Fog Exposure Study.
[d]Results of the ANOVA; NS = not significant at $p<0.05$.
[e]C.V. = coefficient of variation.

Table 26. Statistical Analysis of the Effects of Ozone on the Conductivity of Needle Segment Extracts of White Fir Pretreated with Acidic Fog[a]

Treatment Variable	Needle Age Class	
	Young	Old
Air Quality[b] (AQ)		
CF	2148 ± 289	1392 ± 247
NF	1900 ± 289	1353 ± 268
L.S.D.[c]	117	---
Fog Pretreatment[d] (FP		
pH 2.0	2042 ± 488 y	1391 ± 132 y
pH 3.0	2105 ± 133 y	1298 ± 186 y
pH 4.0	1792 ± 179 z	1113 ± 74 z
No Fog	2158 ± 214 y	1686 ± 184 x
L.S.D.	165	121
AQ/FP		
CF/pH 2.0	2473 ± 254 w	1364 ± 170 y
NF/pH2.0	1611 ± 84 z	1418 ± 91 y
CF/pH 3.0	2161 ± 128 x	1452 ± 100 y
NF/pH 3.0	2048 ± 125 xy	1144 ± 90 z
CF/pH 4.0	1854 ± 182 yz	1079 ± 65 z
NF/pH 4.0	1730 ± 172 z	1148 ± 71 z
CF/No Fog	2104 ± 200 x	1671 ± 143 x
NF/No Fog	2211 ± 236 x	1702 ± 235 x
L.S.D.	233	171
Anova Effect[e]		
AQ	***	NS
FP	***	***
AQ x FP	***	**
C.V. (%)[f]	8.9	9.7

[a]Mean ± one standard deviation in µmhos cm^{-1} g^{-1} dry weight. Sample sizes of 20, 10, and 5 were used to examine the AQ, FP, and AQ x FP effects, respectively. Dissimilar letter designations for each needle age class separately, indicate significant differences at $p<0.05$ (Duncan's New Multiple Range Test).

[b]CF = charcoal-filtered air; NF = nonfiltered air.

[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).

[d]Fog treatment applied in the Spring Fog Exposure Study.

[e]Results of the ANOVA; NS - not significant at $p<0.05$, ** = significant at $p<0.01$, *** = significant at $p<0.001$.

[f]C.V. = coefficient of variaiton.

Table 27. Statistical Analysis of the Effects of Ozone on K^+ Leakage from Needle Segments of White Fir Pretreated with Acidic Fog[a]

Treatment Variable	Needle Age Class: Young		Needle Age Class: Old	
Air Quality[b] (AQ)				
CF	13298 ± 1790		9587 ± 1420	
NF	11350 ± 1370		9418 ± 1130	
L.S.D.[c]	560		---	
Fog Pretreatment[d] (FP)				
pH 2.0	13370 ± 2770	x	10452 ± 1040	x
pH 3.0	11258 ± 1450	z	9287 ± 1200	y
pH 4.0	12105 ± 179	y	8265 ± 844	z
No Fog	12563 ± 1110	y	10006 ± 837	xy
L.S.D.	792		837	
AQ/FP				
CF/pH 2.0	15914 ± 982	v	10838 ± 664	w
NF/pH 2.0	10826 ± 319	yz	10066 ± 1270	wxy
CF/pH 3.0	12237 ± 919	wx	9673 ± 248	wxy
NF/pH 3.0	10278 ± 1220	z	8901 ± 1670	y
CF/pH 4.0	13049 ± 507	w	7568 ± 373	z
NF/pH 4.0	11160 ± 677	xyz	8962 ± 497	xy
CF/No Fog	11992 ± 954	wxy	10268 ± 1130	wx
NF/No Fog	13134 ± 1010	w	9743 ± 443	wxy
L.S.D.	1120		1183	
ANOVA Effect[f]				
AQ	***		NS	
FP	***		***	
AQ x FP	***		*	
C.V. (%)	7.1		9.7	

[a]Mean (± one standard deviation) in µg K^+ g^{-1} dry weight. Sample sizes of 20, 10, and 5 were used to examine the AQ, FP, and AQ x FP effects, respectively. Dissimilar letter designations for each needle age class separately, indicate statistically significant differences at $p<0.05$ (Duncan's New Multiple Range Test).

[b]CF = charcoal filtered air; NF = nonfiltered air.

[c]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).

[d]Fog treatment applied in the Spring Fog Exposure Study.

[e]Results of the ANOVA; NS = not significant at $p<0.05$, * = significant at $p<0.05$, *** = significant at $p<0.001$.

[f]C.V. = coefficient of variation.

Table 28. Statistical Analysis of the Effects of Ozone on the Chlorophyll Content of White Fir Needles Pretreated with Acidic Fog[a]: June 1987

Treatment Variable	Needle Age Class	
	Young	Old
Air Quality[b] (AQ)		
CF	1.90 ± 0.61	4.15 ± 0.76
NF	1.63 ± 0.38	4.38 ± 0.80
Fog Pretreatment[c] (FP)		
pH 2.0	1.99 ± 0.26 y	4.39 ± 0.52
pH 3.0	1.88 ± 0.75 y	4.01 ± 1.06
pH 4.0	1.27 ± 0.27 z	4.42 ± 0.84
No Fog	1.93 ± 0.31 y	4.25 ± 0.60
L.S.D.[d]	0.51	---
AQ x FP		
CF/pH 2.0	2.00 ± 0.31	4.20 ± 0.50 yz
NF/pH 2.0	1.97 ± 0.23	4.58 ± 0.49 y
CF/pH 3.0	2.12 ± 0.98	3.29 ± 0.78 z
NF/pH 3.0	1.63 ± 0.31	4.73 ± 0.80 y
CF/pH 4.0	1.35 ± 0.31	4.43 ± 0.43 y
NF/pH 4.0	1.19 ± 0.21	4.38 ± 1.16 y
CF/No Fog	2.13 ± 0.17	4.68 ± 0.52 y
NF/No Fog	1.73 ± 0.30	3.83 ± 0.30 yz
L.S.D.	---	1.41
ANOVA Effect[e]		
AQ	NS	NS
FP	*	NS
AQ x FP	NS	*
C.V. (%)[f]	12.7	9.3

[a]Mean ± one standard deviation in mg g^{-1} dry weight. Sample sizes of 32, 16, and 8 were used to examine the significance of the AQ, FP, and AQ x FP effects, repsectively. Means followed by dissimilar letter designations indicate statistically significant differences at $p < 0.05$ using Duncan's New Multiple Range Test.

[b]CF = charcoal-filtered air; NF = nonfiltered air.

[c]Fog treatment applied in the Spring Fog Exposure Study.

[d]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).

[e]Results of the ANOVA; NS = not significant at $p < 0.05$, * = significant at $p < 0.05$.

Table 29. Statistical Analysis of the Effects of Ozone on the Chlorophyll Content of White Fir Needles Pretreated with Acidic Fog[a]: September 1987

Treatment Variable	Needle Age Class	
	Young	Old
Air Quality[b] (AQ)		
CF	2.77 ± 0.95	3.79 ± 0.96
NF	2.67 ± 0.99	3.99 ± 0.91
Fog Pretreatment[c] (FP)		
pH 2.0	2.85 ± 1.06	4.01 ± 0.84
pH 3.0	2.72 ± 0.86	3.83 ± 1.17
pH 4.0	2.73 ± 1.20	3.99 ± 0.55
No Fog	2.58 ± 0.74	3.74 ± 1.10
AQ x FP		
CF/pH 2.0	2.75 ± 0.93	3.86 ± 0.85 xyz
NF/pH 2.0	2.94 ± 1.23	4.17 ± 0.84 xy
CF/pH 3.0	2.57 ± 0.55	3.15 ± 0.58 z
NF/pH 3.0	2.87 ± 1.11	4.50 ± 1.24 x
CF/pH 4.0	2.96 ± 1.57	4.14 ± 0.55 xy
NF/pH 4.0	2.49 ± 0.71	3.83 ± 0.55 xyz
CF/No Fog	2.80 ± 0.53	4.00 ± 1.42 xyz
NF/No Fog	2.35 ± 0.88	3.48 ± 0.65 yz
L.S.D.[d]	---	0.81
ANOVA Effect[e]		
AQ	NS	NS
FP	NS	NS
AQ x FP	NS	*
C.V. (%)[f]	41.2	23.4

[a]Mean ± one standard deviation in mg g^{-1} dry weight. Sample sizes of 32, 16, and 8 were used to examine the AQ, FP, and AQ x FP effects, respectively. Dissimilar letter designations indicate significant differences at $p<0.05$ (Duncan's New Multiple Range Test).

[b]CF = charcoal-filtered air; NF = nonfiltered air.

[c]Fog treatment applied in the Spring Fog Exposure Study.

[d]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).

[e]Results of the ANOVA; NS = not significant at $p<0.05$, * = significant at $p<0.05$.

[f]C.V. = coefficient of variation.

Table 30. Statistical Analysis of the Effects of Ozone on the Carotenoid Content of White Fir Needles Pretreated with Acidic Fog[a]: June 1987

Treatment Variable	Needle Age Class	
	Young	Old
<u>Air Quality</u>[b] (AQ)		
CF	0.35 ± 0.07	0.48 ± 0.14
NF	0.33 ± 0.10	0.50 ± 0.11
<u>Fog Pretreatment</u>[c] (FP)		
pH 2.0	0.38 ± 0.11	0.55 ± 0.08 y
pH 3.0	0.32 ± 0.09	0.42 ± 0.13 z
pH 4.0	0.30 ± 0.05	0.45 ± 0.12 z
No Fog	0.35 ± 0.06	0.54 ± 0.12 y
L.S.D.[d]	---	0.06
<u>AQ x FP</u>		
CF/pH 2.0	0.36 ± 0.04	0.53 ± 0.10
NF/pH 2.0	0.40 ± 0.15	0.57 ± 0.05
CF/pH 3.0	0.35 ± 0.10	0.38 ± 0.13
NF/pH 3.0	0.29 ± 0.08	0.46 ± 0.13
CF/pH 4.0	0.29 ± 0.05	0.44 ± 0.10
NF/pH 4.0	0.31 ± 0.04	0.46 ± 0.15
CF/No Fog	0.38 ± 0.03	0.56 ± 0.16
NF/No Fog	0.32 ± 0.07	0.53 ± 0.06
<u>ANOVA Effect</u>[e]		
AQ	NS	NS
FP	NS	**
AQ x FP	NS	NS
C.V. (%)[f]	28.0	25.9

[a]Mean ± one standard deviation in mg g^{-1} dry weight. Sample sizes of 32, 16, and 8 were used to examine the AQ, FP, and AQ x FP effects, respectively. Dissimilar letter designations indicate significant differences at $p < 0.05$ (Duncan's New Multiple Range Test).
[b]CF = charcoal-filtered air; NF = nonfiltered air.
[c]Fog treatment applied in the Spring Fog Exposure Study.
[d]L.S.D. = least significant difference between a pair of means in the set (2-sample t-test, 5% level).
[e]Results of the ANOVA; NS = not significant at $p < 0.05$, ** = significant at $p < 0.01$.
[f]C.V. = coefficient of variation.

f. Assessment of White Fir Responses to Ozone

Although injury responses were not affected by O_3 exposures in white fir, significant increases in stem and needle dry weight were found in plants grown in NF air compared to those grown in CF air. The increase in stem dry weight correlated closely with a significant increase in stem diameter. Biochemically, some support for the observed growth responses is found in membrane permeability responses, where NF plants exhibited lower conductivity and K^+ leakage responses. The disruption of membrane integrity evidenced by the changes in total ion and K^+ efflux may be reflective of incipient changes at the cellular level of organization that were coincident with impacts on growth in white fir.

White fir seedlings appeared to be sensitive to chamber effects which could confound assessments of O_3 impacts. Plants in outside plots exhibited significantly lower conductivity and higher chlorophyll responses than NF/No Fog plants. Thus, enclosing plants in chambers appears to deleteriously impact white fir biochemistry, and could increase the apparent phytotoxic impacts of O_3.

7. Effects of Fog Pretreatment on White Fir

a. Needle Injury

(1) Old Needles

In June, the trees pretreated with pH 2.0 fog were most severely injured (Appendix A-12). By July, the number of trees with >10% injury was also increasing in the pH 3.0 fog group as well (Appendix A-13). In August, seedlings with >10% injury were found in almost all treatment groups (Appendix A-14).

(2) Young Needles

There was a trend toward greater numbers of trees with >10% injury in the pH 2.0 and 3.0 fog pretreatments, however, no obvious differences between the NF and CF trees were observed (Appendices A-15 to A-17).

b. Growth Responses

There were no significant fog pretreatment effects observed on growth (Table 23) or stem dimension (Appendix B-24; Table 24) responses.

c. Physiological Responses

(1) Old Needles

Gas exchange rates in old needles were not measured during the summer.

(2) Young Needles

Gas exchange rates were not significantly affected by fog pretreatment (Table 25).

(3) Roots

Fog pretreatment had no significant effect on mycorrhizal colonization (Appendix B-25).

d. Biochemical Responses

(1) Old Needles

Fog pretreatment had a significant effect on needle extract conductivity and K^+ leakage in old needles of white fir (Tables 26; 27). Seedlings pretreated with pH 4.0 fog displayed the lowest conductivity and K^+ leakage responses, whereas unfogged plants tended to exhibit the highest values for both parameters. Pretreatment with acidic fog did not induce any significant alterations in foliar chlorophyll (Appendices B-26, B-27; Table 28,29) or starch (Appendix B-31). Foliar carotenoid levels were significantly higher in seedlings pretreated with pH 2.0 fog relative to levels in plants previously exposed to pH 3.0 or 4.0 fog in June (Table 30), but differences among fog pretreatment groups were not significant in the other three months of monitoring (Appendices B-28 to B-30).

(2) Young Needles

Needle extract conductivity was significantly affected by fog pretreatment (Table 26). Seedlings previously exposed to pH 4.0 fog exhibited the lowest conductivity relative to plants in the other fog pretreatment groups. The impacts on conductivity were not fully explained by alterations in K^+ leakage, however, as the loss of K^+ was greatest in pH 2.0 fog pretreated plants, and lowest in pH 3.0 plants (Table 27). In June, the seedlings previously exposed to pH 4.0 fog exhibited significantly lower chlorophyll levels than plants in the other fog pretreatment groups (Table 28). In the other months, no significant treatment effects were observed (Appendices B-26, B-27; Table 29). Moreover, no significant fog pretreatment effects on foliar carotenoid content occurred (Table 30; Appendices B-28 to B-30).

e. <u>Assessment of White Fir Responses to Fog Pretreatment</u>

Aside from injury responses in both needle age classes, fog pretreatment had only a slight impact on white fir. Plants pretreated with pH 2.0 fog exhibited a tendency toward greater injury than plants in the other fog pretreament groups, but differences in injury amounts tended to decrease with time. Temporally, the incidence of higher injury amounts in early summer could have long-term impacts insofar as whole-plant productivity. Despite the fact that physiological process rates are low during the summer, the photosynthate produced by uninjured tissues in early summer could provide added resources for O_3 detoxification and storage that could not be provided by fog-damaged needles. Relative to biochemical responses, seedlings exposed to pH 2.0 fog displayed higher carotenoid levels than pH 4.0 fog-exposed plants, however, this response did not parallel fog pretreatment effects on needle extract conductivity which was reduced by pH 2.0 fog exposure compared to pH 4.0 fog exposure. There was no evidence of fog pretreatment effects on growth, gas exchange, mycorrhizal infection, leaf chlorophyll or starch responses. Thus, similar to ponderosa pine, there does not appear to be extended impacts of fog on white fir.

8. <u>Interactive Effects of Ozone and Fog Pretreatment on White Fir</u>

There were no significant [air quality x fog pretreatment] interactive effects on growth (Table 23), stem dimension (Appendix B-24; Table 24) gas exchange (Table 25), mycorrhizal colonization (Appendix B-25), foliar carotenoid (Appendices B-28 to B-30; Table 30) or needle starch (Appendix B-31) responses.

a. <u>Old Needles</u>

Needle extract conductivities were significantly higher in plants previously exposed to pH 3.0 fog in CF air than in plants grown in NF air (Table 26). This response was not coincident with similar trends in K^+ leakage responses, but the same tendency was observed in plants pretreated with pH 4.0 fog (Table 27). In June, July, and September, plants previously exposed to pH 3.0 fog exhibited chlorophyll levels that were significantly higher (<u>i.e.</u>, 43 to 46%) in NF air than in CF air (Tables 28, 29; Appendix B-26). The only other significant [air quality x fog pretreatment] interactive effect was in plants pre-treated with pH 2.0 fog in July, where those grown in NF air had 31% higher chlorophyll levels than those in CF air (Appendix B-26).

b. Young Needles

In plants pretreated with pH 2.0 fog, needle extract conductivity was significantly higher by 54% in CF air plants relative to NF air plants (Table 26). A tendency toward higher conductivities in the CF air plants was observed in all acidic fog treatments. Similar trends of higher membrane permeability in CF air plants were correlated to changes in K^+ leakage (Table 27).

c. Assessment of White Fir Responses to Ozone and Fog Pretreatment In Combination

The combined effects of acidic fog pretreatment and ambient O_3 were not significant with respect to white fir injury, growth, gas exchange, mycorrhizal colonization, leaf carotenoid, or starch responses. However, significant effects were observed insofar as membrane permeability impacts, where old needles pretreated with pH 3.0 fog were found to have higher conductivity and lower chlorophyll levels when grown in CF air than comparably fog-pretreated plants grown in NF air. Relative to conductivity responses in young needles, similar responses were exhibited by seedlings pretreated with pH 2.0 fog (i.e., higher in CF than NF air). Moreover, K^+ leakage from young needles was consistently lower for the NF air plants than for the NF plants across all the acidic fog pretreatments. However, overall, no clear trend was identifiable given the scarcity and lack of consistency in plant responses to O_3 in combination with fog pretreatment.

9. Assessment of Coniferous Tree Seedling Responses to Acidic Fog in the Spring

Multiple acidic fog exposures did not cause significant alterations in any measures of growth, in either tree species. Despite the occurrence of visible injury in new needles, the reduction of the total plant biomass was slight since new needle biomass comprised a small portion of the whole-tree seedling. In comparison, acidic rain applications have been shown to increase white pine growth (Reich et al., 1987), but acidic mists were reported to have no effect on Scots pine growth (Skeffington and Roberts, 1985).

In the present investigation, highly acidic fog exposures (i.e., pH 2.0) caused foliar injury in young and old needles of ponderosa pine and white fir. Of the two tree species, white fir was more sensitive to acidic fog induced injury, but damage to foliage was not coincident with alterations in physiological, biochemical, or growth responses in either species. While both tree species intercepted fog equally on a needle area basis (Table 4), inherent leaf surface buffering capacities were ineffective insofar as neutralizing acid inputs from pH 2.0 fog. On leaf surfaces, pH decreases with time have also been observed in herbaceous crops exposed to pH 2.5 rain (Adams and Hutchinson, 1984), where the rate of droplet evaporation was suggested to be faster than the rate of acid neutralization. Thus, plant surface buffering capacities only appeared to be exceeded, as the neutralization of acid inputs was actually taking place at a faster rate than on surfaces exposed to less acidic rain treatments.

Differences in plant sensitivity to fog-induced injury are likely to have a similar basis to injury caused by acidic rain. Structurally, factors that increase foliar wettability, increase internal leaf cell exposure to acids, and favor wet-deposited particle retention are of prime importance with respect to a plant's ability to neutralize acidic depositions (Shriner and Johnston, 1986). Moreover, surface buffering capacities are also known to be influenced by wax and trichome development (Capron and Hutchinson, 1986), and possibly by the presence of surface-deposited salts and exchangeable cation pools from the leaf interior (Adams and Hutchinson, 1984). In view of this, the sensitivity of young white fir needles to acidic fog-induced injury may be analogous to the sensitivity of cabbage cotyledons to acid rain injury (Capron and Hutchinson, 1986). Specifically, poor development of surface waxes was hypothesized to be the primary reason for the high incidence of injury to cabbage cotyledons compared to true leaves. Wax erosion by acidic rain has been demonstrated in other plant species (Baker and Hunt, 1986), and may also be relevant with respect to the effects of acidic fog on conifers. Alternatively, consideration of the functional status of internal ion exchange capacities, and the loss of surface neutralizing substances by leaching is also warranted.

Although pine injury development from pH 2.0 fog was less severe than in white fir, distinct increases in membrane permeability indicated that incipient subcellular changes had occurred. The most evident impact of acidic fog on the biochemical responses of ponderosa pine was the alteration of membrane permeability responses (Table 8). While white fir was not significantly affected, both pine needle age classes exhibited increased K^+ leakage, and total conductivity in young needles was also significantly increased. Acid rain and mist exposures have been reported to increase the rate of foliar leaching (Evans, 1984; Jacobson, 1984; Scherbatskoy and Klein, 1983), but whether the substances leached were from leaf surfaces or leaf cell interiors has not been clearly defined. Inasmuch as both surfaces and cells provide inorganic and organic chemical inputs to leachates, the contribution of materials from cells is indicative of substances being removed from the cell vacuole or cytoplasm. Conceivably, the leaching of surface salts by acidic fog may precede material losses from leaf cell interiors. H^+ inputs from subsequent fog exposures could possibly weaken cell membranes leading to the disruption of membrane integrity and the inability to regulate ion fluxes.

Fog at pH 2.0 had no significant effects on young needle pigment levels of both species (Tables 9, 14). In contrast, foliar pigment levels in old pine needles were significantly increased by exposure to pH 2.0 fog, despite the fact that injury amounts were slight (Appendix B-3). In comparison, acidic fog effects on foliar pigment levels in crops have been found to be variable (Olszyk et al., 1987). Nevertheless, there was no evidence of a relationship between leaf pigment concentration and injury development in the two tree species examined in the present study.

The depression of photosynthesis was consistently (but not significantly) observed in ponderosa pine exposed to pH 2.0 fog (Appendix B-5), and in white fir exposed to pH 2.0 and 3.0 fog (Appendix B-9) on individual sampling days. The range in response (as influenced by sampling date and plant age) contributed greatly to a lack of a significant acidic fog treatment effect on whole-study average gas exchange rates (Tables 7, 12).

Gas exchange rates in conifers are as much as two orders of magnitude lower than those of crops (Kramer and Kozlowski, 1979). Since the average process rates observed in the present study were low (in an absolute sense), the impact of acidic fog may not have been discernible because of natural plant-to-plant variability. In other words, the extent to which acidic fog altered gas exchange rates of ponderosa pine and white fir was of similar magnitude to the inherent variability in plant gas exchange responses. Thus, the severity of the applied fog stress may not have been great enough to induce a statistically substantive alteration. In red spruce, pH 3.6 mist did not significantly alter photosynthesis or transpiration (Taylor et al., 1986), and pH 3.0 rain treatments enhanced photosynthesis in white pine (Reich et al., 1987). Broadleaf tree photosynthesis has been reported to be unaffected by pH 3.0 rain applications (Reich et al., 1986), but significantly inhibited by pH 2.0 rain (Neufeld et al., 1985). While acidic fog treatments did not significantly alter photosynthesis on a leaf area basis in either tree species in the spring (Tables 7, 12), the starch content of old white fir needles was significantly lowered by pH 2.0 fog (Table 14). The enhanced depletion of carbohydrate reserves (in the absence of marked photosynthetic depressions) suggests that fog treatment may alter carbon allocation patterns in coniferous tree foliage by increasing energy demands for repair and detoxification processes (Taylor et al., 1986; McLaughlin and Shriner, 1980). Moreover, the reduced availability of needle starch reserves could have a pronounced effect in subsequent flush periods, when storage carbohydrates are mobilized for new tissue growth.

Acidic rain has been shown to detrimentally affect mycorrhizal infection in a number of tree species (Reich et al., 1985, Reich et al., 1986; Shafer et al., 1985; Stroo and Alexander, 1985). Since the time between mycorrhizal spore inoculation and the post-fog tree harvest was short, the alterations in soil chemistry or plant vitality that are thought to precede effects on mycorrhizal infection may not have occurred, and the impacts of acidic fog on this important plant root/rhizosphere interaction could not be reliably examined. The total loading of acidic inputs from fog was low in the present study (Tables 2, 4), and probably could not bring about significant changes in the soil pH or ion composition.

10. Assessment of Coniferous Tree Seedling Responses to Ozone in the Summer

Ozone exposures during the summer had no significant effects on the growth responses of ponderosa pine, but white fir seedlings grown in NF air exhibited increased needle and stem dry weight (Table 23), and stem diameter (Table 24) compared to seedlings grown in CF air. At this stage of development, young needles may require more photosynthates for growth than they are able to produce via photosynthesis (Kramer and Kozlowski, 1979). Thus, carbohydrate allocations from older needles (which may be the primary source of photoassimilates) or from non-photosynthetic storage tissues may be required for the development of new needles. In this regard, the reduction of living new needle tissue might reduce the demand for photoassimilates from old needles, and so may not have a profound impact on growth in the present season. However, if new needle growth is inhibited in several subsequent seasons, detectable changes may occur. In other studies on conifer seedlings, O_3 has been reported to decrease radial increment growth in Jeffrey pine (Peterson et al., 1987), reduce slash pine stem dimension and organ dry weight (Hogsett et al., 1985), but have no significant effect on white pine (Reich et al., 1987) or Scots pine (Skeffington and Roberts, 1985).

The greater organ dry weights of white fir grown in NF air paralleled lowered membrane permeability. While no other measured biochemical parameter was consistently altered by ambient O_3, the co-occurrence of these effects indicates that white fir may be more responsive to the effects of O_3 than ponderosa pine. Although the enhancement of plant responses by O_3 is not a common phenomenon, other workers have reported similar findings in other plant species (Bennett et al., 1974; Rajput and Ormrod, 1986). Relative to this, the increased deposition of airborne particulates (i.e., SO_4^{2-}, NO_3^-, NH_4^+) to plants in NF air plots compared to CF air plots has been found to be an important consideration along with O_3 air quality in field studies using open-top chambers (Bytnerowicz and Olszyk, 1987).

Exposure to O_3 has been shown to alter membrane permeability (Beckerson and Hofstra, 1980; Elkiey and Ormrod, 1979; Evans and Ting, 1973), leading to increased electrolyte leakage. In the present study, exposure to ambient O_3 increased ion leakage in ponderosa pine, but decreased leakage rates in young fir needles (Tables 19, 20, 26, 27). Inherent differences in O_3-sensitivity could likely play an important role in the clearly disparate responses exhibited by the two coniferous tree species examined in the present study. In this regard, if the total O_3 dose applied was greater than the tolerance threshold for ponderosa pine, but less than that for white fir, the observed membrane permeability responses would be expected.

Exposure to O_3 resulted in occasional alterations in pigment levels that were statistically significant. In this regard, O_3 has been found to decrease (Johnston et al., 1986), increase (Rajput and Ormrod, 1986), or have no effect (Pratt and Krupa, 1982) on crop leaf chlorophyll responses.

In summer, average gas exchange rates were markedly lower than in the spring (Tables 17, 25). Although the number of measurements were increased, significant air quality effects were not observed in any gas exchange parameter, in either tree species. While O_3 exposures have been shown to induce stomatal closure in a number of plant species (Jensen and Roberts, 1986; Matsushima et al., 1985; Temple, 1986), lower stomatal conductance rates may also have been a consequence of seasonal climatic changes or plant developmental factors (Kramer and Kozlowski, 1979). Nevertheless, decreased O_3 uptake is a beneficial outcome of reduced stomatal conductance, but is offset by lowered carbon assimilation rates (Jensen and Roberts, 1986; Reich, 1987).

Foliar starch concentrations were 70 to 80% lower than in the spring. Inasmuch as these levels reflect the influences of seasonal climate and plant developmental factors, exposure to O_3 may also have been a contributing factor (Constantinidou and Kozlowski, 1979; Ting and Mukerji, 1971). However, since starch levels were extremely low, substantive effects by O_3 would likely be undetectable.

In the present study, ponderosa pine seedlings grown in NF air exhibited higher amounts of mycorrhizal infection than plants grown in CF air, which has also been reported for white pine (Reich et al., 1986), and red oak (Reich et al., 1985). Alternatively, O_3 exposures did not affect mycorrhizal infection responses in loblolly pine (Mahoney et al., 1985). The responses of ponderosa pine in the present study suggest that exposures to O_3 may have enhanced mycorrhizal infection, and it seems evident that root system responses are sensitive to the effects of O_3, at least in ponderosa pine.

In consideration of O_3 concentrations measured in mountain sites typical for ponderosa pine (i.e., 1200 to 2400 m elevation), and the symptomology of O_3 injury development in pine seedlings, the lack of significant impacts on growth in the present study is not surprising. While Miller et al. (1986) reported that maximum 1 h average O_3

concentrations at Sky Forest and Barton Flats in the San Bernardino Mountains were similar to levels found in Riverside in the present study (i.e., 190 to 200, 150 to 160, and 100 to 270 ppb O_3, respectively), 24 h average concentrations in the mountains were nearly equal to or higher than the 12 h average O_3 concentration measured during the summer of 1987 in Riverside. One contributing factor to higher 24 h average O_3 levels in the mountains is the occurrence of 40 to 70 ppb O_3 levels at night (Miller et al., 1986). Since O_3 levels at night are typically at or near zero in Riverside, the 24 h average O_3 level could conceiveably be as much as 50% lower than the reported 12 h average.

The negative effects of O_3 on forests in the Sierra Nevada and San Bernardino Mountains of California, expressed as increased foliar injury and reduced amounts of chlorophyll in ponderosa pine (Miller and Millecan, 1978; Miller et al., 1982), reduced photosynthesis in ponderosa pine (Coyne and Bingham, 1982), or reduced growth of Jeffrey pine (Peterson et al., 1987) have been described. It is important to recognize that the aforementioned studies were performed on mature forest trees which were exposed to ozone for many seasons. In short-term experiments (i.e., shorter than a single-growing season), visible, histological, and biochemical alterations in ponderosa pine foliage due to ozone have been found only when tree seedlings or branches of trees were enclosed in plastic bags and exposed to higher than ambient concentrations of ozone (Miller et al., 1963; 1969; 1983; Evans and Miller, 1972; 1975). Richards et al. (1968) found that exposures to 200 ppb O_3 failed to produce needle injury in four-year old ponderosa pine seedlings, even after eight months of treatment. Moreover, when injury to current year foliage was induced by exposures to higher O_3 dosages (i.e., 500 to 3000 ppb), necrosis or chlorotic mottle became evident in mid-August or early September, and older foliage exhibited oxidant damage symptoms in late October or early November. In a 1982 study performed in open-top chambers located in Riverside, no significant increase of injury to ponderosa pine seedlings was found during 97 days of exposure to at 200 ppb O_3 (7.5 h/day) (Taylor et al., 1986b). In the same study, root growth was stimulated in seedlings exposed to 100 ppb O_3, and the growth of roots in seedlings exposed to 200 ppb O_3 and clean air were the same (Taylor et al., 1986b). Compared to the present study, the age of the seedlings, time of the year, and general condition of seedling propagation were different than in the study by Taylor and co-workers. Seedlings of ponderosa, Jeffrey and Coulter pine exposed to ambient levels of ozone for a single field-season in the San Gabriel Mountains (12 h daily average concentration of 117 ppb, and maximum 1 h average concentrations reaching 295 ppb) did not inhibit significant changes in injury, growth, or physiology (Bytnerowicz et al., in press). Temple (1988) found that after one season of exposures under greenhouse conditions, O_3 at 100 or 200 ppb did not cause significant growth changes in Jeffrey pine and giant sequoia seedlings. However, during the second year of exposure, significant reductions in growth were evident. To our knowledge, no evidence of significant effects from ambient levels of ozone during a single growing season on western forest trees has yet been reported. Recent field studies on the effects of ambient or slightly elevated concentrations of ozone (i.e., levels similar to the present study) on conifers typical for the eastern United States support the findings of this study. In the aforementioned studies, one season of ozone exposure generally did not produce significant changes in growth or physiology of the treated seedlings (Shafer et al., 1987; Adams et al., 1988; Laurence et al., 1989). It is concluded that the results of the present study, in which few significant alterations due to ozone exposure were found are not surprising. There is a clear indication that longer term experiments must be undertaken to accurately evaluate the effects of ambient levels of ozone on trees.

11. Summary of Chamber Effects on Coniferous Tree Seedlings in the Summer

No alterations in growth responses due to chamber effects in either tree species were found. However, there was evidence of alterations in biochemical responses in both tree species. Although the biochemical changes were not coincident with macroscopic alterations, it is clear that incipient biochemical impacts occurred which could influence plant responses to pollutant stresses. Specifically, increased conductivity and K^+ leakage responses in old needles of NF/No Fog plants may be indicative of membrane permeability changes due to higher daytime temperatures in the field chambers. If the observed chamber-related effects on membranes facilitated plant cell membrane disruption by O_3, pollutant effects could be greater in chambers than in chamberless plots. Moreover, the leakiness of organelle membranes may also be increased (i.e., plastids), which could contribute to changes in foliar pigment and starch levels.

12. Overview of the Potential Impacts of Sequential Exposures to Acidic Fog Followed by Ozone in Coniferous Tree Seedlings

Although fogs occur throughout the year in southern California, their frequency is higher during the spring and fall. During these two seasons, the dissipation of fog in the mid-morning hours can be followed by episodes of O_3, which has been suggested to be especially injurious to vegetation (Temple et al., 1987). While a "fog-smog-fog" cycle may exist in nature (Munger et al., 1983), the combined effects of acidic fog and ambient O_3 has been found to be additive in a variety of agronomic crop species (Olszyk et al., 1987; Takemoto et al., 1988b; Temple et al., 1987). Despite the lack of evidence with respect to interactive effects, the question of whether acidic fog exposures can predispose plants to the phytotoxic effects of O_3 is still a valid concern. Specifically, wet depositions have been shown to cause macroscopic and metabolic changes in a wide range of plant taxa, that may reduce the overall vitality of exposed plants (Evans, 1984; Jacobson, 1984). If whole-plant vigor is reduced to the extent that the plant is less able to continue development at a normal rate, the plant may also be more susceptible to the adverse impacts of other environmental stresses (i.e., photochemical oxidants). In view of this, H^+ inputs from acidic fog could have chronic effects on coniferous tree growth that may only become evident if followed by a prolonged stress of similar severity.

The effects of fog pretreatment on white fir injury persisted throughout the summer, but there was no evidence that fog-injured trees were more sensitive to the effects of O_3 than uninjured trees. Since O_3 phytotoxicity is dependent upon O_3 uptake through stomata, any impacts of fog on cuticle erosion, membrane permeability, and/or leaf cell acidification did not appear to have significant consequences with respect to enhancing O_3-related injury development in the present study.

In ponderosa pine, significant disruption of membrane permeability by pH 2.0 fog in the spring or ambient O_3 in the summer, did not correlate well with effects on injury or growth responses. Possibly, the effects on membrane integrity were not of the severity to cause more substantive impacts leading to altered growth responses in the current season. In this regard, since tree growth is known to be greatly influenced by growing conditions in the previous year (Kramer and Kozlowski, 1979), it may be useful to also examine growth responses at appropriate post-treatment intervals. Some support for the importance of assessing post-treatment growth responses can be found in the present study with respect to the reduction of needle starch levels in white fir exposed to pH 2.0 fog. The lowering of needle starch reserves could limit foliar growth in 1988 flush periods (Kramer and Kozlowski, 1979) or delay and reduce bud break, an effect which may occur in O_3-exposed ponderosa pine seedlings.

The complex nature of tree responses to air pollution was evident from the results of the present study. While there was some evidence of significant treatment effects in both the Spring Fog and Summer Ozone Exposure Studies, a definitive causal relationship between alterations in physiological and/or biochemical responses to injury or growth could not be identified. Alternatively, the veritable lack of significant treatment effects suggests that the trees were tolerant of the applied stresses or the stresses were not severe enough to cause significant changes in tree responses (among other explanations). Although clearly distinct impacts on tree growth by either stress agent were not observed in the present study, the presence of detectable alterations is perhaps indicative of incipient pollutant effects. It is conceivable that if the observed physiological and biochemical changes persisted (i.e., due to prolonged exposures to O_3 at current or higher concentrations), significant alterations in tree seedling growth could occur. For example, permanent changes in membrane permeability could lead to losses of important nutrients from foliage and facilitate the entry of ozone and other gaseous toxicants into plant cells. Reduced amounts of leaf pigments could decrease photosynthetic activity and the growth of trees. Changes such as these could cause a general deterioration of individual trees within forests, which in turn could increase tree sensitivity to the harmful effects of insects and microbial diseases. Monocultures of Norway spruce and Scots pine in forests of Central Europe have been reported to have experienced changes similar to those previously described.

High plant-to-plant variability, small sample sizes, the use of moderately severe pollution stresses, relatively short study duration, and low rates of physiological activity, are the factors that can influence the ability to detect air pollution impacts on plants. It is recommended that future studies aimed at examining the responses of trees to wet or dry deposited air pollutants should consider the complexity of tree responses along with these factors, and also include post-treatment monitoring as a research focus.

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