Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume III of III
Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume III

National Center for Environmental Assessment-RTP Office
Office of Research and Development
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PREFACE

National Ambient Air Quality Standards (NAAQS) are promulgated by the United States Environmental Protection Agency (EPA) to meet requirements set forth in Sections 108 and 109 of the U.S. Clean Air Act (CAA). Sections 108 and 109 require the EPA Administrator (1) to list widespread air pollutants that reasonably may be expected to endanger public health or welfare; (2) to issue air quality criteria for them that assess the latest available scientific information on nature and effects of ambient exposure to them; (3) to set “primary” NAAQS to protect human health with adequate margin of safety and to set “secondary” NAAQS to protect against welfare effects (e.g., effects on vegetation, ecosystems, visibility, climate, manmade materials, etc); and (5) to periodically review and revise, as appropriate, the criteria and NAAQS for a given listed pollutant or class of pollutants.

In 1971, the U.S. Environmental Protection Agency (EPA) promulgated National Ambient Air Quality Standards (NAAQS) to protect the public health and welfare from adverse effects of photochemical oxidants. The EPA promulgates the NAAQS on the basis of scientific information contained in air quality criteria issued under Section 108 of the Clean Air Act. Following the review of criteria as contained in the EPA document, Air Quality Criteria for Ozone and Other Photochemical Oxidants published in 1978, the chemical designation of the standards was changed from photochemical oxidants to ozone (O$_3$) in 1979 and a 1-hour O$_3$ NAAQS was set. The 1978 document focused mainly on the air quality criteria for O$_3$ and, to a lesser extent, on those for other photochemical oxidants (e.g., hydrogen peroxide and the peroxycetyl nitrates), as have subsequent revised versions of the document.

To meet Clean Air Act requirements noted above for periodic review of criteria and NAAQS, the O$_3$ criteria document, *Air Quality Criteria for Ozone and Other Photochemical Oxidants*, was next revised and released in August 1986; and a supplement, *Summary of Selected*...
New Information on Effects of Ozone on Health and Vegetation, was issued in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O₃ was not appropriate at that time. That decision, however, did not take into account newer scientific data that had become available after completion of the 1986 criteria document. Such literature was assessed in the next periodic revision of the O₃ air quality criteria document (O₃ AQCD) which has completed in 1996 and provided scientific bases supporting the setting by EPA in 1997 of the current 8-h O₃ NAAQS.

The purpose of this revised air quality criteria document for O₃ and related photochemical oxidants is to critically evaluate and assess the latest scientific information published since that assessed in the above 1996 O₃ AQCD, with the main focus being on pertinent new information useful in evaluating health and environmental effects data associated with ambient air O₃ exposures. However, other scientific data are also discussed in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O₃ and related photochemical oxidants and their precursors in the environment. The document mainly assesses pertinent literature published through 2004, but also includes assessment of a few additional important studies published or accepted for publication in 2005.

A First External Review Draft of this O₃ AQCD (dated January 2005) was released for public comment and was reviewed by the Clean Air Scientific Advisory Committee (CASAC) in May, 2005 to obtain. Public comments and CASAC recommendations were then taken into account in making revisions to the document for incorporation into a Second External Review Draft (dated August, 2005), which underwent further public comment and CASAC review at a December, 2005 public meeting. Public comments and CASAC advice derived from review of that Second External Review Draft were considered in making revisions incorporated into this final version of the document (dated February, 2006). Evaluations contained in the present document will be drawn on to provide inputs to associated O₃ Staff Paper analyses prepared by EPA’s Office of Air Quality Planning and Standards (OAQPS) to pose options for consideration by the EPA Administrator with regard to proposal and, ultimately, promulgation of decisions on potential retention or revision, as appropriate, of the current O₃ NAAQS.

Preparation of this document was coordinated by staff of EPA’s National Center for Environmental Assessment in Research Triangle Park (NCEA-RTP). NCEA-RTP scientific staff, together with experts from other EPA/ORD laboratories and academia, contributed to
writing of document chapters. Earlier drafts of document materials were reviewed by non-EPA experts in peer consultation workshops held by EPA. The document describes the nature, sources, distribution, measurement, and concentrations of O₃ in outdoor (ambient) and indoor environments. It also evaluates the latest data on human exposures to ambient O₃ and consequent health effects in exposed human populations, to support decision making regarding the primary, health-related O₃ NAAQS. Lastly, the document also evaluates ambient O₃ environmental effects on vegetation and ecosystems, surface level solar UV radiation flux and global climate change, and man-made materials to support decision making on secondary O₃ NAAQS.

NCEA acknowledges the valuable contributions provided by authors, contributors, and reviewers and the diligence of its staff and contractors in the preparation of this document.
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Ozone Review Panel

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### ABBREVIATIONS AND ACRONYMS

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<tr>
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<td>ABI2</td>
<td>phospho-tyrosine-specific protein phosphatase</td>
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<td>ACC</td>
<td>1-aminocyclopropane-1-carboxylate</td>
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<tr>
<td>ACS</td>
<td>1-aminocyclopropane-1-carboxylase synthase</td>
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<td>$A_{\text{max}}$</td>
<td>maximum photosynthesis rate</td>
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<td>ANN</td>
<td>artificial neural network</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>Acadia National Park</td>
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<td>AOS</td>
<td>allene oxide synthase</td>
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<td>AOT40</td>
<td>seasonal sum of the difference between an hourly concentration at the threshold value of 40 ppb, minus the threshold value of 40 ppb</td>
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<td>$A_{\text{OT}_x}$</td>
<td>family of cumulative, cutoff concentration-based exposure indices</td>
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<td>ascorbate peroxidase</td>
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<td>AQCD</td>
<td>Air Quality Criteria Document</td>
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<td>$A_{\text{sat}}$</td>
<td>photosynthetic assimilation in saturating light</td>
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<td>blue copper binding protein</td>
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<td>cDNA</td>
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<td>CF</td>
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<td>continuous stirred tank reactor</td>
</tr>
<tr>
<td>CU</td>
<td>cumulative uptake</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>cyt</td>
<td>cytochrome</td>
</tr>
<tr>
<td>DG</td>
<td>diacylglycerol</td>
</tr>
<tr>
<td>DGDG</td>
<td>digalactosyldiacylglycerol</td>
</tr>
<tr>
<td>DHA</td>
<td>dehydroascorbate</td>
</tr>
<tr>
<td>DMPO</td>
<td>dimethylphrrolise 1-oxide; 5,5-dimethyl-1-pyrrole N-oxide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECM</td>
<td>ectomycorrhizal fungi</td>
</tr>
<tr>
<td>EDU</td>
<td>ethylenediurea</td>
</tr>
<tr>
<td>EEA</td>
<td>essential ecological attribute</td>
</tr>
<tr>
<td>EMEP</td>
<td>European Monitoring and Evaluation Program</td>
</tr>
<tr>
<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
</tr>
<tr>
<td>EPO</td>
<td>epoxyconazole</td>
</tr>
<tr>
<td>EPR</td>
<td>electron paramagnetic resonance; ESR</td>
</tr>
<tr>
<td>ERD1</td>
<td>ethylene response</td>
</tr>
<tr>
<td>ESPACE-wheat</td>
<td>European Stress Physiology and Climate Experiment on the Effects of Carbon Dioxide and Oxygen on Spring Wheat</td>
</tr>
<tr>
<td>ESR</td>
<td>electron spin resonance; EPR</td>
</tr>
<tr>
<td>ET</td>
<td>ethylene</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>$\phi_{PSII,max}$</td>
<td>maximum light-adapted apparent quantum efficiency of Photosystem II</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>FA</td>
<td>fatty acid</td>
</tr>
<tr>
<td>FACE</td>
<td>free-air carbon dioxide enrichment (system)</td>
</tr>
<tr>
<td>FFAs</td>
<td>free fatty acids</td>
</tr>
<tr>
<td>FHM</td>
<td>Forest Health Monitoring (assessment)</td>
</tr>
<tr>
<td>FLAG</td>
<td>Federal Land Managers’ Air Quality Related Values Workgroup</td>
</tr>
<tr>
<td>FPM</td>
<td>Forest Pest Management</td>
</tr>
<tr>
<td>G</td>
<td>plants rooted in ground</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>GGGGT</td>
<td>galactolipid:galactolipid galactosyltransferase</td>
</tr>
<tr>
<td>GHG</td>
<td>greenhouse gas</td>
</tr>
<tr>
<td>GPx</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>GR</td>
<td>glutathione reductase</td>
</tr>
<tr>
<td>GRSM</td>
<td>Great Smoky Mountains National Park</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>GSMNP</td>
<td>Great Smoky Mountains National Park</td>
</tr>
<tr>
<td>GSSG</td>
<td>glutathione disulfide</td>
</tr>
<tr>
<td>GST</td>
<td>glutathione synthase</td>
</tr>
<tr>
<td>H⁺</td>
<td>hydrogen ion</td>
</tr>
<tr>
<td>2HDM, 2ndHDM</td>
<td>second-highest daily maximum 1-h concentration</td>
</tr>
<tr>
<td>HF</td>
<td>hydrogen fluoride</td>
</tr>
<tr>
<td>HNO₃</td>
<td>nitric acid</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>HO₃•</td>
<td>protonated ozone radical</td>
</tr>
<tr>
<td>HO•</td>
<td>hydroxyl radical</td>
</tr>
<tr>
<td>HO₂⁻</td>
<td>hydroperoxyl; hydroperoxy radical; protonated superoxide</td>
</tr>
<tr>
<td>HPOT</td>
<td>13-hydroperoxide linolenic acid</td>
</tr>
<tr>
<td>HR</td>
<td>hypersensitive response</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>ICP Forests</td>
<td>International Cooperative Programme on Assessment of Air Pollutant Effects on Forests</td>
</tr>
<tr>
<td>IPCC</td>
<td>Intergovernmental Panel on Climate Change</td>
</tr>
<tr>
<td>JA</td>
<td>jasmonic acid</td>
</tr>
<tr>
<td>$J_{\text{max}}$</td>
<td>maximum rate of electron transport for the regeneration of RuBP</td>
</tr>
<tr>
<td>$J_{\text{sat}}$</td>
<td>saturating light</td>
</tr>
<tr>
<td>KROFEX</td>
<td>Krauzberg Ozone Fumigation Experiment</td>
</tr>
<tr>
<td>LAI</td>
<td>leaf area index</td>
</tr>
<tr>
<td>LOX1</td>
<td>lipoxygenase</td>
</tr>
<tr>
<td>M7</td>
<td>7-hour seasonal mean</td>
</tr>
<tr>
<td>mAOT</td>
<td>modified accumulated exposure over the threshold</td>
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<tr>
<td>MDA</td>
<td>malonaldehyde</td>
</tr>
<tr>
<td>MDGD</td>
<td>monogalactosyldiacylglycerol</td>
</tr>
<tr>
<td>MGDG</td>
<td>monogalactosyldiacylglycerol</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>MT1</td>
<td>mitochondria</td>
</tr>
<tr>
<td>MV</td>
<td>methyl viologen</td>
</tr>
<tr>
<td>NAAQS</td>
<td>National Ambient Air Quality Standards</td>
</tr>
<tr>
<td>NAD</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>reduced nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADP$^+$</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NADPH, NAD(P)H</td>
<td>reduced nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NaE</td>
<td>sodium erythorbate</td>
</tr>
<tr>
<td>NCLAN</td>
<td>National Crop Loss Assessment Network</td>
</tr>
<tr>
<td>n.d.</td>
<td>no data</td>
</tr>
<tr>
<td>NDF</td>
<td>neutral detergent fiber</td>
</tr>
<tr>
<td>NF</td>
<td>national forest</td>
</tr>
<tr>
<td>NF</td>
<td>non-filtered</td>
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NH₃
(NH₄)₂SO₄
N₂O
NO
NO₂
NO₃⁻
NOₓ
NP
NPP
n.s.
O₂⁻
O₂⁺
¹O₂
O₃
OD
OTC
p, P
P
PAD
PAL
PAN
PAR
PC
PE
PFD
PG
PGSM
PI

ammonia
ammonium sulfate
nitrous oxide
nitric oxide
nitrogen dioxide
nitrate
nitrogen oxides
national park
net primary productivity
nonsignificant
superoxide
superoxide radical
singlet oxygen
ozone
outer diameter
open-top chamber
probability value
plants grown in pots
pollutant applied dose
phenylalanine lyase
peroxyacetyl nitrate
photosynthetically active radiation
phosphatidylcholine
phosphatidylethanolamine
photosynthetic flux density
phosphatidylglycerol
Plant Growth Stress Model
phosphatidylinositol
POD peroxidase
ppb parts per billion
ppm parts per million
PQH$_2$ plastoquinone
PR pathogenesis-related (protein)
PR-1 promotor region 1
PRYL predicted relative yield (biomass) loss
PSII Photosystem II
Pxase peroxidase
qP photochemical quenching
$r^2$ correlation coefficient
$R^2$ multiple regression correlation coefficient
$rbcL$ Rubisco large subunit
$rbcS$ Rubisco small subunit
RH relative humidity
RNA ribonucleic acid
ROG reactive organic gases
ROS reactive oxygen species
Rubisco ribulose-1,6-P$_2$-carboxylase/oxygenase
RuBP ribulose bisphosphate
SA salicylic acid
SAB Science Advisory Board
SAG21 senescence
SAR systemic acquired resistance
SD standard deviation
SE standard error
SHEN Shenandoah National Park
SIGMOID sigmoid weighted summed concentration

III-xxx
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Term</th>
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<tr>
<td>SLA</td>
<td>specific leaf area</td>
</tr>
<tr>
<td>SMD</td>
<td>soil moisture deficit</td>
</tr>
<tr>
<td>SNAAQS</td>
<td>Secondary National Ambient Air Quality Standards</td>
</tr>
<tr>
<td>SO₂</td>
<td>sulfur dioxide</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>sulfate</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>SUM00</td>
<td>sum of all hourly average concentrations</td>
</tr>
<tr>
<td>SUM06</td>
<td>seasonal sum of all hourly average concentrations ≥ 0.06 ppm</td>
</tr>
<tr>
<td>SUM08</td>
<td>seasonal sum of all hourly average concentrations ≥ 0.08 ppm</td>
</tr>
<tr>
<td>TMPO</td>
<td>tetramethylphrrolise 1-oxide</td>
</tr>
<tr>
<td>TNC</td>
<td>total nonstructural carbohydrate</td>
</tr>
<tr>
<td>UDG/T</td>
<td>UDP galactose-1,2,-diacylglycerol galactosyltransferase</td>
</tr>
<tr>
<td>UNECE</td>
<td>United Nations Economic Commission for Europe</td>
</tr>
<tr>
<td>UN ECE ICP-Vegetation</td>
<td>United Nations Economic Commission for Europe International Cooperative Programme on effects of air pollution and other stresses on crops and non-woody plants (UN/ECE-Vegetation; formerly -Crops)</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nations Environment Program</td>
</tr>
<tr>
<td>UDP</td>
<td>uridine diphosphate</td>
</tr>
<tr>
<td>USDA</td>
<td>U.S. Department of Agriculture</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>UV-B</td>
<td>ultraviolet radiation of wavelengths from 280 to 320 nm</td>
</tr>
<tr>
<td>VOC</td>
<td>volatile organic compound</td>
</tr>
<tr>
<td>VPD</td>
<td>vapor pressure deficit</td>
</tr>
<tr>
<td>W95</td>
<td>cumulative integrated exposure index with a sigmoidal weighting function</td>
</tr>
<tr>
<td>W126</td>
<td>cumulative integrated exposure index with a sigmoidal weighting function</td>
</tr>
<tr>
<td>ZAPS</td>
<td>Zonal Air Pollution System</td>
</tr>
</tbody>
</table>
ANNEX AX9. ENVIRONMENTAL EFFECTS: OZONE EFFECTS ON VEGETATION AND ECOSYSTEMS

AX9.1 METHODOLOGIES USED IN VEGETATION RESEARCH

AX9.1.1 Introduction

The scale of investigations evaluating the direct effects of O$_3$ on plant response ranges from subcellular to cellular, organismal, population, community, and ecosystem levels, with each level having its own particular experimental methodologies and specialized instrumentation, equipment, facilities, and experimental protocols. These investigations generate data. Other types of methodologies exist for the handling of data and statistical analysis as well as the utilization of data in developing the different exposure metrics or indices used to define exposure, quantitative exposure-response relationships, and computer simulation models of these exposure-response relationships. The objective of this section is not to provide an updated encyclopedia of all the methods that have been used but rather to focus on approaches that have

(1) led to an improved understanding of the quantitatively measurable growth and development responses of plants and plant communities to O$_3$, or

(2) provided information about the extent and geographic distribution of the responses of herbaceous and woody plants, both cultivated and native, to ambient O$_3$ exposures.

The first part of the objective is essential for determining dose-response functions used in developing impact and risk assessments of the effects of O$_3$; it usually involves treating plants to a range of artificial O$_3$ exposures. The second part of the objective is essential for determining the geographic distribution of the risk; it usually involves subjecting plants to ambient air O$_3$ exposures.

The types of methodologies used by biochemists, molecular biologists, or plant physiologists, whose interests lie in determining effects on specific constituents or in understanding the mode of action of O$_3$, are not discussed here but are addressed in Section AX9.2. Methods used to characterize the O$_3$ content of ambient air and to define exposure and exposure-response relations are discussed in Sections AX9.4 and AX9.5, respectively.
The methodologies for exposure-response studies have involved many different types of exposure facilities and protocols and have employed a range of statistical approaches in the analysis and interpretation of the data. Most of the studies have been conducted using major agricultural crop species. The methodologies have improved over the years as a result of the development, availability, or application of new or improved instrumentation, physical systems, and numerical approaches to data analysis. Yet equally important to the roles played by these advances has been the clearer understanding that has emerged from earlier work identifying the type of experimentation needed to achieve realistic assessments of the magnitude and extent of the impact of O$_3$ on vegetation of all types. As a result, significantly increased attention is now being paid to field observations and biomonitoring, particularly to the responses of forest trees and native vegetation.

Other than in various exploratory studies that have used chamber-based steady-state exposure concentrations (so-called “square-wave” exposures), the trend in experimental exposure protocols has been to attempt to expose plants under conditions as natural as possible to temporal profiles that simulate the real world, either by conducting experiments in the field or in elaborately controlled environment facilities that provide simulated field conditions.

Previous Air Quality Criteria Documents for Ozone and Other Photochemical Oxidants (U.S. Environmental Protection Agency, 1986, 1996) described the time course for these methodological developments. Although this section provides a brief overview of the methodologies used in the past and their limitations, it focuses mainly on those techniques that have come into prominence over the last decade. This focus has been aided considerably by several compilations of experimental methodologies and facilities, such as the earlier comprehensive review for the U.S. Environmental Protection Agency/National Acid Precipitation Assessment Program by Hogsett et al. (1987a,b), Manning and Krupa (1992) and by more recent reviews by Musselman and Hale (1997) and Karnosky et al. (2001b).

**AX9.1.2 Methods Involving Experimental Exposures to Ozone**

**AX9.1.2.1 “Indoor”, Controlled Environment, and Greenhouse Chambers**

The earliest experimental investigations of the effects of O$_3$ on plants utilized simple glass or plastic-covered chambers, often located within greenhouses, into which a flow of O$_3$-enriched air or oxygen could be passed to provide the exposure. The types, shapes, styles, materials of
construction, and locations of these chambers were as numerous as the different investigators and, in spite of providing little resemblance to real-world conditions, they yielded much of the basic information on the visible and physiological effects on plants. The construction and performance of more elaborate and better instrumented chambers dating back to the 1960s has been well-summarized in Hogsett et al. (1987a), including those installed in greenhouses (with or without some control of temperature and light intensity).

One greenhouse chamber approach that continues to yield useful information on the relationships of $O_3$ uptake to both physiological and growth effects employs continuous stirred tank reactors (CSTRs) first described by Heck et al. (1978). Although originally developed to permit mass-balance studies of $O_3$ flux to plants, their use has more recently widened to include short-term physiological and growth studies of $O_3 \times CO_2$ interactions (e.g., Costa et al., 2001; Heagle et al., 1994b; Loats and Rebbeck, 1999; Rao et al., 1995; Reinert and Ho, 1995; and Reinert et al., 1997), and of surveys of native plant responses to $O_3$ (Orendovici et al., 2003). In many cases, supplementary lighting and temperature control of the surrounding structure have been used to control or modify the environmental conditions (e.g., Heagle et al., 1994a).

Many investigations have utilized commercially available controlled environment chambers and walk-in rooms adapted to permit the introduction of a flow of $O_3$ into the controlled air-volume. Such chambers continue to find use in genetic screening and in physiological and biochemical studies aimed primarily at improving our understanding of modes of action. For example, some of the ongoing studies of the $O_3$ responses of *Plantago major* populations have been conducted in controlled environment chambers (Reiling and Davison, 1994; Whitfield et al., 1996b).

The environmental conditions provided by indoor chambers of any type will always preclude the use of the information obtained with such chambers in predicting $O_3$ effects in the natural environment, because the environmental conditions will always be measurably different from field conditions. However, highly sophisticated controlled environment chambers such as those described by Langebartels et al. (1997), which are subdivided into aerial and root compartments with dynamic control of light intensity and photoperiod, air and soil temperature, humidity, soil moisture, wind speed, and exposure to $O_3$, may come close to simulating specific natural conditions. Such chambers have provided meaningful insights into a wide array of the early biochemical responses of plants to $O_3$. They can also minimize confounding factors that
make indoor chamber studies only rarely able to be extrapolated to field conditions, e.g., that shoots and roots develop under different temperature regimes. The applicability of the results of many chamber studies may be further limited by their use of container-grown plants. Most of the concerns over the applicability of CO$_2$ enrichment studies, as discussed in Section AX9.5.7.1, may also be relevant to O$_3$ enrichment studies, as suggested by Whitfield et al. (1996a).

Whitfield et al. (1996a) reported significant interactive effects between O$_3$ and soil volume on the growth of *Plantago major*. They noted that although container size may limit root and, hence, plant growth, the reverse may also be true for single plants in large containers, which do not experience typical field competition for resources. Other studies found little or no effect of rooting volume on plant response to O$_3$. Heagle et al. (1979a,b; 1983) found that four wheat cultivars (*Triticum aestivum* L.) had similar proportional suppression of seed yield to season-long O$_3$ exposure whether plants were grown in the ground or in 3.8-L pots. Similarly, proportional O$_3$ injury and yield response of field corn (*Zea mays* L.) (Heagle et al., 1979a) and soybean (*Glycine max* (L.) Merr.) (Heagle et al., 1983) was similar whether the plants were grown in 15-L pots or in the ground. In a two-year experiment with soybean, the relative effects of CO$_2$ and O$_3$ on above-ground biomass and seed yield were similar whether the plants were grown in pots (15 and 21 L) or grown in the ground (Booker et al., 2005). Collectively, the results suggest that while planting density and rooting environment affect plant morphology and growth, the relative responses of seed yield to elevated O$_3$ may be similar whether plants are grown in pots or in the ground.

**AX9.1.2.2 Field Chambers**

Although some types of closed field chambers have largely fallen out of favor in recent years, closed “Solardome” field chambers (Lucas et al., 1987; Rafarel and Ashenden, 1991) have been successfully used in studies of O$_3$ × acid mist interactions (Ashenden et al., 1995, 1996).

Concern over the need to establish realistic plant-litter-soil relationships as a prerequisite to studies of the effects of O$_3$ and CO$_2$ enrichment on ponderosa pine (*Pinus ponderosa*) seedlings led Tingey et al. (1996) to develop closed, partially environmentally controlled, sun-lit chambers (“terracosms”) incorporating 1-m-deep lysimeters containing forest soil in which the appropriate horizon structure was retained.
In general, field chamber studies are dominated by the use of various versions of the open-top chamber (OTC) design, first described by Heagle et al. (1973) and Mandl et al. (1973). Most chambers are ~3 m in diameter with 2.5-m-high walls. Hogsett et al. (1987a) described in detail many of the various modifications to the original OTC designs that appeared subsequently, e.g., the use of larger chambers to permit exposing small trees (Kats et al., 1985a) and grapevines (Mandl et al., 1989), the addition of a conical baffle at the top to improve ventilation (Kats et al., 1976), a frustrum at the top to reduce ambient air incursions, and a plastic rain-cap to exclude precipitation (Hogsett et al., 1985b). All of these modifications included the discharge of air via ports in annular ducting or interiorly perforated double-layered walls at the base of the chambers to provide turbulent mixing and the upward mass flow of air.

Wiltshire et al. (1992) described a large OTC suitable for small trees with roll-up sides that permitted the trees to be readily subjected from time to time to episodic, normal, “chamberless” environmental conditions. In the 6-m-high OTCs described by Seufert and Arndt (1985) used with Norway spruce (Picea abies) trees, a second zone of annular enrichment was also provided between 4 and 5 m. The use of OTCs was adopted for the large European Stress Physiology and Climate Experiment on the effects of CO\textsubscript{2} and O\textsubscript{3} on spring wheat (ESPACE-wheat), conducted over 1994 to 1996 at field sites in eight countries (Jäger et al., 1999). However, typical European chambers have the introduction of O\textsubscript{3}-enriched air at or above canopy height. The relatively low costs of fabrication, operation, and maintenance has favored OTC use in field studies (Fangmeier et al., 1992; Musselman and Hale, 1997). The air supplied to the chambers can be readily filtered through activated charcoal to reduce the O\textsubscript{3} concentration, or it can be enriched with O\textsubscript{3} to provide a range of exposures.

All field chambers create internal environments that differ from ambient air, giving rise to so-called “chamber effects” with the modification of microclimatic variables, including reduced and uneven light intensity, uneven rainfall, constant wind speed, reduced dew formation, and increased air temperatures (Fuhrer, 1994; Manning and Krupa, 1992). Because of the constant wind speed and delivery systems, OTCs can provide a more definable exposure than free-air systems can due to the lack of “hot-spots”, where exposures are essentially undefined, in free-air systems. Nonetheless, there are several characteristics of the OTC design and operation that can lead to unrealistic exposures. First, the plants are subjected to constant turbulence, which, through increased uptake resulting from the consequently low boundary layer resistance to
diffusion, may lead to overestimation of cause-effect relationships (Krupa et al., 1995; Legge et al., 1995). However, in at least one case where canopy resistances were quantified in OTCs and in the field, it was determined that gaseous pollutant exposure to crops in OTCs was similar to that which would have occurred at the same concentration in the field (Unsworth et al., 1984a, 1984b).

A second concern is that the introduction of the O$_3$-enriched air into the lower part of chambers as described by Heagle et al. (1973) and Mandl et al. (1973) results in a O$_3$ concentration gradient that decreases with increasing height, the converse of the situation observed in ambient air in which the O$_3$ concentration decreases markedly from above a plant canopy to ground level (Grünhage and Jäger, 1994; Pleijel et al., 1995b, 1996). Concern that studies conducted in such OTCs may somewhat overestimate the effects of O$_3$ led to the European design, which provides a decreasing downward gradient. It seems unlikely that the “chamber effects” produced by the two designs will be the same. These issues are discussed more fully in Section AX9.1.2.4.

It should also be noted that, although OTCs were originally developed for exposing row crops in the field, many recent studies employing OTCs have used potted plants in order to include or control edaphic or nutritional factors or water relations within the experimental design. Therefore, some caution should be used when extrapolating results of pot studies to the field as noted above (Section AX9.2.2.1).

The difficulties faced in the experimental exposure of forest trees to air pollutants in chambers (e.g., Seufert and Arndt [1985]) led to the development of branch chambers such as those described by Ennis et al. (1990), Houpis et al. (1991), and Teskey et al. (1991). These chambers are essentially large cuvettes without temperature control and, as noted by Musselman and Hale (1997), share many of the characteristics of CSTRs, i.e., transparent walls, internal fans, and inlet and outlet monitors to permit the determination of O$_3$ uptake, CO$_2$ exchange, and transpiration. Although they make it possible to expose whole branches to different O$_3$ regimes, the relevance of the data they yield in regard to the whole tree may be questionable. As noted by Saxe et al. (1998), the inevitable change in environmental conditions resulting from the isolation of the branch may cause different responses from those that would be obtained if the whole tree had been subjected to the same environmental conditions.
### AX9.1.2.3 Plume Systems

Plume systems are chamberless exposure facilities in which the atmosphere surrounding plants in the field is modified by the injection of pollutant gas into the air above or around them from multiple orifices spaced to permit diffusion and turbulence so as to establish relatively homogeneous conditions as the individual plumes disperse and mix with the ambient air. As pointed out by Manning and Krupa (1992), they can only be used to *increase* the O$_3$ levels in the ambient air. The volume of air to be modified is unconfined, and three approaches have been used to achieve desired pollutant concentrations in the air passing over the plants, producing various systems that

1. achieve a concentration gradient, in most instances dependent upon the direction of the prevailing wind;
2. achieve spatially uniform concentrations over a plot, dependent upon wind direction; and
3. seek to achieve spatially uniform concentrations over a plot, independent of wind speed and direction.

Gradient systems created by dispensing a pollutant gas into the air at canopy level from perforated horizontal pipes arranged at right angles to the prevailing wind were described for SO$_2$ studies in the early 1980s. A modified gradient system for O$_3$ was used by Bytnerowicz et al. (1988) to study effects on desert species, but there appear to have been no recent applications of the method. A gradient O$_3$-*exclusion* system is discussed in Section AX9.2.3.1.

Systems designed to achieve spatially uniform pollutant levels by ensuring that the release of a pollutant is always on the upwind side of the study site were also originally described for SO$_2$ studies (e.g., Greenwood et al. [1982]). However, the adaptation of these concepts as introduced by McLeod et al. (1985) in constructing a large circular field site for exposing crops to SO$_2$ led to the subsequent development of both the large-scale O$_3$ and SO$_2$ fumigation system for forest trees in the United Kingdom in 1985 (the Liphook Forest Fumigation Project) (1992), the smaller system for O$_3$ fumigation constructed at Kuopio, Finland in 1990 (Wulff et al., 1992), and the free-air carbon-dioxide enrichment (FACE) systems of gas dispersal over crops (Hendrey and Kimball, 1994) and forest trees (Hendrey et al., 1999). Although originally designed to provide chamberless field facilities for studying the CO$_2$ effects of climate change, large forest tree FACE systems have recently been adapted to include the dispensing of O$_3$.
Volk et al. (2003) recently described a system for exposing grasslands that uses 7-m diameter plots. FACE systems discharge the pollutant gas (and/or CO\textsubscript{2}) through orifices spaced along an annular ring (or torus) or at different heights on a ring of vertical pipes. Computer-controlled feedback from the monitoring of gas concentration regulates the feed rate of enriched air to the dispersion pipes. Feedback of wind speed and direction information ensures that the discharges only occur upwind of the treatment plots, and that discharge is restricted or closed down during periods of low wind speed or calm conditions. The diameter of the arrays and their heights (25 to 30 m) in some FACE systems requires large throughputs of enriched air per plot, particularly in forest tree systems. The cost of the throughputs tends to limit the number of enrichment treatments, although Hendrey et al. (1999) argued that the cost on an enriched volume basis is comparable to that of chamber systems.

An alternative to the FACE system to free-air fumigation uses a horizontal grid system through which pollutant-enriched air is discharged over the canopies of plants in field plots. The original design, termed the Zonal Air Pollution System (ZAPS), was developed for studying the effects of SO\textsubscript{2} on native grasslands (Lee et al., 1975), and it was later modified by Runeckles et al. (1990) by randomly dividing each of three treatment plots into four subplots, each with different numbers of discharge orifices to provide various levels of O\textsubscript{3} enrichment. With the ZAPS system, changes in wind direction and speed result in varying degrees of carryover from subplot to subplot, effectively resulting in 12 stochastically different seasonal exposures. The system was used for studies of growth effects on field crops and 2- to 4-year old Douglas fir (*Pseudotsuga menziesii*) saplings (Runeckles and Wright, 1996). A larger ZAPS design was used by Wilbourn et al. (1995) on a grass (*Lolium perenne*)-clover (*Trifolium repens*) mixture and by Ollerenshaw et al. (1999) on oilseed rape (*Brassica napus*), whereby four replicate field plots were exposed to intermittent constant additions of O\textsubscript{3} to ambient air. A ZAPS design with eight spatially separated treatment plots was also developed to obtain crop response data used in assessing crop losses in the Fraser Valley, British Columbia, Canada (Runeckles and Bowen, 2000).

Another recent adaptation of the FACE design was constructed to fumigate soybean with CO\textsubscript{2} and O\textsubscript{3} in combination (Morgan et al., 2004; Rogers et al., 2004). This modified FACE design was based on those of Miglietta et al. (2001) and does not force air through the canopy; instead, it relies on wind to disperse air across the fumigation plot.
The FACE-type facility developed for the Kranzberg Ozone Fumigation Experiment (KROFEX) in Germany begun in 2000 (Werner and Fabian, 2002; Nunn et al., 2002) to study the effects of O$_3$ on mature stands of beech (Fagus sylvatica) and spruce (Picea abies) trees is more truly a zonal system that functions independently of wind direction. The enrichment of a large volume of the ambient air immediately above the canopy takes place via orifices in vertical tubes suspended from a horizontal grid supported above the canopy.

Recognizing the difficulties of modifying the aerial environments of large trees, Tjoelker et al. (1994) devised a free-air system for exposing branches of sugar maple (Acer saccharum) trees to O$_3$. Near the ends of up to 10 branches, enriched air was discharged through small holes in 38-cm-diameter loops of 0.635-cm-OD (outer diameter) teflon tubes positioned 20 to 30 cm below the terminal foliage cluster.

Although plume systems make virtually none of the modifications to the physical environment that are inevitable with chambers, their successful use depends on selecting the appropriate numbers, sizes, and orientations of the discharge orifices to avoid hot-spots resulting from the direct impingement of jets of pollutant-enriched air on plant foliage (Werner and Fabian, 2002). However, because mixing is unassisted and completely dependent on wind turbulence and diffusion, local gradients are inevitable even in large-scale FACE systems. Both FACE and ZAPS systems have provisions for shutting down under low wind speed or calm conditions and for an experimental area that is usually defined within a generous border in order to strive for homogeneity of the exposure concentrations within the treatment area. They are also both dependent upon continuous computer-controlled feedback of the O$_3$ concentrations in the mixed treated air and of the meteorological conditions.

**AX9.1.2.4 Comparative Studies**

All experimental approaches to the exposure of plants to O$_3$ have shortcomings. The use of laboratory, greenhouse, or field chambers raises concerns for the roles of chamber effects on micrometeorology, as well as the constant turbulence over and within the plant canopy during chamber operation, in modifying O$_3$ uptake and subsequent plant response. In contrast, plume systems suffer from relatively poor control of exposure levels and an inability to reduce O$_3$ levels below ambient in areas where O$_3$ is phytotoxic.
Although chamber effects vary, one concern is the rise in temperature associated with enclosing plants in a chamber. Still, it is not clear whether these effects are directly related to temperature or are the result of temperature interactions with other environmental variables. For example, Olszyk et al. (1992) undertook a 3-year study of the impact of O$_3$ on Valencia orange trees (Citrus sinensis (L.) Osbeck) in large OTCs to determine if “insidious differences in microclimatic conditions could alter plant growth responses and susceptibility to pollutant stress.” Nonfiltered chambers were found to have somewhat lower average O$_3$ concentrations than the ambient air, and fewer hourly exceedances of 100 ppb. In cool seasons, stomatal conductance was also lower, implying lower O$_3$ uptake. However, the cumulative fruit yields were doubled in the chamber trees even though photosynthetically active radiation was consistently reduced by about 19% while leaf temperatures averaged more than 2 °C higher. These data may be somewhat extreme, but they emphasize the need to be cautious when interpreting OTC yield response data, particularly since, as in this study, no O$_3$ enrichment was involved as a complicating factor.

While it is clear that chambers can alter some aspects of plant growth, the question to be answered is whether or not these differences affect plant response to O$_3$. As noted in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996), evidence from the comparative studies of OTCs and from closed chamber and O$_3$-exclusion exposure systems on the growth of alfalfa (Medicago sativa) by Olszyk et al. (1986a) suggested that, since significant differences were found for fewer than 10% of the growth parameters measured, the responses were, in general, essentially the same regardless of exposure system used and chamber effects did not significantly affect response. In 1988, Heagle et al. (1988) concluded: “Although chamber effects on yield are common, there are no results showing that this will result in a changed yield response to O$_3$.” A more recent study of chamber effects examined the responses of tolerant and sensitive white clover clones (Trifolium repens) to ambient O$_3$ in greenhouse, open-top, and ambient plots (Heagle et al., 1996). For individual harvests, greenhouse O$_3$ exposure reduced the forage weight of the sensitive clone 7 to 23% more than in OTCs. However, the response in OTCs was the same as in ambient plots. Several studies have shown very similar yield response to O$_3$ for plants grown in pots or in the ground, suggesting that even such a significant change in environment does not alter the proportional response to O$_3$, at least as long as the plants are well watered (Heagle, 1979; Heagle et al., 1983).
Recent evidence obtained using free-air exposure systems and OTCs supports results observed previously in OTC studies (Table AX9-16, Figure AX9-1). Specifically, a series of studies undertaken using free-air O₃ enrichment in Rhinelander, WI (Isebrands et al., 2000, 2001) showed that O₃-symptom expression was generally similar in OTCs, FACE, and ambient-O₃ gradient sites, supporting the previously observed variation among trembling aspen clones (*Populus tremuloides* L.) using OTCs (Karnosky et al., 1999). The FACE study evaluated the effects of 3 years of exposure to combinations of elevated CO₂ and O₃ on growth responses in mixture of five trembling aspen clones (Isebrands et al., 2000, 2001). Height, diameter, and stem volume (diameter² × height) were decreased by elevated O₃. On average for all clones, stem volume was decreased by 20% over the 3 years in the elevated O₃ treatment as compared with the 1×-ambient treatment. This FACE facility study is important, because it confirms responses reported previously with the same clones grown in pots or soil in OTCs without the alterations of microclimate induced by chambers. Currently, this is the only U.S. study using this technology to have examined the effects of O₃ under these conditions. This study is also significant, because the elevated O₃-exposure pattern used was intended to reproduce the 6-year average pattern from Washtenaw County, Michigan (Karnosky et al., 1999).

Chambered systems such as OTCs provide a charcoal-filtered (CF), clean-air control for O₃ experiments, while FACE and some other plume systems do not. Depending on experimental intent, a replicated, clean-air control treatment is an essential component in many experimental designs. This control cannot be provided by FACE systems where ambient O₃ levels are phytotoxic. This is especially relevant in CO₂ × O₃ experiments in which phytotoxic effects of ambient O₃ can be suppressed due to CO₂-induced reductions in stomatal conductance and O₃ uptake (Booker et al., 1997, 2004, 2005; Heagle et al., 1998b; Fiscus et al., 1997, 2002, 2005).

Plume systems avoid chamber effects, but because they rely solely upon diffusion and natural turbulence to modify the ambient O₃ concentration, they may fail to achieve homogeneity of the air to which the plants are exposed and may give rise to hot spots in which the enriched air jets are inadequately diluted and impinge directly on foliage. A further deterrent to their widespread use is the large-scale generation of O₃ needed, which has, in most cases, limited the numbers of treatments that can be included in an experimental design.

AX9-11
In spite of the various advantages and disadvantages of the two systems, there is still little experimental evidence that allows a direct comparison of OTCs to the free-air plume systems or a determination of the degree to which chamber effects alter plant response to O₃. The evidence that is available suggests that chamber effects do not fundamentally alter the response of plants to O₃; therefore, chambers remain a useful tool for testing species sensitivity and developing O₃-response relationships. However, chamber effects have the potential to alter O₃ uptake (Nussbaum and Fuhrer, 2000), so it is important to fully characterize temperature, light, turbulence, and other chamber characteristics during exposure to allow extrapolation of the results.

**AX9.1.2.5 Ozone Generation Systems**

Two approaches have been used to generate the O₃ needed for enrichment from air or oxygen: (1) high-voltage static discharge and (2) high-intensity UV-irradiation. Using gaseous oxygen as feedstock, both generate O₃-enriched oxygen, free from other impurities. However, the use of high-voltage discharge equipment with air as feedstock requires that the output be scrubbed with water to remove appreciable amounts of the higher oxides of nitrogen (especially nitric acid vapor) that form concurrently with O₃ (Brown and Roberts, 1988; Taylor et al., 1993).

**AX9.1.2.6 Experimental Exposure Protocols**

A few recent chamber studies of physiological or biochemical effects have continued to use square-wave exposure profiles typified by a rapid rise to, and falling off from, a steady target concentration. However, during the last 20 years, most approaches into studying O₃ effects on plant growth and development have employed either simulations of the diurnal ambient O₃ profile or enhancement/reduction of the ambient O₃ concentrations.

Hogsett et al. (1985b), Lefohn et al. (1986), and others have described the use in controlled chambers of daily exposure profiles based on observed ambient O₃ profiles. Such profiles were used in the elaborately controlled chamber studies of Langebartels et al. (1997), while several recent chamber studies have used simpler computer-controlled half- or full-cosine wave profiles to simulate the typical daily rise and fall in ambient O₃ levels (Mazarura, 1997; McKee et al., 1997a,b).
The early studies with OTCs involved adding constant levels of O\textsubscript{3} to ambient air O\textsubscript{3} concentrations, but all recent studies have used enrichment delivery systems that maintain proportionality to, and track, ambient O\textsubscript{3} concentrations to produce levels that more closely resemble field observations. Both FACE and ZAPS studies have used proportional enrichment to provide a range of treatments, although Wilbourn et al. (1995) and Ollerenshaw et al. (1999) adjusted their systems manually to obtain a relatively constant target concentration during exposure episodes.

AX9.1.3 Methods Involving Exposures to Ozone in Ambient Air

The experimental methods discussed above are largely aimed at developing quantitative growth-response functions to permit the estimation of the effects of different ambient O\textsubscript{3} scenarios. Because such methodologies usually involve exposures to higher than ambient O\textsubscript{3} levels, the applicability of the functions obtained may, to some extent, be relevant only to locations that are naturally subjected to high ambient O\textsubscript{3} levels. Furthermore, as pointed out in Section AX9.4, the response functions that they generate rarely incorporate other environmental, genetic, and physiological factors, many of which can severely modify the magnitude of the response to O\textsubscript{3}. The consequences of ignoring such modifications have been well stated by De Santis (1999). The European level for protecting crops (based on the AOT40 index; see Section AX9.4) was derived from OTC studies of O\textsubscript{3}-induced yield loss in wheat observed in experiments conducted mostly in non-Mediterranean locations. However, the impact of ambient O\textsubscript{3} on wheat yields in the Po Valley of northern Italy is much less than the devastatingly high loss (>60%) suggested by the seasonal exceedances of the level. On a similar note, Manning (2003) has recently urged the absolute necessity of seeking “ground truth” as verification of the nature and magnitude of impacts on vegetation as suggested by response functions using ambient O\textsubscript{3} monitoring data.

Such concerns clearly show that attention needs to be focused on incorporating consideration of environmental and other factors into the response functions upon which standards are based. This will require the development of improved simulation response models. These concerns have also led to increasing attention being paid to seeking and developing alternative approaches to assessing of impact, and the geographic extent of such impact—approaches that are based on in situ exposures to ambient or sub-ambient O\textsubscript{3} levels.
Although one approach, the use of air-exclusion systems, requires experimental facilities, the other approaches are generally based on simple field observations or measurements and, hence, can be undertaken on a wide geographic scale.

**AX9.1.3.1 Air-Exclusion Systems**

The term, air-exclusion system, usually refers to a chamberless field system specifically designed to protect plants from exposure to polluted air by blowing filtered air through their canopies. Hogsett et al. (1987a,b) described several dedicated systems developed in the 1960s and 1970s, but there appear to have been no recent O$_3$-exclusion studies using systems specifically designed for this purpose since those described by Olszyk et al. (1986a,b). Their system, a modification of the earlier system of Jones et al. (1977), consisted of perforated 31.8-cm OD inflatable polyethylene tubes laid between crop rows and supplied with CF air. By increasing the size of the orifices progressively in sections along the 9-m length of the tubes, an exclusion gradient was created with a progressive decrease in O$_3$ levels in the air surrounding the crop from one end of the system to the other. The system was used for studies on alfalfa comparing plant response in OTCs, closed field chambers, the air-exclusion system, and ambient air plots (as discussed above in Section AX9.1.2.4).

An air-exclusion component has also been part of the overall design of many OTC experiments which added CF air or mixtures of CF and ambient air to chambers as part of the overall design.

**AX9.1.3.2 Natural Gradients**

Naturally occurring O$_3$ gradients hold potential for the examination of plant responses along the gradient. However, few such gradients can be found which meet the rigorous statistical requirements for comparable site characteristics such as soil type, temperature, rainfall, radiation, and aspect (Manning and Krupa, 1992); although with small plants, soil variability can be avoided by the use of potted plants. The use of soil monoliths transported to various locations along natural O$_3$ gradients is another possible approach to overcome differences in soils; however, again this approach is limited to small plants.

Studies in the 1970s used the natural gradients occurring in southern California to assess yield losses of alfalfa and tomato (*Lycopersicon esculentum* L.) (Oshima et al., 1976, 1977).
A transect study of the impact of O$_3$ on the growth of white clover and barley (*Hordeum vulgare* L.) in the United Kingdom was confounded by differences in the concurrent gradients of SO$_2$ and NO$_2$ pollution (Ashmore et al., 1988). Studies of forest tree species in national parks in the eastern United States (Winner et al., 1989) revealed increasing gradients of O$_3$ and visible foliar injury with increased elevation.

**AX9.1.3.3 Use of Chemical Protectants**

The use of protective chemicals is a relatively inexpensive, promising alternative to experimental field exposures in chambers or free-air systems for determining plant response to O$_3$. These chemicals have recently been used in studies of different plant species, both in the United States (Bergweiler and Manning, 1999; Kuehler and Flagler, 1999) and in Europe (Bortier et al., 2001a; Pleijel et al., 1999; Wu and Tiedemann, 2002), to determine if ambient O$_3$ concentrations affect plant growth and productivity or are just exacerbating foliar injury. Several chemical compounds (e.g., antioxidants, antisenescence agents, fungicides, pesticides) have been known for many years to provide plants some protection from photochemical oxidants such as O$_3$ (Manning and Krupa, 1992). Most of these chemicals were originally used as a one-time application to reduce visible injury caused by acute O$_3$ exposures. The most widely used and popular of these has been ethylenediurea (EDU). Carnahan et al. (1978) reported that EDU protected pinto bean (*Phaseolus vulgaris*) from acute O$_3$ injury. After this initial investigation, EDU was shown to suppress visible O$_3$ injury on several species of plants under both controlled and field conditions (Brennan et al., 1987; Clarke et al., 1983). However, due to lack of a commercial market for this product, its commercial manufacture was largely discontinued. Other chemicals, including benomyl (Manning et al., 1974), carboxin (Rich et al., 1974), ascorbic acid (Dass and Weaver, 1968), and others (Manning and Krupa, 1992), also exhibited some beneficial effects in reducing visible O$_3$ injury.

Several recent studies have used EDU in assessing the response of several plant species to O$_3$ to help validate the proposed critical level (AOT40 = 3000 ppb·h; see Section AX9.5) for crop protection in Europe (Ball et al., 1998; Ribas and Penuelas, 2000; Tonneijck and Van Dijk, 2002a,b). EDU appeared to provide protection from visible foliar injury, but the results regarding yield and biomass reductions were mixed. In a 3-year study over 12 sites throughout Europe, Ball et al. (1998) used the ratio of EDU-treated versus non-treated white clover biomass
and did not find a significant relationship between biomass reductions and AOT40 level. However, an artificial neural network (ANN) model including vapor pressure deficit (VPD), temperature, longitude, year, and altitude explained much more of the variance ($r^2 = 0.79$). The authors suggested that the greater sensitivity at certain sites in Germany may have been due to occurrence of other pollutants. This meta-analysis indicates that EDU effects may be influenced substantially by environmental factors.

In another study, Tonneijck and Van Dijk (2002b) assessed the relationship of visible injury of subterranean clover (*Trifolium subterraneum*) to ambient O$_3$ at four sites over three growing seasons in the Netherlands, using EDU-treated and nontreated plants. Visible injury varied by site and year, but was reduced to near zero by EDU treatment. However, no relationship indicative of a protective effect of EDU with this plant species was observed for biomass. Tonneijck and Van Dijk (2002a) also reported similar results with pinto bean. Both EDU-treated and nontreated plants were exposed to ambient O$_3$ at three locations in Spain over one growing season (Ribas and Penuelas, 2000). Reductions in yield and biomass were correlated with O$_3$ concentration and EDU provided some protective effect, although results varied by location and with meteorological conditions.

Chemicals have also been used to assess the effects of O$_3$ on tree species. Bortier et al. (2001b) injected seedlings of an O$_3$-sensitive poplar (*Populus nigra*) clone with EDU and measured growth over a 1-year period at a field site near Brussels, Belgium. Over the growing season, stem diameter increment was significantly higher (16%), biomass was increased (9%), and foliar O$_3$ symptoms were slightly less for the EDU-treated seedlings. Ozone levels were reported to be low (AOT40 = 6170 ppb-h, May to September) during the exposure period. In another study, Manning et al. (2003) applied EDU (foliar spray) and sodium erythorbate (NaE) at various concentrations, biweekly for three growing seasons to loblolly pine (*Pinus taeda*) at a field site in east Texas. After 3 years, the trees were harvested and biomass measured. Neither EDU nor NaE prevented foliar O$_3$ injury, but EDU applications at 450 ppm resulted in increases both in stem diameter and height and in total above-ground biomass. These measures of growth also tended to slightly increase with applications of NaE, but the effects were statistically nonsignificant.

The mechanisms by which protective chemicals, especially EDU, protect plants are poorly understood. However, Wu and von Tiedemann (2002) reported that applications of two recently
developed fungicides (azoxystrobin and epoxiconazole) provided protection to spring barley to relatively high \( O_3 \) exposures (150 to 250 ppb, 5 days, 7 h/day) and resulted in increases in leaf soluble protein content as well as the activity of several antioxidative enzymes (e.g., superoxide dismutase, catalase, ascorbate-peroxidase, and glutathione reductase). In addition, the increase in these enzymes reduced superoxide levels in the leaves.

Despite advances in the use of protective chemicals, a number of hurdles remain in using them for assessing \( O_3 \) effects. The phytotoxicity of EDU is well known, and the point has been made repeatedly that for a particular species or cultivar, tests under a range of environmental conditions and \( O_3 \) exposures must be made to establish the efficacy of EDU for quantifying \( O_3 \) effects (Heggestad, 1988; Kostka-Rick and Manning, 1992a). Unfortunately, although many studies with EDU have been conducted in recent decades, very few have used multiple EDU application levels along with multiple \( O_3 \) exposures to characterize the EDU system for a given plant species.

Recent studies have also shown that EDU does not always have greater effects at higher \( O_3 \) exposures. For pinto bean grown in pots in studies in Spain and in the Netherlands, EDU increased pod yield (Ribas and Penuelas, 2000; Tonneijck and Van Dijk, 1997). However, this effect was not greater at sites with higher \( O_3 \) exposure despite consistent experimental protocols at all sites, including growing the same cultivar in pots with adequate water (Ribas and Penuelas, 2000; Tonneijck and Van Dijk, 1997). Such results suggest that it may be difficult to quantify ambient \( O_3 \) effects using EDU, because the amount of plant growth or yield expected at a low (background) \( O_3 \) concentration cannot be inferred from EDU-treated plants grown at locations with higher \( O_3 \) exposures.

Several studies suggest that EDU has effects other than its antioxidant protection and phytotoxicity and show that environmental conditions affect the degree of protection afforded by protective chemicals. In one study, even low concentrations of EDU (8 to 32 mg L\(^{-1}\) soil), decreased soybean yield under low \( O_3 \) exposure (7-h mean of 19 ppb) in CF OTCs (Miller et al., 1994). This study also demonstrated that phytotoxicity (both foliar symptoms and growth effects) can differ even in the same series of experiments, apparently due to changes in environmental conditions, and that EDU can suppress yield at application rates that do not always cause foliar symptoms (Miller et al., 1994). Finally, this study found that EDU altered biomass partitioning by increasing vegetative growth and decreasing reproductive growth.
A study of pinto bean grown in OTCs in Germany found that EDU treatment in CF OTCs significantly increased yield, while EDU had no significant effect on yield in other O₃ treatments (Brunschen-Harti et al., 1995). In this study, O₃ significantly reduced the mass of pods, shoots, and roots. EDU increased root, leaf, and shoot mass across O₃ treatments. However, the only statistically significant interaction occurred with O₃ × root mass. This study indicates that EDU can stimulate above-ground growth and/or delay senescence regardless of O₃ treatment.

The EDU approach for assessing the impact of ambient O₃ exposures is potentially useful, because it provides a separate line of evidence from other methods. Before using these chemicals in a field setting, preliminary investigations under controlled conditions (e.g., chambers) should be done to evaluate the methods and timing of application, as well as proper application rates, so as to avoid any potential toxic effects (Manning, 2000; Manning and Krupa, 1992). Unfortunately, such characterization has so far been limited, although substantial progress has been made for radish (Raphanus sativus L.) (Kostka-Rick et al., 1993; Kostka-Rick and Manning, 1992a, 1993). Thus, it is difficult to use data from existing EDU studies to develop exposure-response relationships or to quantify the effects of ambient O₃ exposure. Despite these limitations, the EDU studies reviewed in previous O₃ AQCDs (U.S. Environmental Protection Agency, 1986, 1996) and the more recent studies summarized in Table AX9-1 (Section AX9.5) provide another line of evidence that ambient O₃ exposures occurring in many regions of the United States may be reducing the growth of crops and trees.

AX9.1.3.4 Biomonitoring

Bioindicators

The use of biological indicators to detect the presence of O₃ injury to plants is a longstanding and effective methodology (Chappelka and Samuelson, 1998; Manning and Krupa, 1992). A bioindicator can be defined as a vascular or nonvascular plant exhibiting a typical and verifiable response when exposed to a plant stress such as an air pollutant (Manning et al., 2003). To be considered a good indicator species, plants must

1. exhibit a distinct, verified response,
2. have few or no confounding disease or pest problems, and
3. exhibit genetic stability.
Table AX9-1. Advantages and Disadvantages of Protective Chemicals Used in Assessment of O$_3$ Effects on Plants

<table>
<thead>
<tr>
<th>Advantages</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No chambers required. Plants exposed to ambient conditions of O$_3$, light,</td>
<td>Can conduct studies “in situ.” Equipment needs are minimal. No</td>
</tr>
<tr>
<td>temperature, etc.</td>
<td>“chamber effects”</td>
</tr>
<tr>
<td></td>
<td>A high degree of replication possible both within and among</td>
</tr>
<tr>
<td></td>
<td>locations</td>
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</table>

<table>
<thead>
<tr>
<th>Disadvantages</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure-response studies require inclusion of other methodologies (OTCs,</td>
<td>Need measurements of ambient O$_3$ and other meteorological</td>
</tr>
<tr>
<td>etc.)</td>
<td>variables (temp, rainfall, etc)</td>
</tr>
<tr>
<td></td>
<td>Many are toxic; have to conduct preliminary toxicology studies to</td>
</tr>
<tr>
<td></td>
<td>determine proper rate, timing etc.</td>
</tr>
<tr>
<td></td>
<td>Species response can vary; need to screen for proper species to</td>
</tr>
<tr>
<td></td>
<td>use</td>
</tr>
<tr>
<td></td>
<td>Mode of action not fully understood; may alter growth and biomass</td>
</tr>
<tr>
<td></td>
<td>partitioning</td>
</tr>
</tbody>
</table>

Sources: Manning and Krupa (1992); Heggestad (1988); Kostka-Rick and Manning (1992b); Miller and Pursley (1994).

Such sensitive plants can be used to detect the presence of a specific air pollutant such as O$_3$ in the ambient air at a specific location or region and, as a result of the magnitude of their response, provide unique information regarding specific ambient air quality. Bioindicators can be either introduced *sentinels*, such as the widely used tobacco (*Nicotiana tabacum*) variety Bel W3, or *detectors*, which are sensitive native plant species (e.g., milkweed [*Asclepias syriaca*]). The approach is especially useful in areas where O$_3$ monitors are not operated (Manning et al., 2003). For example, in remote wilderness areas where instrument monitoring is generally not available, the use of bioindicator surveys in conjunction with the use of passive samplers (Krupa et al., 2001) is a particularly useful methodology (Manning et al., 2003). However, the method requires expertise or training in recognizing those signs and symptoms uniquely attributable to exposure to O$_3$ as well as in their quantitative assessment.

Since the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996), many new sensitive species have been identified from controlled exposure studies and verified in the field (Flagler, 1998; Innes et al., 2001). In addition, several new uses of this methodology have been demonstrated, including a national O$_3$ bioindicator network, studies in wilderness areas, and
mature tree studies. Although it has been difficult to find robust relationships between the foliar injury symptoms caused by O$_3$ and effects on plant productivity or ecosystem function, visible injury correlations with growth responses have been reported (Table AX9-2) (1998) (2003) (2003). One workshop on the utility of bioindicators of air pollutants led to a useful series of peer-reviewed publications in *Environmental Pollution* (Skelly, 2003).

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>No chambers required. Plants exposed to ambient conditions of O$_3$, light, temperature, etc.</td>
<td>Results are generally correlative in nature with no true control</td>
</tr>
<tr>
<td>Relatively inexpensive. Equipment needs are minimal. No “chamber effects”</td>
<td>Individuals need to be trained and experienced in O$_3$ symptom recognition</td>
</tr>
<tr>
<td>A high degree of replication possible (sentinels) both within and among locations</td>
<td>Need adequate numbers of plants (detectors) to ensure valid results</td>
</tr>
<tr>
<td></td>
<td>Need preliminary tests to insure a constant symptomology of material used</td>
</tr>
<tr>
<td></td>
<td>Need to use more than one indicator species (detector) per area if possible</td>
</tr>
<tr>
<td></td>
<td>Need to quantify site characteristics (soils, light) that may influence symptom expression</td>
</tr>
<tr>
<td></td>
<td>Need measurements of ambient O$_3$ (active or passive) and other meteorological variables (temp, rainfall, etc)</td>
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<tr>
<td></td>
<td>Need to ensure that cultural (sentinels) practices (soil, irrigation, fertilization, etc.) are similar among sites</td>
</tr>
</tbody>
</table>

**National network**

The U.S. Forest Service in cooperation with other federal and state agencies developed a network of O$_3$ bioindicators to detect the presence of O$_3$ in forested systems throughout the United States (Smith et al., 2003). This ongoing program was initiated in 1994; and 33 states currently participate. In a coordinated effort, a systematic grid system is used as the basis of plot...
selection, and field crews are trained to evaluate O$_3$ symptoms on sensitive plant species within the plots (Coulston et al., 2003; Smith et al., 2003).

The network has provided evidence of O$_3$ concentrations high enough to induce visible symptoms on sensitive vegetation. From repeated observations and measurements made over a number of years, specific patterns of areas experiencing visible O$_3$ injury symptoms can be identified. Coulston et al. (2003) used information gathered over a 6-year period (1994 to 1999) from the network to identify several species that were sensitive to O$_3$ over a regional scale including sweetgum (*Liquidambar styraciflua*), loblolly pine, and black cherry (*Prunus serotina*).

**Wilderness areas**

The use of bioindicator species as detectors has proven to be an effective technique for deriving a relative estimate of O$_3$ injury in wilderness areas in both the United States and Europe (Chappelka et al., 1997, 2003; Manning et al., 2002). However, to be truly effective, these regional and national bioindicator studies need the inclusion of air quality data and related growth studies to determine effects on productivity and ecosystem function (Bytnerowicz et al., 2002; Manning et al., 2003; Smith et al., 2003). In addition, O$_3$ often co-occurs with other air borne pollutants, so it is important to consider that, in some areas, other pollutants may be playing a role as well.

Chappelka et al. (1997, 2003) conducted surveys of foliar injury on several native plant species throughout the Great Smoky Mountains National Park (GRSM), including black cherry (*Prunus serotina*), tall milkweed (*Asclepias exaltata*), cutleaf coneflower (*Rudbeckia laciniata*), and crownbeard (*Verbesina occidentalis*). Visible foliar symptoms were prevalent throughout the Park, indicating that injury-producing O$_3$ levels were widespread in GRSM.

Manning et al. (2002) recently summarized a multiyear (1993 to 2000) bioindicator project in the Carpathian Mountain range in eastern Europe. They evaluated numerous trees, shrubs, forbs, and vines for possible symptoms of O$_3$ injury. Observations were made at plots located in the vicinity of either active or passive O$_3$ monitors (Bytnerowicz et al., 2002). Approximately 30 species of native plant detectors were identified as possible bioindicators, the majority of which (21) were shrubs (Manning et al., 2002). Based on these observations, it was concluded that O$_3$ concentrations were sufficiently high to impact ecosystems in the region. Similar
investigations regarding the sensitivity of native species have been conducted in Switzerland (Novak et al., 2003) and Spain (Orendovici et al., 2003).

**Mature tree detectors**

Many studies have reported visible injury of mature coniferous trees caused by O$_3$, primarily in the western United States (Arbaugh et al., 1998) and, to a lesser extent, to mature deciduous trees in eastern North America. In an effort to determine the extent and magnitude of visible injury in mature tree canopies, Hildebrand et al. (1996) and Chappelka et al. (1999b) conducted independent studies in the GRSM and the Shenandoah National Park (SHEN). The species examined were sassafras (*Sassafras albidum*), black cherry, and yellow-poplar (*Liriodendron tulipifera*) in GRSM and white ash (*Fraxinus americana*), black cherry, and yellow-poplar in SHEN. Protocols were similar at both parks, and trees were located near O$_3$ monitors at three different areas in each park. Results from both studies indicated that symptoms of O$_3$ injury were present in the trees and correlated with O$_3$ exposure both spatially and temporally. Ozone injury tended to be most severe at the highest elevation, except with yellow-poplar.

Hildebrand et al. (1996) observed significant O$_3$ exposure-plant response relationships with black cherry. The best relationships were found between foliar injury and the cumulative exposure statistics SUM06 and W126 (see Section AX9.5), indicating that higher O$_3$ concentrations were important in eliciting a response in black cherry. No O$_3$ exposure-plant response relationships were found with any species tested in GRSM (Chappelka et al., 1999b); but, when the data were combined for both parks, a significant correlation ($r = 0.72$) with black cherry was found for both SUM06 and W126, and injury was the greatest ($r = 0.87$) at the higher elevations (Chappelka et al., 1999a).

Based on a study in which visible symptoms of O$_3$ injury were characterized for large, mature yellow-poplar and black cherry trees in GRSM (Chappelka et al., 1999a), Somers et al. (1998) compared radial growth differences among trees classified as sensitive or nonsensitive based on the severity of visible foliar injury observed over a 3-year period (1991 to 1993). Significantly more radial growth was observed over both a 5- and a 10-year period for the nonsensitive compared to the sensitive trees. No significant relationship was found for black cherry tree growth.
Vollenweider et al. (2003a), using data collected from continuous forest inventory (CFI) plots across Massachusetts, compared growth rates among either symptomatic or asymptomatic mature black cherry trees. Of the 120 trees sampled in 1996, 47% exhibited visible foliar injury. Using CFI data, growth rates were compared over a 31-year period. The growth rates for symptomatic trees were reduced by 28% compared with asymptomatic trees.

Because these studies (Somers et al., 1998; Vollenweider et al., 2003a) were not controlled studies and used a small sample of trees, they cannot validly be used to characterize cause and effects related to the visible symptoms and radial growth they describe. However, the results indicate the possibility that O$_3$ is correlated with growth losses in some sensitive genotypes, illustrating the potential usefulness of this visible O$_3$ injury methodology in assessing effects on the growth rates of mature deciduous trees.

**Cultivar comparisons**

The idea of using cultivars or isogenic lines of crop species that differed in O$_3$ sensitivity as sentinels to determine the ambient effects of O$_3$ in the field was presented in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996). The rationale was that comparing the ratio of injury scores or some measure of growth between two different cultivars varying in O$_3$ sensitivity should be indicative of the relative amount of ambient stress to plants at a given location. A sensitive:resistant ratio close to unity would indicate relatively low O$_3$ concentrations; and a low ratio, higher O$_3$ levels. Results from locations differing in O$_3$ concentrations could be evaluated to develop exposure-response models. The original protocol was derived using two isogenic lines of white clover (Trifolium repens) differing in O$_3$ sensitivity (Heagle et al., 1994b, 1995).

This white clover model system has been used in several multi-location studies in the United States (Heagle and Stefanski, 2000) and Europe (Ball et al., 2000; Bermejo et al., 2002; Mills et al., 2000). Heagle and Stefanski (2000) compared results from eight sites over a 2-year period with various exposure indices (SUM00, SUM06, W126, and others) to determine a best-fit regression. They found that most of the indices preformed similarly. The highest $r^2$ values (0.87 to 0.93) were obtained using only the later harvests and a 6 h day$^{-1}$ index (1000 to 1600 h). Similar multiple-comparison studies conducted in Europe using the AOT40 index (Ball et al., 2000; Mills et al., 2000) yielded poorer $r^2$ values. Factors such as air temperature, NO$_x$...
(high levels at some sites), and lower O$_3$ concentrations in Europe were suggested to account in part for the differences between U.S. and European study results. Bermejo et al. (2002), in a study in Spain, improved the model by comparing the biomass ratio of these white clover isolines to measures of O$_3$ uptake (flux) rather than an exposure index (AOT40). Together, these studies indicate that systems such as the white clover model can help reveal O$_3$ exposure-response relationships and provide valuable information regarding ambient O$_3$ conditions in a given location. Table AX9-3 lists the advantages and disadvantages of the use of cultivar comparisons in assessing O$_3$ effects of plants.

<table>
<thead>
<tr>
<th>Table AX9-3. Advantages and Disadvantages of Cultivar Comparisons Used in Assessment of O$_3$ Effects on Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>No chambers required. Plants exposed to ambient conditions of O$_3$, light, temperature, etc.</td>
</tr>
<tr>
<td>Relatively inexpensive. Equipment needs are minimal. No “chamber effects”</td>
</tr>
<tr>
<td>A high degree of replication possible both within and among locations</td>
</tr>
<tr>
<td>Can conduct studies “in situ”</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
</tr>
<tr>
<td>Need preliminary tests to insure sensitivity and growth patterns of genotypes used are consistent</td>
</tr>
<tr>
<td>Need measurements of ambient O$_3$ and other meteorological variables (temp, rainfall, etc)</td>
</tr>
<tr>
<td>Have to ensure cultural practices (soil, irrigation, fertilization, etc.) are similar among sites</td>
</tr>
<tr>
<td>Need to closely monitor plants for presence of other factors that may cause a misinterpretation of results</td>
</tr>
</tbody>
</table>

**Dendrochronological techniques**

It has been difficult to determine whether O$_3$ significantly affects tree growth and productivity in the field, because O$_3$ concentrations are omnipresent and tree response to this pollutant is altered by many factors. The use of dendrochronological techniques to answer questions regarding ambient O$_3$ effects on forest growth and ecosystem function has recently emerged as a very useful biomonitoring methodology (Cook, 1990; McLaughlin et al., 2002).
The technique is useful when either instrument or passive O$_3$ monitoring methods are used to
determine ambient O$_3$ conditions.

Initial experiments were primarily correlative in nature and attempted to relate symptoms
of visible injury with growth losses as revealed by tree ring analysis (Arbaugh et al., 1998;
Benoit et al., 1983; Peterson et al., 1995; Somers et al., 1998; Swank and Vose, 1990). These
studies evaluated radial growth patterns determined by cores removed from trees in the presence
or absence of overt O$_3$ injury symptoms.

The method has also been adapted to better understand forest ecosystem function
(McLaughlin and Downing, 1995; Bartholomay et al., 1997; McLaughlin et al., 2003). The
response of mature loblolly pine growing in eastern Tennessee to ambient O$_3$ and moisture stress
was evaluated by McLaughlin and Downing (1995, 1996). They made radial growth
measurements from 12 to 37 times per year using dendrometer bands and determined
relationships between O$_3$, moisture stress, and radial growth. Exposures to O$_3$ concentrations
$\geq 0.04$ ppm with high temperatures and low soil moisture resulted in short-term depression in
radial growth. Reductions in growth were estimated to vary from 0 to 15% per year and
averaged approximately 5% per year.

Bartholomay et al. (1997) examined white pine (Pinus strobus) radial growth in eight
stands throughout Acadia National Park, Maine over a 10-year period from 1983 to 1992. They
related growth rates to several factors, including O$_3$ concentration. Ozone levels were negatively
correlated with radial growth in seven of the eight stands. Site characteristics were important in
the relationship: stands growing on shallow, poorly drained soils were most sensitive to O$_3$ in
the late portion of the growing season, possibly due to premature senescence of foliage.
However, litterfall measurements were not reported. Trees growing on better sites were more
sensitive to O$_3$ during the entire growing season, indicating the possibility of high O$_3$ uptake
rates throughout the growing season. Although these field studies (Bartholomay et al., 1997,
1996; McLaughlin and Downing, 1995) did not compare the direct effects of O$_3$ on the two pine
species, they indicate that potential interactions exist among O$_3$ and other climatic and edaphic
factors, such as temperature and soil moisture.

Using both automated and manual dendrometer bands, McLaughlin et al. (2002) examined
the growth response of yellow-poplar trees recently released from competition. In addition to
measuring growth, sap flow measurements were conducted and soil moisture was measured in
the vicinity of the trees. They were not able to detect O<sub>3</sub> effects in this 1-year study. Advantages and disadvantages of dendrochronology techniques for evaluating whole-tree physiological responses for individual trees and forest stands are listed in Table AX9-4.

### Table AX9-4. Advantages and Disadvantages of Various Dendrochronological Techniques Used in Assessment of O<sub>3</sub> Effects on Plants

<table>
<thead>
<tr>
<th><strong>Advantages</strong></th>
<th><strong>Disadvantages</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Provide information regarding growth effects under ambient conditions</td>
<td>Results are generally correlative in nature with no true control</td>
</tr>
<tr>
<td>Good historical information regarding O&lt;sub&gt;3&lt;/sub&gt; effects</td>
<td>Need background O&lt;sub&gt;3&lt;/sub&gt; and meteorological data (historical records)</td>
</tr>
<tr>
<td>Can provide data on daily and seasonal growth and O&lt;sub&gt;3&lt;/sub&gt; patterns and correlate with physiological function</td>
<td>Need to account for other factors such as competition, in analyzing data</td>
</tr>
<tr>
<td>Provide information on forest function related to ambient O&lt;sub&gt;3&lt;/sub&gt; concentrations</td>
<td>Individuals need to be trained in counting growth rings</td>
</tr>
<tr>
<td>Can link data with process-level growth models</td>
<td>Replication can be difficult (expensive and technological limitations)</td>
</tr>
<tr>
<td></td>
<td>Complicated statistical analyses are sometimes required</td>
</tr>
<tr>
<td></td>
<td>Can be expensive, especially if using automated growth (dendrometer) bands</td>
</tr>
</tbody>
</table>

The use and evolution of various dendrochronological methods in the field of air pollution effects research is reviewed in detail by McLaughlin et al. (2002). Automated dendrometer bands provide a powerful tool for measuring radial growth responses of trees on an hourly or daily basis. Diurnal patterns of growth can be related to water use and O<sub>3</sub> concentrations using time-series analyses. The major drawbacks of the method are that it is expensive and time consuming.
**AX9.1.3.5 Calibrated Passive Monitors**

Many studies have used passive monitors in the mapping of ambient O₃ concentrations, especially in remote areas (Cox and Malcolm, 1999; Grosjean et al., 1995; Krupa et al., 2001). Because they are cumulative recording devices, they do not record short-term variations in O₃ concentration but only the total exposure over a given interval, usually between 1 to 4 weeks. Thus, they produce a measurement that resembles the instrumentally derived exposure index SUM00. However, it is common to divide the cumulative exposure by the number of hours of exposure to get an hourly average. In addition, Krupa et al. (2001, 2003) were able to estimate the underlying frequency distribution of hourly O₃ concentrations from passive samplers using models based on a collocated O₃ monitor, showing the potential for passive samplers to provide estimates beyond total O₃ sum.

Runeckles and Bowen (2000) used the ZAPS system described in Section AX9.2.2.3 to subject both crops and passive monitors (Williams, 1994) to a range of exposures. Passive monitors were also exposed at 16 agricultural field sites along a transect through the Fraser Valley, British Columbia, Canada. Most field sites were downwind of the Greater Vancouver metropolitan area. All passive monitors were replaced at weekly intervals and the data from those in the ZAPS plots were “calibrated” to crop responses by means of Weibull exposure-response functions. Since the meteorological conditions throughout the valley were reasonably consistent from site to site, the use of these functions with data from the network passive monitors as inputs permitted the estimation of crop losses at the network sites. The overall method was, thus, a hybrid of several methodologies.

Although based on a single study, the use of passive monitors has potential for assessing crop losses at sites removed from locations with known ambient O₃ concentrations. Provided that the network and calibration sites have similar meteorological conditions, the method yields crop loss estimates that are responses to local ambient O₃ levels as influenced by local meteorological conditions.

**AX9.1.4 Numerical/Statistical Methodologies**

Proper experimental design strategies including replication, randomization, and experimental protocols are paramount in O₃-effects research. These have been discussed in detail in previous O₃ AQCDs (U.S. Environmental Protection Agency, 1996, 1986), as have the
different statistical analytical procedures used to determine the probable significance of results. However, new investigative approaches have demanded the adoption of new analytical methods. For example, the use of dendrochronological techniques has led to the use of time-series analysis (McLaughlin et al., 2003) and linear aggregate models (Cook [1990], as reviewed by McLaughlin et al. [2002]).

In spite of the rigors of the analyses, many differences occur in the published literature for almost any plant response to O₃ stress. Differences inevitably result from different researchers studying different locations, using different experimental methodologies and genetically different plant material even when using a common species. The techniques of meta-analysis can be used to consolidate and extract a summary of significant responses from a selection of such data.

Despite the differences in responses in the 53 primary studies used, a recent meta-analysis by Morgan et al. (2003) of the effects of O₃ on photosynthesis, growth, and yield of soybean showed “overwhelming evidence for a significant decrease in photosynthesis, dry matter production and yield. . . .across all the reported studies on effects of chronic O₃ treatment.” The meta-analysis defined O₃ stress as exposure to ~70 ppb O₃ for at least 7 days and found average shoot biomass and seed yield decreases of 34% and 24%, respectively. Furthermore, although other stress factors such as drought and UV-B did not affect the O₃ responses, elevated CO₂ was found to significantly decrease O₃-induced losses.

The meta-analysis method clearly has the potential to consolidate and refine the quantitative exposure-response models for many species. The majority of the reported growth and physiological responses related to O₃ stress are for individual plants, primarily in various types of exposure chambers. It is difficult to extrapolate these responses to stand/community, ecosystem, or region-wide assessments, particularly in view of the importance of the significant interactions that may occur between plant responses O₃ and other environmental stresses. Along with the shift in effects research to a more ecological approach, these concerns necessitate a move from simple regression analysis to more complex mathematical approaches to handle a wider array of independent input variables than O₃ exposure alone. Other independent input variables that must be accounted for include air and soil temperatures, soil moisture, relative humidity, wind speed, and, particularly in the case of natural systems, biotic factors such as pests and pathogens, plant density/spacing, and measures of plant competition.
Artificial neural network methodology was used by Balls et al. (1995) for “unraveling the complex interactions between microclimate, ozone dose, and ozone injury in clover” and in the study with the protectant chemical EDU, discussed in Section AX9.2.3.3 (Ball et al., 1998). The multi-factor model for predicting the effects of ambient O₃ on white clover developed by Mills et al. (2000) utilized both ANN and multiple linear regression methods.

Models incorporating ANNs are of the “regression” type (Luxmoore, 1988) in contrast to “mechanistic” or “phenomenological” models which have wider applicability. Process-level models of either type have been developed at the organelle, individual plant (Constable and Taylor, 1997; Weinstein et al., 1998), canopy (Amthor et al., 1994), and stand level (Ollinger et al., 1997; Weinstein et al., 2001) and provide estimates of the rate of change of response variables as affected by O₃ over time. However, as pointed out in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996), mechanistic process models lack the precision of regression models as well as their ability to estimate the likelihoods of responses. In their extensive reviews, Kickert and Krupa (1991) and Kickert et al. (1999) summarized the advantages and shortcomings of many different models and made the important point that most of the models that have been described provide consequence assessments that quantify the magnitudes of effects, but not risk assessments that quantify the likelihoods of such effects. Descriptions of several specific models are provided in other sections of this criteria document, and advantages and disadvantages of modeling techniques used in assessing O₃ effects on plants are summarized in Table AX9-5.

<table>
<thead>
<tr>
<th>Table AX9-5. Advantages and Disadvantages of Modeling Techniques Used in Assessment of O₃ Effects on Plants</th>
</tr>
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<tbody>
<tr>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>• Provide an understanding of cause-effect relationships over time</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
</tr>
<tr>
<td>• Have to make assumptions based on a paucity of data</td>
</tr>
<tr>
<td>• Most models are very complex and difficult to understand</td>
</tr>
<tr>
<td>• Need to be evaluated for predictive validity</td>
</tr>
</tbody>
</table>
AX9.1.5 Improved Methods for Defining Exposure

Ambient air quality is defined in terms of the measured O$_3$ concentrations in the air at plant height above ground level. Compilations of such concentration data have long been used as surrogates of the exposures to which plants are subjected. However, as long ago as 1965, field research provided evidence that plant response was a function, not of ambient O$_3$ concentration per se, but of the estimated flux of O$_3$ to the plant canopy (Mukammal, 1965). Subsequently, Runeckles (1974) introduced the term “effective dose” to define that part of the ambient exposure that was taken up by a plant. Fowler and Cape (1982) later referred it as “pollutant applied dose” (PAD), defined as the product of concentration, time and stomatal (or canopy) conductance, with units g m$^{-2}$. Such estimates of O$_3$ uptake or flux provide a more biologically relevant description of exposure than the simple product of concentration and time alone, and they formed the basis of Reich’s 1983 “unifying theory” of plant response to O$_3$ (Reich, 1983).

However, it was not until the early 1990s that the inherent advantages of using O$_3$ flux rather than O$_3$ concentration as a basis for determining response effects began to be widely accepted, as demonstrated by the subsequent increase in publications involving flux measurements and modeling (e.g., Fuhrer et al. [1997]; Grünhage and Jäger [2003]); Grünhage et al. [1993; 1997]; Massman et al. [2000]); Musselman and Massman [1999]; Pleijel [1998]). A key requirement for flux determination is the measurement of stomatal or canopy conductances, using established porometer/cuvette techniques or eddy correlation methods. The usefulness and relevance of flux as a measure of exposure are discussed in detail in Section AX9.4.

Efforts to develop regional-scale models of O$_3$ deposition and stomatal uptake are currently under way with a view to providing improved assessments of the risks to vegetation across Europe (Emberson et al., 2000; Simpson et al., 2001, 2003).

AX9.2 SPECIES RESPONSE/MODE-OF-ACTION

AX9.2.1 Introduction

The evaluation of O$_3$ risk to vegetation requires fundamental understanding of both the functioning of the vegetation and how external environmental influences can alter that function. For biological organisms subjected to atmospheric O$_3$, those alterations can be complex and
multiple. In addition, biological organisms have plasticity to external interactions due to their complex internal, self-correcting systems, making the task of identifying their “correct” functioning difficult. This section emphasizes reactions of O\textsubscript{3} with the cell and tissue, rather than the whole plant, to describe the fundamental mechanisms known to govern the response of the plant to O\textsubscript{3} exposure.

The many regulatory systems contained in leaves change both as a function of leaf development and in response to various environmental stresses. Leaves function as the major regulators of anatomical and morphological development of the shoot and control the translocation of carbohydrates to the whole plant (Dickson and Isebrands, 1991). This section discusses the movement of O\textsubscript{3} into plant leaves and their biochemical and physiological responses to O\textsubscript{3}.

The 1996 criteria document (U.S. Environmental Protection Agency, 1996) assessed the information available at that time concerning the biochemical and physiological responses to the movement of O\textsubscript{3} into plant leaves. This information continues to be valid. Ozone uptake in a plant canopy is a complex process involving adsorption to surfaces (leaves, stems, and soil) and absorption into leaves (Figure AX9-1). However, the initial biochemical changes that result within leaf cells after the entry of O\textsubscript{3} and how these changes interact to produce plant responses remain unclear. The response of vascular plants to O\textsubscript{3} may be viewed as the culmination of a sequence of physical, biochemical, and physiological events. Only the O\textsubscript{3} that diffuses into a plant through the stomata (which exert some control on O\textsubscript{3} uptake) to the active sites within a leaf impairs plant processes or performance. An effect will occur only if sufficient amounts of O\textsubscript{3} reach sensitive cellular sites that are subject to the various physiological and biochemical controls within the leaf cells. Ozone injury will not occur if (1) the rate and amount of O\textsubscript{3} uptake is small enough for the plant to detoxify or metabolize O\textsubscript{3} or its metabolites or (2) the plant is able to repair or compensate for the O\textsubscript{3} impacts (Tingey and Taylor, 1982; U.S. Environmental Protection Agency, 1996). Therefore, a precondition for O\textsubscript{3} to affect plant function is that it must enter the stomata and be absorbed into the water lining the mesophyll cell walls. The response of each plant is determined by the amount of O\textsubscript{3} entering the leaves, which varies from leaf to leaf.

Some potentially significant processes have been investigated since the 1996 criteria document, especially detoxification and compensatory processes. The role of detoxification in
Figure AX9-1. Ozone uptake from the atmosphere. Ozone moves from the atmosphere above the canopy boundary layer into the canopy primarily by turbulent air flow. Canopy conductance, controlled by the complexity of the canopy architecture, is a measure of the ease with which gases move into the canopy. Within the canopy, O\textsubscript{3} is adsorbed onto surfaces as well as being absorbed into the foliage. Foliage absorption is controlled by two conductances, leaf boundary layer and stomatal, which together determine leaf conductance. The solid black arrows denote O\textsubscript{3} flow; dotted arrows indicate processes affecting uptake or response to O\textsubscript{3}. Boxes at the left with double borders are those processes described in the figure.

providing a level of resistance to O\textsubscript{3} has been investigated; however, it is still not clear as to what extent detoxification can protect against O\textsubscript{3} injury. Data are needed especially on the potential rates of antioxidant production and on the subcellular localization of the antioxidants. Potential rates of antioxidant production are needed to assess whether they are sufficient to detoxify the O\textsubscript{3} as it enters the cell. The subcellular location(s) is needed to assess whether the antioxidants are in cell wall or plasmalemma locations that permit contact with the O\textsubscript{3} before it has a chance to damage subcellular systems. Although these processes divert resources away from other sinks,
detoxification and compensation processes may counteract the reduction in canopy carbon fixation caused by O$_3$. The quantitative importance of these processes requires investigation.

As a result of the research since the 1996 criteria document (U.S. Environmental Protection Agency, 1996), the way in which O$_3$ exposure reduces photosynthesis, especially its effects on the central carboxylating enzyme, Rubisco (ribulose-1,5-bisphosphate/carboxylase), is better understood. The rate of leaf senescence has been shown to increase as a function of increasing O$_3$ exposure. The mechanism of the increased senescence is not known, and, hence, it deserves further study.

Finally, the role that changes in allocation of resources play in plant response to O$_3$ is now better understood. Most studies have shown that O$_3$ decreases allocation of photosynthate to roots. In some cases, allocation to leaf production has increased. Whether these changes are driven entirely by changes in carbohydrate availability or are controlled by other factors (e.g., hormones) is not known. Physiological effects within the leaves inhibit photosynthesis; alter the assimilation of photosynthate and shift its allocation patterns; and can lead to reduced biomass production, growth, and yield (U.S. Environmental Protection Agency, 1986, 1996).

The major problem facing researchers trying to predict long-term O$_3$ effects on plants is determining how plants integrate the responses to O$_3$ exposures into the overall naturally occurring responses to environmental stressors. Little is now known about how plant responses to O$_3$ exposures change with increasing age and size, but this information is crucial to predicting the long-term consequence of O$_3$ exposure in forested ecosystems.

This section focuses on reactions of O$_3$ within cells and cellular tissue, in order to explain known mechanisms that govern plant responses. The processes that occur at cell and tissue levels within the leaf will be divided into several steps beginning with O$_3$ uptake and its initial chemical transformations into a series of currently unknown, but suspected toxic, chemicals (Figure AX9-2). The discussion will then focus sequentially upon various cell regions, their general physiology, and the changes that may occur within a plant after O$_3$ exposure. This is important because the varying responses of the different plant species in a community ultimately lead to an ecosystem response. Finally, a general summary is presented that discusses the known or suspected changes that occur within the whole plant.
Absorption and transformation of O$_3$ within the leaf. The varied processes are broken down into smaller mechanistic steps that lead from uptake of atmospheric O$_3$ into the alterations which may occur within the individual plant. Each plant responds to the O$_3$ level and therefore interacts with the total ecological setting to generate an ecosystem response due to the O$_3$.

**AX9.2.2 Mechanisms of Ozone-Induced Plant Alterations**

Plants can survive O$_3$ stress through exclusion or tolerance mechanisms (Levitt, 1972) (Tingey and Taylor, 1982). Ozone may be excluded from tissues or cells via stomatal closure, by extracellular oxidants, or by membrane impermeability to O$_3$ or its products. Past investigations of O$_3$ injury have indicated that physiological and metabolic changes occur (Harris and Bailey-Serres, 1994; Heath, 1988; Heath and Taylor, 1997; Reddy et al., 1993). Many of these changes are likely initiated via gene expression. During the last decade, our understanding of the cellular processes within plants has increased. Although the fundamental hypotheses concerning O$_3$-induced changes in physiology have not changed, a more complete development of the theories is now nearing possibility.
AX9.2.2.1 Changes in Metabolic Processes: Current Theories

The current hypotheses regarding the biochemical response to O$_3$ exposure revolve about injury and its prevention. These are well discussed by Pell et al. (1997) and are listed below in no order of importance. Although they are listed separately, some may be interlinked and related to each other.

1. **Membrane Dysfunction.** The membrane is altered by O$_3$, principally via protein changes not involving the lipid portions of the membrane (except at extremely high levels of O$_3$). These alterations involve increased permeability with perhaps lessened selectivity, declines in active transport, and changes in the trigger mechanisms of signal transduction pathways such that the signals are no longer suitable for the state of the cell. The cellular pools and transport systems of Ca$^{2+}$/K$^+$/$H^+$ are the primary suspects.

2. **Antioxidant Protectants.** Varied antioxidants (both as metabolites and enzyme systems) can eliminate the oxidant or its products, if present at time of fumigation and in sufficient abundance. However, oxidant entry that occurs rapidly can overwhelm the antioxidant response.

3. **Stress Ethylene Interactions.** Visible injury is caused by the interaction of O$_3$ with stress-induced ethylene, either by direct chemical transformation to a toxic product or by alteration of the biochemical relations at the ethylene binding site.

4. **Impairment of Photosynthesis.** A product of O$_3$ (and less probably, O$_3$ itself) enters the cell, causing a decline in the mRNA for Rubisco (especially the message RNA species of rbcS and rbcL) such that Rubisco levels slowly decline within the chloroplast, leading to a lowered rate of CO$_2$ fixation and productivity. This process is very similar to early senescence and may be linked to general senescence. Alternatively, a false signal is generated at the cell membrane which lowers the transcription of DNA to mRNA. Ozone alters the normal ionic and water relations of guard cells and subsidiary cells, causing the stomata to close and limit CO$_2$ fixation. In any case, the response of the stomata to the current environment does not promote efficient photosynthesis.

5. **Translocation Disruption.** One of the biochemical systems most sensitive to O$_3$ exposure is the translocation of sugars, such that even a mild exposure inhibits the translocation of carbohydrate (Grantz and Farrar, 1999, 2000).

6. **General Impairment/Disruption of Varied Pathways of Metabolism.** This is the oldest and most vague concept of how O$_3$ alters metabolism. It is based upon early work in which the enzymes and metabolites that could be assayed were. Thus, these results were based upon what could be done, rather than on a coherent hypothesis. The best examples are listed in Dugger and Ting (1970).
The latter two theories can be restated as a loss of productivity with three possible somewhat-independent causes: (a) a reduced production of the basic building blocks of growth and, hence, a slowing of growth in at least one organ; (b) a reduced ability to reproduce, leading to a decreased production of viable seeds or of fruits and nuts; and (c) a decreased ability to mount a defense against pathogens or insects, leading to weaker plants, which are more liable to be overcome by other stresses. It is important to separate out effects that may be detrimental or disfiguring, such as the production of visible injury, but which have not been shown to lead directly to a loss of productivity due to possible compensation by the remaining tissue.

AX9.2.2.2 Modifications of Plant Physiological Processes

The discussion that follows will focus on physiological processes rather than on species-specific responses; in most cases, the mechanisms of response are similar regardless of the degree of sensitivity of the species. Therefore, *Arabidopsis*, whose physiology and genome continue to be studied and described by a large number of scientists is an appropriate model plant for studying O₃ injury. Though the responses of mature trees and understory plants are critical to understanding plant interactions at an ecosystem level, the time required for trees to reach maturity makes using them to study biological mechanisms an inefficient choice.

The high levels of O₃ used for some investigations do not automatically invalidate the results obtained in those studies. Typically when a new hypothesis is being investigated, extreme levels of the toxicant are used to determine its effects clearly. The older studies that used concentrations as high 1 ppm, an extreme level, helped to define current studies. Later experiments have used concentrations nearer ambient levels. Many of the current studies on physiology use exposures between 0.15 and 0.25 ppm, which though higher than ambient levels in some areas of the country, bypass confounding changes but allow for rapid experiments.

Three forms of air pollutant-induced injury patterns are currently known to exist: (a) acute stress, generated by high atmospheric concentrations of pollutants for short periods of time; (b) chronic stress, generated by lower concentrations of pollutants for long periods of time; and (c) accelerated senescence, generated by very low concentrations of pollutants for very long periods of time. At higher levels, distinct visible injury generally occurs due to cellular and tissue death of regions of leaf mesophyll cells. This leads to a decline in the total area of metabolically active tissue, with consequent loss of membrane integrity, loss of metabolites into
the extracellular tissue space, and formation of oxidative products. When no visible injury is observed, lowered rates of photosynthesis or productivity are often used to document injury. Under these conditions, metabolism is altered and the pool sizes of many metabolites are changed. More importantly, the altered biochemical states within the tissue lead to the inability of the plant to respond properly to existing environmental conditions and to other stressors (Heath, 1988, 1994b; Koziol and Whatley, 1984; Manning and Keane, 1988; Schulte-Hosted et al., 1988).

**AX9.2.3 Ozone Uptake by Leaves**

Plants respond to O₃ similarly to other stressors on the levels of exclusion, tolerance, and repair (Levitt, 1972). The response mechanism depends upon the O₃ concentration, environmental conditions, and the developmental and metabolic state of the plant (Guzy and Heath, 1993). These responses are detrimental to plant productivity, because they cost the plant metabolic resources. In some cases, the stomata close under the O₃ exposure, excluding the pollutant from the leaf interior and preventing injury. However, if this happens too often, CO₂ fixation is also inhibited and plant productivity suffers.

Atmospheric O₃ does not cause injury, but rather it is the O₃ that enters the plant that causes an effect (Guzy and Heath, 1993; Tingey and Taylor, 1982). Three well-defined, sequential processes control the movement of O₃ from the atmosphere into the sites of action within the leaf and must occur to trigger O₃ stress (Heath, 1980). The processes are (1) entry of O₃ into the leaf, (2) reactions of O₃ and its possible reaction product(s) in the water phase at cell surfaces, and (3) movement of an O₃ reaction product(s) into the cell with enzymatic or chemical transformation of those products in the cell.

**Process 1. Entry of O₃ into the leaf.** Often incorrectly, the external concentration of O₃ is used to give an indication of “dose” (Heath, 1994a). Ozone-induced changes on a plant’s cuticle are minimal, and O₃ does not penetrate the cuticle (Kerstiens and Lendzian, 1989) to cause an effect. As O₃ has no easily measured isotope, virtually no measurements have been done on an actual dose of O₃, i.e., the amount of O₃ which reacts with individual biochemicals in the leaf. Yet the measurement of dose will be the amount of O₃ expected to penetrate into the tissue through the stomata. Dose is expressed as a rate of delivery to a surface area (mol/m² s⁻¹).
Whether dose or total accumulation (mole/m², rate integrated over exposure time) is most critical for the development of injury remains a major question.

Ozone uptake includes gaseous diffusion through the leaf boundary layer and stomata into the substomatal cavity (Figure AX9-3). Although the movement of pollutants through a boundary layer into the stomata region is known to be important, and even rate limiting in many cases of low wind velocity, its description has been defined from aeronautical concepts and usually relates to smooth surfaces that are not typical of leaf-surface morphology; however, it is nearly the only treatment available (Gates, 1968). Once through the boundary layer, the gas must enter the leaf through the stomata. The entry of gases into a leaf is dependent upon the physical and chemical processes of gas phase and surfaces and is a well-defined path that approximately follows a linear flux law of:

$$j = g(C_o - C_i)$$  \hspace{1cm} (AX9-1)

where the flux, $j$, into the internal space of a leaf is related to the conductance, $g$, through the boundary layer and stomata and the gradient of concentration of gas from the outside, $C_o$, inward, $C_i$. This formulation has been used for years for both water and CO$_2$ (Figure AX9-4), and for regions of varied CO$_2$ concentration that correspond to $C_o$ (CO$_2$ of the atmospheric air, below the leaf proper) and $C_i$ (CO$_2$ near the leaf’s spongy mesophyll cells) (Ball, 1987; Farquhar and Sharkey, 1982).

Figure AX9-3. The uptake of O$_3$ into the leaf. Each of the individual concentration layers of O$_3$ represents a different process of movement and of plant/microenvironmental interaction. This figure leads into Table 9-6, in which the amounts of O$_3$ along the pathway are calculated.
In the past, the internal concentration of O$_3$ has been assumed to be zero (Laisk et al., 1989), due to early studies that found that virtually no O$_3$ could pass through a leaf. That was expected because O$_3$ is extremely reactive with cellular biochemicals. If the assumption that the internal concentration zero is correct, then the effective delivery rate for O$_3$ is given as g × C$_o$, with stomatal conductance being the major regulatory control (Amiro et al., 1984; Taylor et al., 1982). However, a recent study by Moldau and Bichele (2002) indicated that the internal O$_3$ concentration may not be zero as previous assumed. Moldau and Bichele (2002) permitted leaves of Phaseolus vulgaris L., which have stomata on both upper and lower leaf surfaces, to take up O$_3$ at a high rate for 3 to 5 min. Exposure of the lower leaf surface resulted in up to 5% of the O$_3$ that was taken up to be diffused through the leaf, emerging from the stomata on the upper surface. This suggested the presence of above-zero concentrations of O$_3$ in the intercellular leaf air spaces. The descriptive calculations and plots of Moldau and Bichele (2002) indicate that the rise in internal O$_3$ level (from both sources of external O$_3$ concentration) within the first few minutes of exposure is due to its reaction with an antioxidant, most probably absorbate, within the apoplastic space of the leaf (Figure AX9-5). The rate of rise is probably
Figure AX9-5. The change in the O$_3$ concentration inside a leaf with time. Data are from O$_3$ exposures at two different concentrations.

Source: Derived from data in Moldau and Bichele (2002).

due to more complete penetration of O$_3$ with a concurrent depletion of the external antioxidant. The rise peaks at about 2 min for 0.82 ppm and 3 min for 0.34 ppm and then falls to a lower level. This may be due to a replenishment of the antioxidant. The authors saw no injury to the plasmalemma (as measured by penetration of a dye) and no change in the stomatal conductance for the lower concentration of O$_3$ (Moldau and Bichele, 2002). The higher level (0.88 ppm) caused the plasmalemma of the mesophyll cells to pass a dye, and a slight decline in stomatal conductance resulted at about 2.5 minutes. These data suggest that the antioxidant hypothesis is correct.

Gaseous pollutants flow from the substomatal cavity within the leaf through the cell wall into the cell. It is suspected that the internal concentration of the pollutant is not uniform within the cavity (Taylor, and Hanson, 1992). From within the wall, an equilibrium between the gas and aqueous phase must occur at the interface where the gaseous species dissolve into the water according to Henry’s Law (Heath, 1980, 1987; Wellburn, 1990). It is important to understand
exactly how much O$_3$ could move into the tissue of the leaves. Calculations in Table AX9-6 give an indication of the amount of O$_3$ which may end up near the surface of cells within the leaf. The calculation is done for a standard temperature (25 °C), an ambient concentration of O$_3$ (0.10 ppm), and for nonspecific leaves. For example, 0.3 ppm would be the same general numbers but multiplied by 3. Similarly for more closed stomata, the value of 1.0 cm/s (equivalent to about 400 µmole$^{-2}$-leaf area s$^{-1}$) for a conductance would be reduced and the smaller values would lead to a smaller amount of O$_3$ moving into the tissue. Nonetheless, these values give some indication of what sort of chemical concentration can be expected. Under these conditions, a delivery rate of O$_3$ into the substomatal cavity near the spongy mesophyll tissue of about 0.42 nmol/(L·h) appears to be reasonable.

**Process 2.** Ozone diffuses into the leaf air spaces and reacts either with varied biochemical compounds that are exposed to the air (path 1) or is solubilized into the water lining the cell wall of the air spaces (path 2). As shown in Figure AX9-6, each reaction has the possibility of transforming O$_3$ into another chemical species (a toxicant) which, in turn, may react with other chemical species and lead to a cascade of reactions.

Within the stomata, gases react with the water at the cell’s surface and generate new species with the components within the cell wall region. The possible varied pathways are depicted in Figure AX9-7. Although these chemical reactions are poorly understood, some of the fundamentals are known (Heath, 1987, 1988; Wellburn, 1990). Ozone reacts with organic molecules at the double bonds to form carbonyl groups and, under certain circumstances, generates peroxides, such as hydrogen peroxides (H$_2$O$_2$), superoxide (O$_2^-$) and its protonated form (HO$_2^+$), hydroxyl radicals (HO$^+$), and peroxy radicals (HO$_2^-$). Other chemicals present in the water phase can lead to many other oxygenated moieties (Figure AX9-6). Each of the steps is generally pH dependent (Jans and Hoigne, 2000; Walcek et al., 1997).

Sulfhydryls are particularly easy targets, with the formation of disulfide bridges or sulfones (Mudd and Kozlowski, 1975). In water, the reactions become more confusing, but some products have been described by Heath and Castillo (1987), such as H$_2$O$_2$, HO$^+$, and O$_2^-$ (Figure AX9-7). Effective detoxification reactions can occur here via antioxidant metabolites and enzymes such as ascorbate, glutathione (GSH), and superoxide dismutase (SOD) if they are present at high enough concentrations (Castillo et al., 1987; Matters and Scandalios, 1987).
Table AX9-6. The Flow of Ozone into a Leaf and Possible Reactions

The level of O$_3$ in the atmosphere is chosen to be close to a standard and yet make calculations to other amounts easy. The same concept will be used for all standard parameters for these calculations.  

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>The atmospheric level of O$_3$ is given as:</td>
<td>O$_{3a}$ = 0.1 ppm</td>
</tr>
<tr>
<td>For an air temperature of:</td>
<td>T$_a$ = 25 °C</td>
</tr>
<tr>
<td>The perfect gas law ($pV = nRT$) is used to convert the O$_3$ level into standard mks. Further, the volume for a mole of gas (V$_0$ = 22400 m$^3$) will be used, from the perfect gas law with R = 8.3144</td>
<td></td>
</tr>
<tr>
<td>Thus, the concentration of O$_3$ within the atmosphere is:</td>
<td>C$_{O3}$ = 4.873 x 10$^{-12}$ moles/m$^3$</td>
</tr>
<tr>
<td>The stomatal conductance of the gas must be chosen to be standard but adjustable. The number should be as large as typically measured but allow for easy conversion, if necessary. For a stomatal conductance of:</td>
<td>gs$_{sw}$ = 1 cm/s</td>
</tr>
<tr>
<td>The amount of O$_3$ that will penetrate inside the leaf (for a typical concentration of nearly zero inside the leaf), is:</td>
<td>O$_{3L}$ = 2.984 x 10$^{-14}$ mol/(m$^2$.s)</td>
</tr>
<tr>
<td>In terms of amount of water within the leaf, we can assume that about 85% of the weight is water and the density of water is 1 g/mL. A typical leaf has a wet weight/area:</td>
<td>FW$_L$ = 30 mg/cm$^2$</td>
</tr>
<tr>
<td>Thus, the square surface area of the leaf will translate into water space (for concentration of chemicals), as:</td>
<td>Ar$_L$ = 0.255 L/m$^2$</td>
</tr>
<tr>
<td>The maximum amount of toxic compound that will be generated, assuming all the O$_3$ is converted, is given below. Here the units of the leaf area weight are converted into the mks system and the water space units are converted into L, such that the concentrations calculated will be in mol/(L.hr). The final units assume that the O$_3$ is present (and no back reactions occur) for one hour (short but typical units of exposure).</td>
<td></td>
</tr>
<tr>
<td>O$<em>{3Lc}$ = (O$</em>{3L}$ / Ar$_L$) x 3600</td>
<td>O$_{3Lc}$ = 4.21 x 10$^{-10}$ mol/(L-h)</td>
</tr>
<tr>
<td>Thus, the maximum amount of toxic chemicals generated per hour in a leaf would be:</td>
<td>O$_{3Lc}$ = 0.42 nmol/(L-h)</td>
</tr>
</tbody>
</table>

Possible errors in these calculations (aside from the input numbers) are (1) the O$_3$ within the leaf does not react uniformly within the leaf space; (2) the O$_3$ within the leaf does not totally convert to any one species; (3) varied products of O$_3$ react, leading to innocuous chemicals; and (4) O$_3$ reactions can be catalytic and generate more reactions by radical reaction cycling.
Figure AX9-6. Possible transformations of O$_3$ within a leaf.

Figure AX9-7. Possible reactions of O$_3$ within water. (a) Ozone reacts at the double bonds to form carbonyl groups. (b) Under certain circumstances, peroxides are generated.
If the levels are low, it is believed that stimulation of their production is a response to O₃, albeit a slow one (Harris and Bailey-Serres, 1994). Certainly it is possible that chemical modification of wall-specific biochemicals (Castillo et al., 1987) such as glucan synthase (Ordin et al., 1969) and diamine oxidase (Peters et al., 1988) occurs.

**Process 3. Movement of reaction product(s) into and enzymatic or chemical transformations within the cell.** It is believed that the initial site of O₃ injury is near or within the plasma membrane. Certainly, membrane functions, such as membrane fluidity (Pauls and Thompson, 1980), permeability (Elkley and Ormrod, 1979), K⁺-exchange via ATPase reactions (Dominy and Heath, 1985), and Ca²⁺ exclusion (Castillo and Heath, 1990), are changed. The similarity of wounding responses (Langebartels et al., 1991) and O₃-induced membrane disruption suggests the induction of normal wound-regulated genes (Mehlhorn et al., 1991; Sandermann, 1998). This implies that O₃ can react with cell-wall components that are connected to the cytoplasm through the cell wall and membrane by membrane-specific proteins that are not directly involved with transport.

Ozone is soluble in water and once having entered the aqueous phase, it can be rapidly altered to form oxidative products that can diffuse more readily into and through the cell and react with many biochemicals. Again, the presence of an internal antioxidant would be critical to reduce the concentration of most oxidants. A toxic product of O₃ may migrate through the cytoplasm to react with photosynthetic processes, or a spurious signal generated at the membrane may affect some control process or signal transduction pathway (Schraudner et al., 1998; Overmyer et al., 2000, 2003; DeCaria et al., 2000; Rao et al., 2002; Booker et al., 2004; Leitao et al., 2003; Rao and Davis, 2001; Sandermann, 2000; Vahala et al., 2003b).

**AX9.2.3.1 Possible Reactions Within the Leaf**

Ozone can react with many compounds within the substomatal cavity of the leaf¹ to produce a variety of oxidizing and toxic chemicals. Some of the possible reactions that will generate H₂O₂, HO⁻, and SO₄²⁻, as well as charged O₃ intermediates, are indicated in Figure AX9-8. Many of these complex reactions have been studied within water solutions through

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¹The volume of the substomatal cavity (that are within the leaf immediately below the stomata) must be regarded as the region in which most O₃ reactions occur. That volume, at a relative humidity of near 100%, possesses many diverse surfaces with varied bonding, which could alter the fate of O₃.
Figure AX9-8a,b. The Crigee mechanism of O₃ attack of a double bond. (a) The typical Crigee mechanism is shown in which several reactions paths from the initial product is shown. (b) Typical reaction of ascorbic acid with O₃.

Source: Adapted from Mudd (1996).
research of O₃-induced water purification and are very dependent upon solutes present with the solutions, including H⁺ (see Von Gunten [2003]). An important point is that in alkaline media, O₃ forms H₂O₂, but in acid media, O₃ is relatively stable in the absence of free metal ions.

The rates of reaction of O₃ with several important compounds, including those with a double bond, the so-called Crigee Mechanism shown in Figure AX9-8, can be calculated from the reaction coefficient as given by Atkinson (1990) (Table AX9-7). The double bond of the ascorbate molecule is particularly sensitive to O₃ attack. Because of the ring formation of the ascorbate molecule, an unstable ozonide product is formed, which then accelerates the breakage of the double bond, leading to the formation of two products. These products are relatively unstable and can lead to further reactions not shown in Figure AX9-8. The rates of reactions can be calculated (Heath, 1987). At a local concentration of 25 µM O₃, it would take 5000 s (83 min) for all of the O₃ to react if there was no further flow of O₃. Clearly, O₃ does not react rapidly with the compounds in Table AX9-7 and, although some of the products would be formed through the Crigee Mechanism (see Figure AX9-8a), they would be low in concentration². While other radicals, such as the hydroxyl radical (see Figure AX9-8b) can attack double bonds, the products differ. Of particular note for later discussion, is the reaction of O₃ with ascorbate (see Mudd (1996) (see Figure AX9-8b), which will cleave the double bond in the ring. Unfortunately, little work has been done to characterize possible products within the leaf (but see next section).

In a paper discussing the stability and reactivity of O₃ in the pulmonary air/tissue boundary, Pryor (1992) calculated that O₃ has a half-life of about 7 × 10⁻⁸ s in a bilayer. However, the transit time through the lung lining fluid layer is about 2 × 10⁻⁶ s, based upon a reasonable estimate for the diffusion of O₃. This means that O₃ would suffer nearly 29 half-lives³ in passage through the layer, reducing it to about 3 × 10⁻⁹ of the original concentration — zero for all practical aspects. In the same publication, Pryor points out that any sulfhydryl or ascorbate would interact strongly with O₃, further reducing its net concentration. The reactivity of cysteine is 10⁹, while the reactivity of tryptophan, methionine, polyunsaturated fatty acids,

²For example, hydroxymethyl hydroperoxide would be expected to be formed by the reaction of O₃ with ethylene and its effects have been tested on peroxidases (Polle and Junkermann, 1994). Unfortunately, the concentration of required for inhibition is much higher than would be expected to be formed within the leaf.

³Here a half-life is the time that it takes the reactive species to travel a distance in which it loses 50% of its initial concentration. Therefore for a 29 half-life, the concentration has been reduced by 2⁻²⁹ or about a 10⁻⁹ decline.
Table AX9-7. Some Rates of Reaction of Ozone With Critical Biochemicals

[a] **Double bond reactions.** The second column is taken from Atkinson (1990) and transformed into Column 3. Those rate coefficients are used to calculate the rate of reaction at a concentration of 10 ppm for the organic and 0.1 ppm for O$_3$ in the air stream within the leaf (localized concentration of about 25 mM, see Table AX9-6).

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\times 10^{-18}$ cm$^3$/molecules s$^{-1}$</th>
<th>Rate coefficient (L/mole s$^{-1}$)</th>
<th>Rate of reaction (M/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethane</td>
<td>1.7</td>
<td>$1.02 \times 10^3$</td>
<td>$4.3 \times 10^{-11}$</td>
</tr>
<tr>
<td>Propene</td>
<td>11.3</td>
<td>$6.80 \times 10^3$</td>
<td>$2.8 \times 10^{-10}$</td>
</tr>
<tr>
<td>1-butene</td>
<td>11</td>
<td>$5.91 \times 10^3$</td>
<td>$2.5 \times 10^{-10}$</td>
</tr>
<tr>
<td>trans-2 Butene</td>
<td>200</td>
<td>$1.20 \times 10^5$</td>
<td>$5.0 \times 10^{-9}$</td>
</tr>
<tr>
<td>$\alpha$-pinene</td>
<td>85</td>
<td>$5.12 \times 10^4$</td>
<td>$2.1 \times 10^{-9}$</td>
</tr>
</tbody>
</table>

[b] **Possible Oxidative Species.** Another possibility is given by the reactions below from Walcek et al. (1997).

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Rate constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) $\text{O}_3 + \text{OH} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 + \text{OH}$</td>
<td>$k_1 = 3.67 \times 10$ mole$^{-1}$ L s$^{-1}$</td>
</tr>
<tr>
<td>(2) $\text{O}_3 + \text{O}_2 \rightarrow \text{HO} + 2 \text{O}_2 + \text{OH}$</td>
<td>$k_2 = 1.26 \times 10^9$ mole$^{-1}$ L s$^{-1}$</td>
</tr>
<tr>
<td>(3) $\text{O}_3 + \text{HO}_2 \rightarrow \text{HO} + \text{O}_2 + \text{O}_2$</td>
<td>$k_3 = 2.09 \times 10^6$ mole$^{-1}$ L s$^{-1}$</td>
</tr>
</tbody>
</table>

c] **Possible Concentrations of Other Oxidative Species.** Table from Heath (1987). Based upon 100 ppm O$_3$ in gas stream.

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration (M)</th>
<th>pH 7</th>
<th>pH 9</th>
<th>Molecules within wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide Radical ($\text{O}_2^\cdot$)</td>
<td></td>
<td>$8.75 \times 10^{-15}$</td>
<td>$1 \times 10^{-12}$</td>
<td>$5.5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Ozone Radical</td>
<td></td>
<td>$4.16 \times 10^{-15}$</td>
<td>$5 \times 10^{-14}$</td>
<td></td>
</tr>
<tr>
<td>Protonated O$_3$ radical ($\text{HO}_3^\cdot$)</td>
<td>$1.48 \times 10^{-16}$</td>
<td>$1 \times 10^{-18}$</td>
<td>$5.5 \times 10^{-6}$</td>
<td>$6.3 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Number of molecules within apoplastic space of (10$^{-12}$ L) at 0.1 ppm O$_3$.

and tyrosine is about $2 \times 10^6$ and that of phenylalanine is only $10^3$. These numbers are similar to what has been found for O$_3$ reactivity with amino acids and proteins in aqueous solutions.

In glycoporphin (Banerjee and Mudd, 1992) and cytochrome C (Mudd et al., 1997b; Mudd et al., 1996) in aqueous solutions, only the methionine was oxidized by O$_3$, producing sulfoxide.

In other proteins lacking methionine, tryptophans were oxidized only if they were in an exposed position on the surface of the proteins (Mudd et al., 1997b). Treatment of red blood cell ghosts
with O₃, oxidized peripheral proteins of the plasma membrane before it oxidized lipids (Mudd et al., 1997a).

**AX9.2.3.2 Toxicants Within the Wall Space**

While Mehlhorn et al. (1990) are often thought to have shown that free radicals were formed in plant leaves under O₃ exposure, careful reading of that paper clearly shows that there was no real evidence of free radicals induced by O₃. Living tissues have many free radical signals, making it difficult to observe changes in free radicals. Further, the work of Grimes et al. (1983) has also been cited as showing the presence of free radicals in living tissues due to O₃ exposure; however, no radical signals were found unless certain organic acids (e.g., caffeic acid) were added to the tissue with the O₃ exposure. They used the radical trap TMPO (tetramethylphrrolise 1-oxide) which reacts with many types of free radicals to form a stable radical that can be used to “trap” or increase the amount of radical present (see Figure AX9-9a). Ozone would directly react with this trap only if it were bubbled into the solution, not passed over the top of the solution. In the presence of sorbitol or caffeic acid, the trap would indicate the presence of OH radical, which would mean that O₃ → HO’. Superoxide dismutase, catalase, or EDU had no effect upon this signal, suggesting O₂ and H₂O₂ were not involved in the above sequence. Both O₃ and O₃ plus caffeic acid had no effect upon the protoplasts’ intactness or viability. Thus, 10⁻⁵ M HO’ and/or 0.30 to 0.40 ppm O₃ did not react with the cell membrane. They found no signal in normal cells after subjecting the leaf to O₃ and concluded that the radicals were produced via a concerted mechanism with the acid. This does not fit with the mechanism postulated by Mehlhorn et al. (1991), which involved a reaction of wound-induced ethylene and O₃ at the wall level to generate some free radicals.

The hypothesis that the production of wound-induced ethylene by O₃ exposure and its reaction with O₃ would result in the production of radicals was tested by Mehlhorn et al. (1990), using electron paramagnetic resonance spectroscopy. After 4 h of 300 ppb, an EPR signal of a compound was detected which resembled a butonyl radical (Figure AX9-9b [Mehlhorn et al., 1990]). Using 70 ppb, the signal was reduced by about one third that of an ethyl radical⁴.

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⁴The reaction would be: O₃ + H₂C = CH₂ → varied C-1 compounds, due to double bond cleavage, at a rate constant of $1.7 \times 10^{18}$ cm³/molecule sec = $1.02 \times 10^3$ M⁻¹ s⁻¹ (Atkinson, 1990). This should be compared with a reaction of the hydroxyl radical with ethylene, which has a rate constant of $8.52 \times 10^{12}$ cm³ molecule⁻¹ s⁻¹, or $10^6$ × faster.
Figure AX9-9. Varied ESR radicals, trapped and not, generated by O$_3$ under somewhat physiological conditions. (a) The generation of a DMPO-trapped radical with caffeic acid in water solution (Grimes et al., 1983). (b) The generation of a DMPO-trapped radical within bean (*Phaseolus vulgaris* cv. Pinto) leaves pretreated with spin trapped PBN after a 4-h fumigation with O$_3$ (0-300 nl/L) (Mehlhorn et al., 1990). (c) The EPR signal produced within a bluegrass leaf exposed to 1000 µg m$^{-3}$ O$_3$ for 1 h (Runeckles and Vaartnou, 1997). Although no trapping agent was used in this experiment, the signal is complex, because of various free radicals normally present within the illuminated leaf.

leading to injury. However, the spraying of the plant with 1-aminoethoxyvinyl-Gly (AVG), which reduces the production of ethylene and visible injury’, had no effect upon the EPR signal, suggesting that the radical is not a direct sequela to visible injury.

Runeckles and Vaartnou (1997) (Figure AX9-9c) discovered a signal by subtracting other EPR signals of the leaf, which seemed to be due to an O$_3$ reaction with plant material, using 0.48 ppm O$_3$. This difference signal looked very much like O$_2^-$. At a lower concentration, they observed that this signal still occurred but accumulated more slowly. Both bluegrass and ryegrass leaves seemed to saturate after about 5 h of exposure at 22 to 28 units of signal, while radish leaves reached a maximum of 7 units at 3 h and then declined. The problem, which is
typical of any of these methods, was that the detached leaf had to be rolled and placed into the EPR detection cavity. Reichenauer et al. (1998) also detected an undefined free radical signal that seemed to be related to a Mn(II) spectrum. The Nandu and Perlo cultivars of wheat were more sensitive to O₃ than Extradur (according to growth rate and closure of stomata under an O₃ exposure of 80 ppb for 8 h/day, 7 days/week over 100 days), and these more sensitive cultivars had a greater, but insignificant (P = 15%), EPR signal. Thus, data showing any production of a free radical must be approached with some skepticism.

With an O₃ delivery rate of about 25 µM/h (Table AX9-6), only 250 µM would be found after a full day, if all of the O₃ were stable. While the use of free radical traps is the best method available to observe any build-up of radicals, the traps are not as specific to individual radicals. Currently, studies should be looking for hydroxyl radicals, superoxide, hyroperoxides, ethylene radicals, and ascorbate radicals.

**AX9.2.3.3 Products of Ozone**

Ozone should reach a certain concentration in the substomatal cavity, which is dependent upon its entry speed and its reactivity with the wall constituents. Once near the apoplastic space, O₃ moves in two different pathways (Figure AX9-6). It can react with constituents that are within the wall as a gas in reaction 1A (path 1); or it can solubilize into a water space and travel to another region within the water space and react through reaction 2A (path 2).

**Hydrogen Peroxide**

Hydrogen peroxide, until recently was thought to be purely a toxic compound for cells. However, it is now clear that it functions as a signaling molecule in plants and mediates responses to abiotic and biotic stressors (Figure AX9-10). Generation of H₂O₂ is increased in response to various stressors, implicating it as a key factor mediating the phenomena of acclimation and cross tolerance, in which exposure to one stressor can induce tolerance of subsequent exposure to the same or different stresses (Neill et al., 2002). The signaling response to attack by invading pathogens using H₂O₂ has been described (Mehdy, 1994; Simon-Plas et al., 1997). The reactions leading to hypersensitive cell death are caused by a pathogen recognition step (Figure AX9-10a), probably due to the plant cell wall releasing oligosaccharides in response to the pathogen enzymatically breaking down the cell wall to penetrate it. A feed-forward
Figure AX9-10. Pathogen-Induced Hypersensitivity. (a) The reactions leading to hypersensitive cell death and the formation of a global response of salicylic acid. (b) The cascade of the elicitor-induced reactions within the cell.

step in which $H_2O_2$ increases the level of benzoic acid leads to the activation of the hydroxylase step in the production of salicylic acid and to a feedback step in which the salicylic acid increases the production of $H_2O_2$ (Leon et al., 1995).
An elicitor, e.g., a bacterial or fungal pathogen, induces a cascade of reactions within a cell (Figure AX9-10b). Some of the lipid reactions are thought to be due to the opening of the Ca\textsuperscript{2+} channels and the alkalination of the cell wall region. The oxidative burst due to H\textsubscript{2}O\textsubscript{2} production is believed to lead to the transformation of a small population of lipids into jasmonic acid, which is a secondary messenger.

Hydrogen peroxide also has an oxidative role in lignification (Schopfer, 1994). In the interaction of lignification and the beginning processes of hypersensitivity, pectinase produced by the pathogen disrupts pectin and dissolves the cell wall. Fragments of the dissolved cell wall trigger an increase in the transcription of peroxidases within the remaining cell wall, leading to signification, which is a cross-linking of the cell wall that does not use pectin. This prevents further pathogen disruption of the wall and reduces its further entry into the plant cell.

It is believed that the first species generated through a one-electron reduction of molecular oxygen is SO\textsubscript{2}\. That generation is carried out using a cytochrome b\textsubscript{6} by the NAD(P)H oxidase located on the cell membrane (Auh and Murphy, 1995). In the acid region of the cell wall, SO\textsubscript{2} is converted by a protonation and dismutation to H\textsubscript{2}O\textsubscript{2}. The induced oxidative burst is believed to play a role in stimulating the Cl\textsuperscript{-} and K\textsuperscript{+} efflux, generating an alkalinization of the extracellular space (Cazale et al., 1998). In the wall region, H\textsubscript{2}O\textsubscript{2} is not especially toxic, as no necrosis was reported in tobacco when 500 mM peroxide was infiltrated into the leaf tissue. However, the production of salicylic acid and benzoic 2-hydroxylase can be induced with only 30 and 0.3 mM H\textsubscript{2}O\textsubscript{2}, respectively, indicating some metabolic signaling (Leon et al., 1995). On the other hand, 1M H\textsubscript{2}O\textsubscript{2} infiltrated into soybean will generate lipid peroxidation after 1 h with a peroxidation rate of 15 nmol/g-FW\textsuperscript{-}h (Degousee et al., 1994). Cells react to the system and generate peroxide scavenging compounds within 1.5 to 2 hours, which appear to “mop up” the excess H\textsubscript{2}O\textsubscript{2} (Baker et al., 1995).

After O\textsubscript{3} exposure in birch, H\textsubscript{2}O\textsubscript{2} has been found in the wall (Pellinen et al., 1999). By using CeCl\textsubscript{2} as a cellular stain for H\textsubscript{2}O\textsubscript{2} (as a cerium perhydroxide precipitate), Liu et al. (1995) observed a gradual development of stain after 8 h of O\textsubscript{3} exposure (at 150 ppb). After 2 h exposure, H\textsubscript{2}O\textsubscript{2} stain was visible on the surfaces of both sets of mesophyll cells. Accumulation of H\textsubscript{2}O\textsubscript{2} stain continued for 16 h after exposure, suggesting a triggered-reaction rather than O\textsubscript{3}.

\footnote{Soybean suspension cells were inoculated with \textit{Pseudomonas syringae pv syringae}, which generate an active oxygen response. Light emission by luminol, reacting with H\textsubscript{2}O\textsubscript{2}, was the assay for the peroxide.}

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decomposition itself. $H_2O_2$ stain was present in the mitochondria, peroxisomes, and cytoplasm but not in the chloroplast. If methyl viologen (MV) was given to the leaves and then the leaves were exposed to light, $H_2O_2$ stain could be observed within the chloroplast. This indicated that the stain worked within the chloroplast if $H_2O_2$ were generated by the Mehler reaction ($MV + O_2^-$). Thus, apparently, for birch, $O_3$ exposure does not generate excess $H_2O_2$ within the chloroplast. Furthermore, these sets of experiments indicate that $O_3$ per se does not generate the $H_2O_2$, but rather triggers stress-related $H_2O_2$ formation similar to what occurs in a pathogen attack (the Reactive Oxidative Species or ROS reaction).

The presence of higher than normal levels of $H_2O_2$ within the apoplastic space is a potential trigger for the normal, well-studied pathogen defense pathway. Figure AX9-10b depicts such a pathway and suggests that all the events and activation of pathways/genes caused by pathogen defense could be observed when plants are fumigated with $O_3$. The events shown in Figure AX9-10b will be alluded to in later sections.

$H_2O_2$ has been linked to the hormone ABA-induced closure of the stomata by activating the calcium influx in guard cells (Pel et al., 2000). The addition of $H_2O_2$ at a level of only 5 mM to a guard cell preparation will cause a dramatic increase (ca. 9×) in electrical current at the hyperpolarizing potential of –200 mV. Amounts as low 50 mM $H_2O_2$ will cause a less, but still sizable, increase. Membrane stability is unaffected by the $H_2O_2$ and the activation of the channel requires only about 2 to 3 minutes. Pel et al. (2000) also found that ABA induced the production of $H_2O_2$ through ROS accumulation (also see Zhang et al. [2001]).

Certain levels of ABA within the leaf lead to stomatal closure. The inactivation of a phospho-tyrosine-specific protein phosphatase (ABI2) is an inhibitor of stomatal opening induced by ABA but that enzyme is inhibited by $H_2O_2$ (Meinhard et al., 2002). This means that $H_2O_2$ shifts the sensitivity of the stomatal opening to ABA (Figure AX9-11), making the stomatal complex more sensitive to ABA. Thus, for a given level of ABA present in the guard cell complex due to environmental factors (e.g., low humidity, high air temperature, or low soil water potential), the generation of $H_2O_2$ would (by inhibiting ABI2) induce a closure of the stomata by increasing the sensitivity of the guard cells to ABA. In the past, it has been difficult to understand why $O_3$ would often decrease conductance in some cases, but not always (Heath, 1994b). This interaction between $H_2O_2$ and ABA could help understand this complexity.
Figure AX9-11. The interaction of H$_2$O$_2$ and Ca$^{2+}$ movements with ABA-induced stomatal closure. It is well known that certain levels of ABA within the leaf lead to closure of the stomata within the leaf. That level, however, can be shifted to make closure more or less sensitive to a given level of ABA. Recently it has been shown that H$_2$O$_2$ (externally or produced by the plant) within the cell wall region can shift that sensitivity. Here ABA stimulates the production of H$_2$O$_2$, which in turn increases the rate of Ca$^{2+}$ moving from the wall region into the cytoplasm. That shift in internal Ca$^{2+}$ level increases the closure of the stomata. Hydrogen peroxide also blocks the activation of a polypeptide (ABI2) that inhibits stomatal closure seemingly induced by ABA.

Sources: From Assmann (2003); Assmann and Wang (2001); and Zhang et al. (2001).

**Ethylene Reactivity**

Ethylene (ET) is produced when plants are subjected to biotic stressors, e.g., attacks by insects, fungi, and bacteria or abiotic stressors such as wounding or environmental stressors such as heat, cold, or oxidative stress and O$_3$. If an O$_3$ stress has induced a wounding response with ET release, then ET within the substomatal cavity could react with O$_3$, generating some relatively noxious chemicals (see Figure AX9-6). The relationship between O$_3$ injury and wounding is supported by the observation by Mehlhorn et al. (1991) that an inhibitor of ET formation, AVG (an inhibitor of ACC synthase, a committed step to ET production), would block ET formation and inhibit visible injury. Other studies with polyamine (which is closely linked to ET production), including those of Ormrod and Beckerson (1986) who fed polyamines to the transpirational stream and prevented visible injury, suggested a close involvement of both...
pathways to the production of visible injury. Both the lack of ET production and an increased level of polyamines slowed or prevented visible injury.

This concept was taken another step by Langerbartles (1991). The linkage to the pathogen wound responses and visible injury is well established (Sandermann, 1996). Sandermann (1998) used a system of Bell B and W3 tobacco, plants with differential O₃ sensitivities, in which the O₃ exposure level was chosen such that the sensitive cultivar was injured, while the tolerant one was not. This led to a marvelous control that could be used to their advantage. They followed a time sequence to show that the rise of varied systems followed the same order as seen for a pathogen attack (Heath, 1994a).

More recent studies, however, indicate that O₃ responses resemble components of the hypersensitive response (HR) observed in incompatible plant-pathogen interactions (Sandermann, 1998). The similarity to the HR response may be related to the occurrence of ROS in the apoplast. The O₃-derived ROS apparently trigger an oxidative burst in the affected cells by an as yet unknown mechanism. An oxidative burst is similar to one of the earliest responses of plants to microbial pathogens and is an integral component in HR-related cell death (Overmyer et al., 2000).

In plants exposed to O₃, ET synthesis is a result of the specific ET induction of the genes encoding 1-aminocyclopropane-1-carboxylase synthase (ACS), one of the fastest and most obvious responses to O₃, which has been mechanistically linked to the regulation of O₃ lesion formation. Biosynthesis of ET inhibited, with ACS inhibitors significantly reduced, the induction of lesion formation in plant leaves exposed to O₃ (Mehlhorn et al., 1991; Mehlhorn and Wellburn, 1987; Vahala et al., 2003b). Ethylene biosynthesis correlates best with O₃ exposure (Overmyer et al., 2000; Vahala et al., 2003b). These data support the concept that elimination of ET formation will prevent visible injury.

**Ethylene-Interaction with Injury and Conductance**

Increased ET production by plants exposed to O₃ stress was identified as a consistent marker for O₃ exposure decades ago (Tingey et al., 1976a). They exposed more than 20 plant species and cultivars to O₃ to determine whether the production of O₃-induced stress-ET could be used to determine differences in plant sensitivity to O₃. Their studies suggested that increased
production of stress-ET correlated well with the degree of foliar injury that developed within hours or days after O₃ exposure. The amount of ET released was exponentially related to the O₃ exposure. Furthermore, the amount of O₃-induced ET declined with repeated exposure, indicating an acclimatization to O₃. This acclimatization effect associated with repeated wounding has not yet been well described. The release of wound-induced ET is not linear with time, but declines after the initial response (Stan et al., 1981), as is also seen after O₃ exposure (Stan and Schicker, 1982). The stress-induced ET production correlates better with O₃ exposure level than with exposure duration. In other words, peaks of high O₃ (rather than accumulated dose) generate a higher rate of ET release, at least for a single O₃ exposure under an acute dose.

The production of ET after an O₃ exposure is thought to be a typical wounding response (Tingey et al., 1975). Prevention of ET release may prevent the formation of visible injury (Mehlhorn and Wellburn, 1987). However, the question arises as to whether this effect was limited to the prevention of visible injury or if the chemicals used to prevent ET release closed the stomata. Using Glycine max L., Taylor et al. (1988b) showed clearly that AVG did not necessarily close stomata nor inhibit carbon assimilation per se.

The correlation of ET release with O₃-induced visible injury was likewise shown in pea cultivars (Dijak and Ormrod, 1982). With O₃ exposure (generally 6 h at 0.3 ppm), the stomata closed by ~50% within 3 h after a dose of 3 × 10⁻⁵ mol cm⁻² (with an average rate of 2 × 10⁻⁹ moles cm⁻² s⁻¹, as calculated from their data). Both sensitive and insensitive cultivars had a visible-linked-injury ET release, but sensitive cultivars scored higher both in visible injury and in ET release after a given exposure.

Gunderson and Taylor (1988, 1991) used exogenous ET to alter the gas exchange of Glycine max and found an exponential, but not simultaneous, decline of both stomatal conductance and carbon assimilation with ET. Interestingly, the exogenous ET caused a slight rise in difference of CO₂ within and without the leaf, indicating a lowering of internal CO₂, which was not observed in the experiments of Farage et al. (1991) for O₃ exposure. Ethylene inhibits both stomatal conductance and carbon assimilation to some extent (Taylor et al., 1988b). Thus, one could postulate that O₃ generates a wounding response with a production of ET, which would, in turn, generate the change in stomatal conductance and photosynthesis. Clearly, these multiple events may have confounded some earlier studies.
AX9.2.3.4 Antioxidants Within the Apoplastic Space

The first line of defense against \( \text{O}_3 \) is a closure of the stomata to exclude its uptake. This is counterproductive for efficient photosynthesis, but some amount of closure limits the rate of \( \text{O}_3 \) deposition into the leaf tissue to allow for a secondary line of defense to detoxify the \( \text{O}_3 \). The secondary line of defense involves a range of antioxidants, which are highly reactive to the types of chemicals that can be generated by \( \text{O}_3 \). Several antioxidant proteins are stimulated by \( \text{O}_3 \) in *Plumbaginifolia*, including glutathione peroxidase (GSH-Px), SOD, and catalase. The timescales for changes in their levels vary: some rise rapidly, while others rise more slowly. The pattern of changes in these particular proteins varies greatly among different species and conditions.

**Ascorbate Within the Cell Wall**

Most of the recent reports indicate that ascorbate within the cell wall is the real first line of all defense. Ascorbate within the wall declines when the tissue is exposed to \( \text{O}_3 \) (Luwe et al., 1993; Moldau, 1998). This decline appears to be closely linked to the amount of \( \text{O}_3 \) penetrating the leaf tissue.

It has long been suspected that intracellular antioxidants play a role in preventing \( \text{O}_3 \)-induced injury to plant cells. Variation in the types of biochemical compounds present in the apoplastic space can give rise to a multiplicity of reactions with \( \text{O}_3 \), but the predominant biochemical species is ascorbate. Ascorbate is water soluble, present in the solution where \( \text{O}_3 \) can dissolve, and is highly reactive. Unfortunately, a variety of antioxidants are found throughout the cell and any measurements of one particular type within the total leaf tissue can give misleading results. For example, ascorbate is present within the cell wall, cytoplasm, and chloroplasts (Burkey, 1999; Moldau, 1998); and ascorbate can move between the cytoplasm and the cell wall with relative ease (Figure AX9-12) (Bichele et al., 2000). The total of all ascorbate pools is measured when the tissue is ground and assayed. If the cell wall ascorbate concentration drops by 50% due to \( \text{O}_3 \) exposure but all other tissue concentrations remain the same, the measurement of the total loss is dependent upon the amount of ascorbate within the cell wall. Turcsányi et al. (2000) showed that, compared to the concentration of apoplastic ascorbate, the rest of the cells contained about 38 times as much. So a 50% loss of apoplastic ascorbate would be converted into only 2 to 3% loss of the total ascorbate.
Figure AX9-12. The reaction of ascorbate within the apoplasm of the cell wall and its ultimate reduction/oxidations.

(a) Movements of reducing power (from Dietz [(1997)]).
(b) The use of glutathione to maintain the level of ascorbate within the cell wall region (from Horemans et al. [2000]).
The ascorbate deficient *Arabidopsis thaliana* mutant has proven to be a powerful tool in furthering the understanding of ascorbate biosynthesis in plants (Smirnoff et al., 2001). Three classes of mutants were formed when *A. thaliana* seed was mutagenized with ethyl methanesulfonate: (a) those deficient in SOD, (b) those that failed to accumulate more antioxidant proteins upon increased O$_3$ exposure, and (c) those that were deficient (but not depleted) in ascorbate. The low-ascorbate mutant type had 50 to 60% less ascorbate than the wild type and displayed more foliar injury. This mutant is involved with the coding of the GDP-D-Mannose pyrophosphorylase enzyme in the Smirnoff-Wheeler pathway for ascorbate biosynthesis. Smirnoff et al. (2001) also suggested that other pathways can produce ascorbate without relying upon the pyrophosphorylase step, but most probably at a slower through-put rate, because any fully ascorbate-deficient mutant would be lethal, perhaps because of ascorbate use as a cofactor rather than its antioxidant properties. In addition to its lowered antioxidant capacity, the ascorbate-deficient *Arabidopsis* mutant vtc1 (Conklin et al., 1996; Conklin and Barth, 2004) may show suppressed growth due to lower mannose levels that are necessary for cell wall formation. Ozone thus may suppress growth in these mutants through interference with cell wall biosynthesis as well as through lower antioxidant protection.

The ascorbate peroxidase (APX, which uses ascorbate to detoxify peroxides) family consists of at least five different isoforms, with isozymes in the apoplastic and cytosolic space. Furthermore, most forms of ascorbate can move through the plasma membrane (Bichele et al., 2000), making the levels of all forms of ascorbate interdependent and able to at least partially influence each other. Dehydroascorbate (DHA) can be broken down into other smaller fragments easily in vivo and represents a continuous loss of ascorbate from varied parts of the cell if ascorbate is allowed to remain in the oxidized form in some regions. In fact, the turnover rate in leaves is estimated to be from 2 to 13% per hour, depending upon species and developmental age (Smirnoff et al., 2001). There are apparently three pathways for ascorbate turnover (Figure AX9-12a). The typical reaction is a reduction of DHA into ascorbate from which an oxidative step generates DHA. Pathway I requires a reductive step using NADH external to the plasma membrane generated from internal malate using a malate/oxaloacetate

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6 EC 2.7.713, Mannose-1 phosphate guanylyltransferase; mannose + GTP → GDP-mannose + ppi; this product leads into cell wall polysaccharide synthesis and protein glycosylation through GDP-galactose and GDP-fucose and, ultimately, through galactose into ascorbate synthesis.
transporter. Pathway II uses a direct transporter of ascorbate/DHA. Pathway III moves the required electron(s) through a cytochrome b system, maintaining two separate pools of ascorbate/DHA (within the cytoplasm and within the wall). Each of the pathways (Dietz, 1997) represented by Roman Numerals in the Figure AX9-12, require only one NAD(P)H molecule to reduce the DHA molecule back to ascorbate. However, the transport properties and redox potential of the cell differ for each pathway. The efficiency of the reduction of DHA is dependent upon the redox coupling and the region in which the chemical species is located.

Tučsányi et al. (2000) exposed broad bean (*Vicia faba*) grown under two regimes in duplicate controlled chambers: charcoal/Purafil filtered air (CFA) or (CFA) plus 0.075 ppm O$_3$ for 7 h/day for 28 days (chronic exposure) or exposed to 0.150 ppm for 8 h (acute exposure). Responses of the two sets of plants were similar except for stomatal conductance, which was 50% lower in the chronically exposed plants. Plants grown under acute exposures developed visible injury, while plants grown under chronic conditions developed no visible injury. Within an hour of the start of the acute exposure, the stomatal conductance was reduced by nearly 40% and assimilation was reduced by nearly 18% in the clean air plants; the reduction in conductance was only 21% and assimilation 16% in the plants subjected to chronic O$_3$ exposures. The assimilation was affected similarly in both cases, while the conductance showed less of a percentage drop in the chronic O$_3$-exposed plants and began at a lower O$_3$ level. The similarity of the assimilation indicated that the stomata were not limiting assimilation in either case before acute exposure. More to the point, the decline in ascorbate in the apoplastic space due to the O$_3$ exposure was “…more often than not, on the borderlines of statistical significance.” However, a 30% decline in ascorbate after 4 h of acute O$_3$ exposure in both cases was observed. This lack of significance may be due to a relatively large standard error of the data, which in turn may be due to the difficulty of extracting and measuring ascorbate from the apoplastic space in quantitative terms.

The chemical reaction of ascorbate and O$_3$ is given by the molecular rate constant of $4.8 \times 10^7$ M$^{-1}$ s$^{-1}$. This is some 50,000× the rate constant for ET. Of course, it depends upon the relative concentration of ascorbate and ET, but it is likely that ascorbate is in higher

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7These chemical rate constants are those constants within a bulk solution. In the apoplasm, the possibility exists for the chemicals to be preferentially oriented near a surface; so the constants may not be the same as for bulk solutions.
concentration than ET. One would then expect that the rate reaction of ascorbate with O$_3$ would greatly dominate any possible reaction of O$_3$ with ET. For a concentration of ascorbate in the range of 1 mM and for an O$_3$ concentration of about 0.1 ppm or $4.2 \times 10^{-9}$ M, the detoxification rate would be $4.8 \times 10^7 \times 10^{-3} \times 4.2 \times 10^{-9} \text{ M s}^{-1} = 2.0 \times 10^{-4} \text{ M s}^{-1}$. Turcsányi et al. (2000) calculated an O$_3$ flux of about $1.6 \times 10^{-9}$ moles m$^{-2}$ sec$^{-1}$. With a wall thickness of $0.12 \times 10^{-6}$ m and all the O$_3$ flux going into the wall region, this would give about $1.3 \times 10^{-2}$ mol m$^{-3}$ s$^{-1}$ or $1.3 \times 10^{-5}$ M s$^{-1}$ flux, which is less than 10% the detoxification rate.

**Glutathione**

Many of the initial studies of O$_3$ exposure used high concentrations and measured only the total sulfhydryl contents of the tissues. For example, in some of the earlier work, exposures of tobacco to 1 ppm O$_3$ for 30 min induced a 15% loss of the total sulfhydryls (0.74 µmole/g-FW) (Tomlinson and Rich, 1968). These results are similar to other studies at high O$_3$ levels (Dugger and Ting, 1970). It is now suspected that the severe injury in their studies resulted in a massive collapse of the cells and release of most of their internal constituents. Much of the oxidation thus observed may have been the result of chemical oxidations of the O$_3$ that subsequently entered the damaged tissue. Even under milder conditions, changes in sulfhydryl components have still been noted and any sulfhydryl on the surface of the cell would be at risk due to its high reactivity with O$_3$ (Mudd et al., 1997b; Mudd et al., 1969). For example, the level of sulfhydryl compounds within the protein of isolated chloroplasts declined about 66% when the chloroplasts were subjected to O$_3$ (about 1 µmole O$_3$) exposure (Mudd et al., 1971).

At this stage, it is important to note that there are inherent problems with metabolic studies of full tissues. The first is that most organs have several different types of tissues. For example, leaves have, at the minimum, epidermal and vascular tissues and two types of mesophyll cells. Each type of cell may be metabolizing quite differently and producing very different levels of metabolites and enzymes. Furthermore, most pathways are well regulated and after any small disruption, the pathway tends to return to near its former stability. Changes in the level of enzymes are likewise difficult to measure. Many enzymes function below their maximum activities. Their speeds of reactions are often increased through regulation, rather than through the production of more enzyme.
Glutathione is a three-amino acid peptide, which has antioxidant properties due to its free reducing sulfhydryl group (G-SH). Glutathione is generally kept in its reduced form by +glutathione reductase (GR) with the reaction:

$$\text{GS} - \text{SG(oxidized)} + 2e^- + 2H^+ \rightarrow 2\text{GSH} \quad \text{(AX9-2)}$$

GR has six isoforms\(^8\) within the chloroplast and six isoforms outside. The optimum activity occurs at pH 7.8, suggesting it is located within the stroma of the chloroplast or in the cytoplasm rather than in the cell wall, which is at pH 4-5 (Madamanchi et al., 1992). Clearly, an increased expression of GR (generated through transgenic implants) is important within the chloroplast to prevent some oxidations\(^9\) (Aono et al., 1995).

**Catalase**

Catalase, even though it breaks down H\(_2\)O\(_2\), does not appear to protect plants from O\(_3\) exposures. Two principal reasons may cause this lack of reactivity: (1) catalase has a high Km for H\(_2\)O\(_2\) and a low rate coefficient, and (2) catalase seems not to occur within the cell wall regions but rather in the cytoplasm and peroxisomes (Buchanan et al., 2000). While a few reports suggest that catalase is increased by exposure to O\(_3\) (Azevedo et al., 1998), Booker et al. (1997) found no effect of catalase activity in soybean until late in the growing season, and others have found decreased catalase activity in wheat in response to O\(_3\) (McKee et al., 1997).

Unfortunately H\(_2\)O\(_2\) induced by some forms of wounding in mesophyll cells can lead to induction of an increase in GSH and the transient production of catalase (Vanacker et al., 2000). In general, it seems that catalase is not really involved primarily in the defense of the cell due to O\(_3\) attack but rather may be a secondary response. The reaction of catalase (Scandalios, 1993) is as follows:

\[^8\]An isoform is the same enzyme, with the same structure and perhaps within the same organelle, but its promoter region has different DNA codes. Thus, each protein segment is induced by different signals, and so its enzyme can be formed in response to different environments. This is in contrast to isozymes, which classically are similarly reacting, but structurally different, enzymes in different compartments.

\[^9\]Typically this protection is observed in the paraquat sensitivity of plants. In this assay, added paraquat, the herbicide which intercepts electrons from the reducing end of photosystem I in the chloroplast, caused oxidations, chlorophyll loss, and death due to the buildup of superoxide and peroxides.

AX9-62
Superoxide Dismutase

The varied compounds that O$_3$ can produce upon entering an aqueous solution are very similar to those involved in the HR when plants are infected by an avirulent pathogen (Figure AX9-10). The sequence of the plant response to the pathogen is (1) recognition of the gene products of the pathogen by the plant (elicitor), (2) generation of an immediate phytoresponse to attempt to localize the attack and its products, and (3) generation of a systemic acquired resistance (SAR) to subsequent attack by the pathogen. Inducible defense responses are phytoalexin synthesis and production of pathogenesis-related proteins (PR). One aspect of this total response is the production of O$_2^-$ and H$_2$O$_2$ by the cell (Lamb and Dixon, 1997). The elicitor can generate a transient alkalinization of the apoplast, up to pH 7.2, caused by a lowering of the H$^+$-pump rate and an increase in the H$^+$-influx/K$^+$-efflux exchange. Other effects include a weak accumulation of transcripts for PAL (phenylalanine lyase); a larger and rapid induction of glutathione S-transferase, GSH-Px; oxidative cross linking of cell wall proteins which is blocked by ascorbate acid; generation of localized apoptosis; and rapid influx of Ca$^{2+}$, which activates apoptosis among other pathways (Lamb and Dixon, 1997). These effects seem to be very similar to those induced by O$_3$ exposure (Sandermann, 1996, 1998).

The putative antioxidant enzyme SOD (Equation AX9-4 and Table AX9-8) catalyzes the oxidoreductase reaction, which eliminates SO$_2^-$ by dismutation (Bowler et al., 1992):

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad K = 1.7 \times 10^{-7} \text{ M}^{-1} \text{ sec}^{-1} \quad (AX9-3)$$

$$2O_2^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2 \quad K = 2.4 \times 10^9 \text{ M}^{-1} \text{ sec}^{-1}. \quad (AX9-4)$$

The number, as well as the activity, of isozymes of each type of SOD in Table AX9-3 can vary with plant species. However, the isozymes that have been tabulated are Cu-Zn SODs, in cytosol and chloroplast; Fe-SOD, active in chloroplast stroma; and Mn-SOD, active in mitochondrial matrix (Karpinski et al., 1993). In the experiment demonstrating the activation of varied SODs, there were three Cu-Zn SODs ($csd1, csd2, csd3$), three Fe-SODs ($fsd1, fsd2, fsd3$), and one Mn-SOD ($msd1$) (Kliebenstein et al., 1998). Ozone sensitivity was determined by...
Table AX9-8. Superoxide Dismutase Isozymes and Isoforms

<table>
<thead>
<tr>
<th>Isozymes</th>
<th>M.W.</th>
<th>Isoforms</th>
<th>Cytolocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu-Zn</td>
<td>20 kDa</td>
<td>csd1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>csd2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>csd3</td>
<td>Plastid</td>
</tr>
<tr>
<td>Fe</td>
<td>23 kDa</td>
<td>fsd1</td>
<td>Mitochondrial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sd2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>fsd3</td>
<td>Plastid</td>
</tr>
<tr>
<td>Mn</td>
<td>23 kDa</td>
<td>msd1</td>
<td>Mitochondrial</td>
</tr>
</tbody>
</table>

exposure of plants to 8 h of 0.33 ppm of O$_3$. csd1 induced by O$_3$ and UV-B was one of the earliest SOD increases and most pronounced responses for mRNA and protein. Also, some increase in csd3 (thought to be peroxisomal) was induced when the plants were exposed to a high-intensity light pulse; msd1 was unresponsive to the environmental stressors used here, including O$_3$; and csd2 (thought to be chloroplast) showed little increase. The fsd1 isozyme (present in the apoplasm) showed a slight decrease. On the other hand, an early report on snap beans in which the experimenters used EDU, N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N-phenylurea (Carnahan et al., 1978; Kostka-Rick and Manning, 1993), to prevent visible injury by O$_3$, 4-h O$_3$ exposure at 0.45 ppm was correlated with an increase in general enzyme activity of SOD, i.e., the level rose nearly 2.5× in 2 weeks at a level of 50 mg EDU per pot (Lee and Bennett, 1982). It is believed that EDU may induce SOD, which then protects the plant. While gross assays of enzyme activity have not proven to be very useful in understanding the mechanism of O$_3$ action, in a well-crafted, long-term study involving ponderosa pine clones. Benes et al. (1995) stated that “changes in antioxidant enzyme activity were not a consistent response to the O$_3$ fumigation, but when observed, they occurred most often in the O$_3$-sensitive clone and in symptomatic, fumigated branches…total (intra- and extracellular) activities of the antioxidant enzymes did not appear to be good indicators of O$_3$ tolerance….”
Ozone exposure (70 ppb for 7 h/day for 14 to 42 days of exposure)\textsuperscript{10} caused an increase in POD and a decline in SOD with no change in APX. No GSH was detected, but the concentration of (ASC + DHA) was at 20 to 25 nmol/g-FW of extracellular fluid, compared with 2.4 to 3.0 mmol/g-FW of cell fluid. Glutathione within the cell was only 100 to 170 nmol/g-FW of cell fluid. While these results are what one might expect for POD, the decline in SOD and lack of change in APX are not what would be expected if protection was provided by SOD and ascorbate. Yet as noted, because the rate of SOD reaction is many times higher than the rate of O\textsubscript{3} entry, there may be no pressure to increase the SOD level.

Some protection against visible injury (induced by 59 ppb daily mean O\textsubscript{3} for 14 h/day for 7 days) was observed in genetically modified tobacco plants with excess chloroplast SOD\textsuperscript{11} (2 to 4 times higher), but less protection was observed in plants that had an excess of mitochondrial SOD (8× higher) (Van Camp et al., 1994). In all lines, the conductance of the leaves dropped about 50%, compared with the unmodified plants. There was a correlation with age of leaf (less injury in younger leaves) that corresponded to that found in spruce trees in which the amount of SOD declined in relation to the longer that needles were held on the tree (Polle et al., 1989). A slightly different study, however, found no O\textsubscript{3} protection with varied SOD within the needles (Polle and Rennenberg, 1991). Interestingly, in maize, the synthesis of SOD (any form) was not stimulated by O\textsubscript{3} exposure (at 0.50 or 0.75 ppm for 8 h, variable times thereafter) but was by exposure to 90% O\textsubscript{2} (Matters and Scandalios, 1987). It may be that this high level of O\textsubscript{3} does not affect the SOD, or perhaps it stimulates and degrades the enzyme simultaneously.

The conclusions to be drawn from these results are not obvious. There seems to be SOD (a Cu-Zn form) present in the apoplastic space of some plants, but it does not necessarily rise with O\textsubscript{3} exposure. Thus, either its concentration is sufficient to provide protection or it is not needed. Over expression of any SOD in other organelles may play a role, especially in the chloroplast (Cu-Zn or Fe forms); however, it may be playing a secondary role due to other effects of O\textsubscript{3} that generate conditions in which light can overload the chloroplast and generate

\textsuperscript{10}At a level of 70 ppb, the concentration of O\textsubscript{3} in air was about $3.06 \times 10^{-6}$ mol/m$^3$, which with the conductance of 0.042 mol/m$^2$s, gives a flux rate of O\textsubscript{3} of $1.27 \times 10^{-8}$ mole/m$^2$s. Converting the SOD rate of 23 units/g-FW into a SOD rate within the apoplastic space of $6.9 \times 10^{-3}$ mol/m$^2$s, or about 500,000 times the entry rate of O\textsubscript{3}.

\textsuperscript{11}The SOD enzymes were from \textit{Nicotiana plumbaginifolia} with appropriate transit sequence for targeting the correct organelle and expressed under control of cauliflower mosaic virus 35S promoter.
detrimental circumstances, including the production of SO₂⁻. In addition, SOD is developmentally expressed in varied concentrations, so that long-term exposure to O₃ may alter each leaf’s developmental age and, in turn, alter what level of SOD is observed. In any case, SOD does not seem to be the primary antioxidant system to protect against O₃.

Changes to the Plasmalemma

Reports of “peroxidation” generally occur within unicellular organisms subjected to very high levels of O₃ (in Chlorella [Frederick and Heath, 1970] and in Euglena [Chevrier et al., 1990]). Heath (1987) determined that by the time biochemical events were altered and MDA was produced in Chlorella, little permeability remained in the cells and most metabolic pathways were greatly disrupted by the subsequent loss of substrates. In fact, MDA production was concurrent with a high O₃ uptake by the cell, indicating a complete opening of the cell and associated with the concurrent inability to plate the cells on a glucose medium (indicative of cell death). Heath (1987) reached the conclusion that no one had proven that lipid oxidation was in any way a part of the initial reactions of O₃ with the cell, a conclusion confirmed by Mudd et al. (1997a). An excellent review regarding the initial action induced by O₃ within a plant (Kangasjärvi et al., 1994) should be consulted. There is little data to show that lipids are attacked by O₃ in any living system that was not previously severely injured by O₃. Most of the data suggesting lipid attack by O₃ has been demonstrated in plants subjected to O₃ concentrations of 0.5 to 1.0 ppm for several hours, during which gross wilting of the plant tissues usually occurs, suggesting extreme water loss. It is not surprising that lipid and protein injury are observed under these conditions. While those reports were useful in the 1960s and 1970s, they are not especially insightful now when ambient levels of O₃ are rarely above 0.2 ppm.

AX9.2.4 Wounding and Pathogen Attack

The decline of an enzyme is more difficult to measure than the rise of a new enzyme; an increase from 0 to 2% may be within the precision of any assay, but a decrease from 100 to 98% is often masked by simple variation of the assay. Thus, measuring enzymes, which are in great abundance in prefumigated tissue, is a risky operation. On the other hand, if O₃ induces a general physiological change that has characteristics similar to other well-studied, stress-induced
changes, then O₃ studies could “piggyback” onto those studies to gain insight into the full scope of metabolic alterations. It is now becoming clear that wounding and pathogen attack of plants are similar to O₃-induced changes in plants, and a reasonable hypothesis is that O₃ must induce one or more of the first steps seen in the wounding/pathogen-attack response.

Systemic acquired resistance (SAR) has been heavily investigated, and DNA probes have existed for some time for a series of expressed genes (see Table AX9-9). Several enzyme classes are associated with O₃ injury, including glucanases and peroxidases and others, such as the PR proteins and chitinases. Thus, strong evidence exists from enzyme function and genetic material that O₃ induces an activation of a SAR-like response.

Table AX9-9. Gene Families and cDNA Clones Used as Probes for SAR (Ward et al., 1991)

<table>
<thead>
<tr>
<th>Probe</th>
<th>Relevant Properties of Encoded Protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-1</td>
<td>Acidic, extracellular; function unknown most abundant PR protein in tobacco; &gt;90% identical to PR-1b and PR-1c</td>
<td>Payne et al. (1989)</td>
</tr>
<tr>
<td>PR-2</td>
<td>Acidic, extracellular b-1,3-glucanase, &gt;90% identical to PR-N and PR-O</td>
<td>Ward et al. (1991)</td>
</tr>
<tr>
<td>PR-3</td>
<td>Acidic, extracellular chitinase; also known as PR-O; &gt;90% identical to PR-P</td>
<td>Payne et al. (1990)</td>
</tr>
<tr>
<td>PR-4</td>
<td>Acidic, extracellular; unknown function; homologous to C-terminal domain of Win1 and Win2 of potato</td>
<td>Friedrich et al. (1991)</td>
</tr>
<tr>
<td>PR-5</td>
<td>Acidic, extracellular; homologous to thaumatin and bifunctional amylase/proteinase inhibitor of maize; also known as PR-R or PR-S</td>
<td>Payne et al. (1989)</td>
</tr>
<tr>
<td>PR-1 basic</td>
<td>Basic isoform of acidic PR-1</td>
<td>Payne et al. (1989)</td>
</tr>
<tr>
<td>PR-O'</td>
<td>Acidic, extracellular b-1,3-glucanase; approximately 55% identical to PR-2 group</td>
<td>Payne et al. (1990)</td>
</tr>
<tr>
<td>Basic, glucanase</td>
<td>Vacuolar; approximately 55% identical to PR-2 group and PR-O’</td>
<td>Shinshi et al. (1988)</td>
</tr>
<tr>
<td>Basic chitinase</td>
<td>Vacuolar; approximately 65% identical to PR-3 group</td>
<td>Shinshi et al. (1987)</td>
</tr>
<tr>
<td>Acidic peroxidase</td>
<td>Extracellular; lignin-forming</td>
<td>Lagrimini and Rothstein (1987)</td>
</tr>
</tbody>
</table>
Mehdy (1994) described a model of how an elicitor produced by the pathogen attack activates a G-protein, which opens the inward-flowing $\text{Ca}^{2+}$ channel. The flow of $\text{Ca}^{2+}$ into the cytoplasm raises the internal level (at the $\mu\text{M}$ level) and activates a protein kinase that increases the activity of the plasma membrane NAD(P)H oxidase and generates $\text{O}_2^-$. Superoxide dismutase converts $\text{O}_2^-$ into $\text{H}_2\text{O}_2$. Both $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ are responsible for the active oxygen species response, which is believed to be a defense mechanism to kill the pathogen. In this normal defensive reaction, a subsequent system induces either localized lipid peroxidation per se or a membrane lipase to produce jasmonic acid or inositol triphosphate, which act as secondary messages to activate the defense gene products.

Booker et al. (2004) found that G-proteins might be involved in the perception of $\text{O}_3$ in the extracellular region using *A. thaliana* G-protein null mutants. The activation of a passive inward flow of $\text{Ca}^{2+}$, e.g., by an $\text{O}_3$-induced response, would serve the same function as activation of the G-protein. Once the level of cytoplasmic $\text{Ca}^{2+}$ rises, all else follows. It is suspected that exposure of plants to $\text{O}_3$ does just that, as Castillo and Heath (1990) demonstrated — the in vivo fumigation of bean plants both inhibits the outward-directed ATP-requiring $\text{Ca}^{2+}$ pump and increases the passive permeability of $\text{Ca}^{2+}$. It was thought that the calcium transporter system has a sensitive sulfhydryl group which, if oxidized, would alter normal $\text{Ca}^{2+}$ movements. In addition, Dominy and Heath (1985) observed that the $\text{K}^+$-activated ATPase (believed to be involved in $\text{K}^+$ transport) was inactivated by in vivo exposure to $\text{O}_3$ and that inactivation was traced to a sensitivity sulfhydryl. Mudd et al. (1996) argued that several amino acids are very sensitive to $\text{O}_3$, including any with an exposed sulfhydryl. Thus, the $\text{O}_3$-induced change in $\text{Ca}^{2+}$ permeability may be the trigger to most, if not all, the wounding responses. However, the difficult problem of proving that the cytoplasmic $\text{Ca}^{2+}$ change is the first event in $\text{O}_3$ injury remains.

Some wound- and pathogen-induced genes that are activated or repressed in *Arabidopsis thaliana* are found with DNA arrays (Cheong et al., 2002). While these responses may not be uniform for all plants, they suggest the possibility of wide-ranging gene changes that may occur with a simple wound and that those changes are wide-ranging and diverse. As an example, these responses are related to hormonal responses that are related to jasmonic acid, ET, and auxin pathways; signal transduction responses; and transcription factors for a variety of pathways. The involvement of ET in wounding and pathogen attacks is discussed in Section 9.2.3.3.
AX9.2.4.1 Peroxidases

Increases in cytosolic and apoplastic peroxidase activity in response to O₃ are often observed, but the reasons and outcomes of these changes have yet to be fully explained. Increased activity is frequently correlated with O₃ injury. Dass and Weaver (1972) observed that increases in peroxidase after O₃ injury was similar to that observed for plant infection by a virus. Tingey et al. (1975) observed a 35% decrease in peroxidase activity immediately following O₃ exposure; however, within 24 to 48 h, activity had increased significantly and was above control level and remained there throughout the remainder of the study. Dijak and Ormrod (1982) also observed increases in peroxidase activity when two O₃-sensitive and two O₃-resistant varieties of garden peas (Pisum sativum) were exposed to O₃. Peroxidase activity was not related to cultivar sensitivity nor to visible injury. Unfortunately, there are many peroxidases (Birecka et al., 1976); therefore, any general increase is not specific. In ET-treated leaves, peroxidase reaction products were found between the plasma membrane and the cell wall, suggesting that ET itself could induce peroxidase activity (Abeles et al., 1989a,b; Birecka et al., 1976).

At the same time, others examined peroxidase reactions in general and found two types of peroxidases (designated as acidic or anionic and basic or cationic, EC 1.11.1.7, but also listed as EC 1.14.18.1). Many types of peroxidases are located in diverse organelles, and each seems to be activated by different conditions (e.g., pH for anionic and cation types and substrates such as guaiacol, syringaldazine, and ascorbate). Peroxidases belong to at least two groups, which catalyze two separate reactions: (1) the reaction of H₂O₂ with ascorbate to form DHA, discussed earlier (Thom and Maretzki, 1985), which is regenerated by plasma membrane electron transport using a dehydrogenase (Gross and Janse, 1977) now believed to be a malate/oxaloacetate shuttle through the membrane coupled to a NAD(P)H-cytochrome-b-reductoxidase; and (2) the reaction with coniferyl alcohol (from phenylalanine through phenylalanine ammonia lyase) to form lignin within the wall. The anionic peroxidase thought to be involved with lignification is within the cell wall (Buchanan et al., 2000; Taiz and Zeiger, 2002). Some basic peroxidases are maintained within the cell, while some are external to the cell. After wounding (Gasper et al., 1985; Lagrimini and Rothstein, 1987), some basic peroxidases can be activated by processes leading to the synthesis of stress ET (Yang and Hoffman, 1984) and/or by excess Ca²⁺ (Gasper et al., 1985). Elicitor treatment of plants change a series of peroxidases, some of which are similar to those seen in O₃-induced changes (see Table AX9-9).
The formation of lignin is due to the phenylpropanoid metabolism (Buchanan et al., 2000). Tyrosine and phenylalanine are converted to cinnamic and \( p \)-coumaric acid, which are, in turn, converted to \( p \)-coumaryl, coniferyl, and sinapyl alcohols, and then into lignins. Hence, the peroxidase activity is often measured by one of these substrates (Espelie et al., 1986; Gasper et al., 1985). However, it is questionable whether apoplastic peroxidase activity is limiting for lignification; laccases have a prominent role as well. Also, availability of monolignols is critical for core lignin formation, and it is unclear whether levels of these metabolites change in response to O\(_3\). Studies by Booker and others (Booker et al., 1991, 1996; Booker, 2000; Booker and Miller, 1998) indicated that O\(_3\) did not increase core lignin concentrations in foliage of loblolly pine, soybean, or cotton; although levels of phenolic polymers and cell wall-bound phenolics were elevated in soybean. Increased phenolic polymers appear to be lignin in acid-insoluble lignin assays and may well be responsible, along with polyphenol oxidase, for the stippling injury observed in O\(_3\)-treated plants. Cell wall function implies the transport of peroxidase molecules out of the cell and, most likely, the regulation of their activities within the wall space. These extracellular peroxidases may be observed by vacuum infiltration of buffer into leaf air spaces and subsequent centrifugation of the tissues to remove the buffer with the apoplastic enzymes that wash out (Castillo and Greppin, 1986). However, exposure to O\(_3\) induces important changes in the plant. For example, extracellular peroxidase activity in Sedum album leaves increased nearly 3-fold over that in the control plants after 2-h exposure to 0.40 ppm O\(_3\) (Castillo et al., 1984). This O\(_3\)-induced increase in extracellular peroxidase appears to be under the control of Ca\(^{2+}\) (Castillo et al., 1984; Heath and Castillo, 1987). Initially, no effect on the anionic activity as measured with syringaldazine (specific electron donor for lignifying peroxidases) was observed, yet 21 h later, the anionic peroxidase activity was increased, whereas the cationic (ascorbate measured) peroxidase activity was decreased, in O\(_3\)-treated plants. This suggests an immediate response (ascorbate peroxidase activation) and a secondary response that activates the lignifying peroxidase via gene activation.

The rapid response of cationic peroxidase after O\(_3\) exposure may not result from de novo protein synthesis but from the secretion and direct activation by Ca\(^{2+}\) ions of enzyme molecules already present in the tissue. Cationic peroxidases might attack the peroxides and, in this manner, act as a detoxifying agent with ascorbate as the substrate in the apoplasmp. The effect of Ca\(^{2+}\) upon peroxidase activity is stronger at low H\(_2\)O\(_2\) concentrations (Penel, 1986). Thus, one
can imagine that, when the H$_2$O$_2$ concentration is low, this peroxidase activation would have a greater in vivo importance. Furthermore, the secretion of cationic peroxidases into the free spaces as a result of O$_3$ treatment is accompanied by a simultaneous release of at least one of its natural substrates (ascorbic acid); this cationic peroxidase exhibits a much higher affinity toward ascorbate (up to 6-fold) than the anionic isozyme (Castillo and Greppin, 1986).

### AX9.2.4.2 Jasmonic Acid and Salicylic Acid

Jasmonic acid (JA) and salicylic acid (SA) are considered to be regulators of the plant defense response (Figure AX9-13) (Buchanan et al., 2000). They tend to respond more slowly than ET, causing widespread effects in the plant tissues. Both seem to be heavily involved in responses of the plant to O$_3$, once again linking the pathogen/wounding defense to O$_3$-induced injury; however, their roles are far from clear.

![Figure AX9-13](image)

**Figure AX9-13.** The pathway leading from phospholipids to jasmonic and traumatic acid. The role of lipoxygenase and the production of a hydroperoxyl moiety from the unsaturated fatty acid are clearly demonstrated. More importantly, several of the enzymes within this pathway have been shown to be activated by oxidative conditions, including O$_3$ exposure. The production of both of these acid species could lead to a general global response of a whole plant to the O$_3$ exposure of a single leaf.

Sources: Howe et al. (2000); Croft et al. (1993); Buchanan et al. (2000).
One of the lipoxidase isoforms is activated by pathogen infection (POTLX-3) within 6 h and accumulates for a week (Kolomiets et al., 2000). This enzyme is the first stage of the JA pathway which leads to 13-hydroperoxide linolenic acid (HPOT) which is converted either to allene oxide through AOS or to C6 aldehydes through hydroperoxide lyase. These aldehydes act as signaling agents via systemic (Sivasankar et al., 2000) or volatile odiferous compounds (oxylipins) that have been implicated as antimicrobial toxins (Froehlich et al., 2001). Interestingly, these compounds seem to target the chloroplast envelop where they interact with its metabolism. As HPOT and AOs are both implicated in plant defense and are activated by O₃, these interactions may be related to how chloroplast enzymes and their mRNAs are involved in O₃-induced injury.

**AX9.2.4.3 Stress-Induced Alterations in Gene Expression**

Early studies addressed the qualitative and quantitative effects of O₃ on protein metabolism (Harris and Bailey-Serres, 1994). Subsequent reports suggested that the physiologic and metabolic consequences of exposure to O₃ were, in part, mediated by increased gene expression. A summary of the gene-linked changes in proteins induced by SAR was provided earlier in Table AX9-9. Of particular note are the productions of PR proteins, chitinase, glucanase, and acidic peroxidases that appear to be common markers used in many O₃ studies. A summary of varied proteins as measured by changes in the mRNA in *A. thaliana* induced by O₃ exposure is shown in Table AX9-10. While studies on *Arabidopsis thaliana* required high concentrations of O₃ to produce a response, the levels reported in most of the studies did not induce visible injury. The types of messages induced included glutathione S-transferase, PAL, ACC synthase, SOD, and some PRs. Slower increases in messages are seen for other PR and SAR-senescence proteins. Declines in messages were observed for varied chloroplast enzymes, including those for Rubisco and chlorophyll binding proteins. A few new proteins were found — a casein kinase and three plasma membrane proteins. It is interesting to note that few messages for “new” proteins were generated by O₃ exposure.

The working hypothesis is that O₃, which is not eliminated by antioxidants in the cell wall, alters the properties of the plasma membrane. Specific polypeptides, indicative of these antioxidants, are induced. If specific receptor molecules or channels on the membrane are affected, the ionic balance within the cytoplasm is changed, leading to altered transcription or
<table>
<thead>
<tr>
<th>Exposure</th>
<th>Physiological Events</th>
<th>Identified Proteins Fast Increase Response</th>
<th>Slow Increase Response</th>
<th>Decline</th>
<th>Examined, But No Change</th>
<th>Unknown Proteins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>150/300 ppb for 6 h daily</td>
<td>Leaf curling; reduced growth</td>
<td>GST, PAL</td>
<td>Pxase, SOD</td>
<td>CAT, LOX1</td>
<td></td>
<td>AtOZI1 &gt;&gt; casein kinase II</td>
<td>Grosjean et al. (1994)</td>
</tr>
<tr>
<td>300 ppb for 6 h daily</td>
<td>10 bands of 10 RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 plasma membrane proteins: 75-, 45-, 35-kDa peptides</td>
<td>Sharma and Davis (1995)</td>
</tr>
<tr>
<td>200/500/1000 ppb for 2 h</td>
<td>Wilting (8 h); premature senescence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 bands of 10 RNA</td>
<td>Tokarska-Schlattner et al. (1997)</td>
</tr>
<tr>
<td>350 ppb for 1-6 h</td>
<td>Ethylene production; downward curvature; water logging</td>
<td>ACS-6</td>
<td></td>
<td>ACS-1, -2, -4, -5</td>
<td></td>
<td></td>
<td>Vahala et al. (1998)</td>
</tr>
<tr>
<td>300 ppb for 6 h</td>
<td>Necrosis in NahG and Cvi-0 (accumulating SA), not in Col-0</td>
<td>Chl SOD, cytAPX, GST1</td>
<td>Chl GPX</td>
<td></td>
<td></td>
<td></td>
<td>Rao and Davis (1999)</td>
</tr>
<tr>
<td>150 ppm for 6 h/8 and 14 days</td>
<td>Downward rolling of leaf; early senescence</td>
<td>BCB, ERD1, SAG21</td>
<td>Cab, rbcS</td>
<td>Atgsr2, MT1, SAG 12, SAG 13, SAG 19, SAG 20</td>
<td></td>
<td></td>
<td>Miller et al. (1999)</td>
</tr>
<tr>
<td>160 ppb for 3-72 h</td>
<td>Early senescence</td>
<td>GST1, VSP2</td>
<td>MT1</td>
<td></td>
<td></td>
<td>CAB</td>
<td>Mira et al. (2002)</td>
</tr>
<tr>
<td>(a) 250 ppb for 8 h; (b) 250 ppb for 2 h; (c) 175 ppb for 8 h/4 days</td>
<td>(a) little chlorosis or lesions; (c) growth retardation</td>
<td>GST Apx, CuZn-SOD</td>
<td>PAT1</td>
<td>Fe-SODl GR, cab, rbs</td>
<td></td>
<td></td>
<td>Conklin and Last (1995)</td>
</tr>
</tbody>
</table>

**Table AX9-10. Proteins Altered by Ozone as Measured by Molecular Biological Techniques as mRNA Level or Other Gene Activity Rather than Enzyme Activity**
Table AX9-10 (cont’d). Proteins Altered by Ozone as Measured by Molecular Biological Techniques as mRNA Level or Other Gene Activity Rather than Enzyme Activity

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Physiological Events</th>
<th>Identified Proteins Fast Increase Response</th>
<th>Slow Increase Response</th>
<th>Examined, But No Change</th>
<th>Unknown Proteins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 ppb for 24 h</td>
<td></td>
<td>PR-1, PR-2a, PR-5, AtEDS1, AtGST1, AtGST2</td>
<td>PR-3b, PR-4</td>
<td>LOX2, AtOZI1, PAL, Lhcb, PAT1, HSP</td>
<td></td>
<td>Matsuyama et al. (2002)</td>
</tr>
<tr>
<td>250 ppb for 6 h</td>
<td>Lesion initiated on margin and spread inward</td>
<td></td>
<td></td>
<td>rcd1, on chromosome 1, single Mendelian trait</td>
<td></td>
<td>Overmyer et al. (2000)</td>
</tr>
</tbody>
</table>

Abbreviations used in Tables 9-7 and 9-10.

GST = Glutathione synthase
PAL = Phenylalanine ligase
PR-1 = Promoter region 1
Pxase = Peroxidase
CAT = Catalase
LOX1 = Lipooxygenase
ACS-6 = ACC synthase
SOD = Superoxide dismutase
CuZn-SOD = cyto SOD
Fe-SOD1 = Chl SOD
cyt APX = Ascorbate peroxidase
Chl GPX = Glutathione peroxidase
Cab mRNA = Chlorophyll a/b binding protein
chl GR = Glutathione reductase
BCB = Blue copper binding protein
ERD1 = Ethylene response
SAG21 = Senescence
rbcS = Rubisco small subunit
MT1 = Mitochondria
translation of the genes controlling those and other types of polypeptides. Once this membrane disruption occurs, the cell must mobilize repair systems to overcome the injury. Thus, carbon and energy sources once destined for productivity, must be used in repair processes. Some of these repairs are thought to result from the induction of specific genes. Photosynthesis is inhibited by direct inhibition of some of the enzymes, through byproducts of O$_3$ attack or by altered ionic balance. At the very least, the decrease in photosynthesis is a result of an O$_3$-induced decrease in rbcS mRNA.

**AX9.2.5 Primary Assimilation by Photosynthesis**

**AX9.2.5.1 Photooxidation: Light Reactions**

Photooxidation refers to the oxidation of chlorophyll within the light reaction due to an imbalance between light absorption and the CO$_2$ use to produce carbohydrates. It was discovered in the 1920s and studied under the concept of chlorophyll bleaching and photoautooxidation (Asada, 1999; Rabinowitch, 1945). What generally occurs is that electron transfer from H$_2$O to NADPH declines, and a light reaction overload occurs. The slowdown of electron transfer may also be due to inhibition of the dark reactions, through the poor use of small molecular weight carbohydrates or a lowered amount of the fundamental substrate CO$_2$.

To counteract these detrimental reactions, a series of “antioxidant” reactions exist, which eliminate the buildup of oxidative intermediates.

A lowered CO$_2$ level, which can be caused by stomatal closure (Heath, 1996), blocks the use of reduced plastoquinone (PQH$_2$) in Photosystem II through NADP reduction in Photosystem I (Hankamer et al., 1997). The buildup of PQH$_2$ reduces the amount of Q$_A$, resulting in a buildup of P$_{680}^+$Pheo$^+$ species (the primary photoact). The inability to reduce this radical leads to injury to the D$_1$ protein (32 kDa) and its fragmentation into 23-, 16-, and 10-kDa fragments (Hankamer et al., 1997). Ozone exposure of bean plants leads directly to the loss of this D$_1$ protein (Pino et al., 1995). The loss of D$_1$ stimulates the production of new D$_1$ (and its mRNA). Also, the production of the oxidized form of P$_{680}$ (P$_{680}^{+}$) is harmful to the plant, because electron flow from water to P$_{680}^+$ is limited, generating a P$_{680}^+$ (the triplet form of P$_{680}$), which is highly oxidizing and can lead to dangerous reactions. One form of protection is the use of β-carotene to convert the triplet form back to its normal state; however, that reaction can lead to the loss of β-carotene. Without the protection of β-carotene, oxygen reacts with oxidized
products to produce singlet state of O$_2$. This, in turn, can react with chlorophyll, leading to ring breakage that, in essence, leads to chlorosis. These types of reactions do not seem to occur often, but chlorosis is one form of visible injury, and loss of β-carotene has been reported.

Using a FACE system to expose soybean to elevated O$_3$, Morgan et al. (2004) found that, in leaves at the top of the canopy, there was no effect on the maximum light-adapted apparent quantum efficiency of PSII ($\phi$PSII,max), electron transport at growth [CO$_2$], and saturating light ($I_{sat}$) nor on the probability of an absorbed photon reaching an open PSII reaction center ($F'_v/F'_m$; the quantum yield). There was a small, but significant, decrease in photochemical quenching (qP) at the top of the canopy. As leaves aged, the decrease in qP was significant as leaves began to senescence, likely due to losses of chlorophyll. The results of these studies and others suggest that alterations to the dark reactions are much more common than to light reactions (Farage et al., 1991; Farage and Long, 1999).

**AX9.2.6 Alteration of Rubisco by Ozone: Dark Reactions**

A large body of literature published since 1996 shows that O$_3$ exposure affects Rubisco concentrations (Pell et al., 1997). Treatment of a variety of plants with O$_3$ at near-ambient levels results in a loss of Rubisco and of the mRNA coding for both subunits of Rubisco ($rbcS$, small and $rbcL$, large). In addition, oxidation of Rubisco by O$_3$-generated ROS may be an important factor in suppressing photosynthesis (Pell et al., 1997). Increased carbonyl concentrations of Rubisco are correlated with O$_3$ injury (Kanoun et al., 2002; Leitao et al., 2003). Because Rubisco plays such an important role in the production of carbohydrates (Figure AX9-14), any loss may have severe consequences for the plant’s productivity.

Chronic O$_3$ exposure, both with and without elevated CO$_2$, significantly lowered assimilation and leaf conductance of soybean in aging mature leaves (Fiscus et al., 1997; Reid and Fiscus, 1998), which was associated with significant decreases in Rubisco content in aging leaves. Noormets et al. (2001b) also found that O$_3$ had the greatest effect on older leaves of aspen clones using a FACE exposure facility in which areas of ambient CO$_2$ (daytime 360 ppm) and ambient with added CO$_2$ (560 ppm), with added O$_3$ (97.8 ppb), and with added CO$_2$ and O$_3$, were used. They found an O$_3$-induced decline in assimilation and in conductance, and subsequently confirmed that the internal CO$_2$ (calculated for within the leaf) is not affected by O$_3$ exposure. Higher levels of CO$_2$ increased the assimilation and lowered the conductance,
Figure AX9-14. The production of Rubisco and its Calvin Cycle pathway reactions. Two peptides are used to build Rubisco: \( rbcS \), the small subunit produced by DNA within the nucleus; and \( rbcL \), the large subunit produced by DNA within the chloroplast itself. Clearly both polypeptides must be closely regulated to produce the enzyme in a coherent manner. Furthermore, at least five isoforms of DNA can produce \( rbcS \), each of which is regulated by a different promoter region.

maintaining the internal to external CO\(_2\) ratio identical to that found with the ambient CO\(_2\) level, corresponding to the theory of Farquhar et al. (1980). More to the point was that the stomatal limitation\(^{12}\) was not altered by O\(_3\) exposure, with or without excess CO\(_2\). It is critical to point out that mesophyll conductance is directly linked to internal CO\(_2\) level\(^{13}\). So if \( C_i/C_o \) (\( C_o = \text{CO}_2 \) outside) is constant and \( g_s \) declines, then \( g_m \) must likewise decline. If, as it is argued, Rubisco levels are constant or at least increasing, then a regeneration of RuBP must be the cause of the

\(^{12}\)The limitation was defined as the ratio of stomatal resistance to the total resistance, which included the operating point of the assimilation (A) versus internal CO\(_2\) concentration (\( C_i \)) curve and the resistance of the boundary layer. The operating point of the curve was defined as the internal CO\(_2\) level, which is calculated by the conductance and assimilation. The resistance of this operating point was calculated as the cotangent of the slope to the operating point. Unfortunately, the slope is not a dimensionless parameter but is rather moles of air per area of leaf’s of time and, thus, it is unclear whether the slope changes with added CO\(_2\) and O\(_3\).

\(^{13}\)Respiration is generally small at saturating A and often is ignored. By transforming the term \( \{A = g_s (C_o - C_i)\} \) into \( \{A = g_t C_o - g_m C_i = (g_t - g_m) C_o\} \) where \( g_m = g_t (C_i/C_o) \) or the mesophyll conductance in earlier literature.
decline in $g_m$. Farquhar et al. (1980) were more concerned with high levels of $CO_2$ and had little to say about $O_3$ exposure.

In a similar study, Morgan et al. (2004) examined elevated $O_3$ using a FACE system to increase $O_3$ by 20% over the entire growing season. They examined $O_3$ effects from emergence through the entire life cycle to senescence. Leaf photosynthetic performance was measured using a LI-COR 6400 with integrated chlorophyll fluorescence capabilities to examine both dark and light reactions. This study found no effect of elevated $O_3$ on newly expanded leaves over the growing season. There were little $O_3$-induced changes in the light reactions; however, as leaves aged, there were significant changes in the dark reactions. For example, there were significant losses in the maximal photosynthetic assimilation in saturating light ($A_{sat}$) and the maximum rate of carboxylation (an in vivo measure of Rubisco efficiency), and maximum rate of electron transport for the regeneration of RuBP ($J_{max}$). The findings showed the greatest impact of $O_3$ on the oldest leaves and demonstrated the significant impact on seed production.

The level of carbohydrate within the cell has an effect upon the amount of mRNA for Rubisco ($rbcS$). Experiments by Krapp et al. (1993) indicated that a decline in carbohydrate levels is probably due to the increased production of control metabolites, such as fructose 2,6-bisphosphate, which can shut down important sugar production pathways. This report also leads to a measure of half-time for the decline in $rbcS$ of about 2 days\(^{14}\) when 50 mM glucose is added to a cell suspension of Chenopodium. Also, the carbohydrate level was increased by cold girdling the petioles of intact tobacco and potato plants. The levels of carbohydrate nearly doubled in 5 days and the level of $rbcS$ declined rapidly (reaching 25% after 12 h). A decline in Rubisco followed, but more slowly (with an estimated half-time of about 108 h after a lag of at least 12 hours). This, of course, is expected; the level of the enzyme would decline slowly with a lag after a loss of the message.

A better estimation of the half-life of $rbcS$ can be found in the Jiang et al. (1993) study of the destabilization of the message by an antisense message. The wild-type $rbcS$ in tobacco had a half-life of about 5 h compared to that in the mutant with the antisense. It was argued that the antisense message increased the degradation of the normal $rbcS$. The estimated half-life of $rbcS$ under $O_3$ fumigation is about 1 h (Pell et al., 1994). Although comparisons of these diverse systems cannot be easily made, the normal half-life of $rbcS$ may be closer to 5 to 10 h; and $O_3$

\(^{14}\)The amount of Rubisco drops from an initial 0.12 to a final amount of 0.04 µmole/g-FW in 6 days.

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fumigation does not simply stop the transcription of DNA, but rather it alters the rate of degradation, either independently of, or simultaneously with, transcription.

Williams et al. (1994) developed a correlation between the levels of ABA after water stress in Arabidopsis thaliana leaves and the loss of rbcS. Although their data were not quantitative, the level of ABA had a half-time rise of about 1 to 2 h and the level of rbcS had a half-life decline of about 2 to 4 h. Their work suggests that drought stress may alter the CO₂ metabolism by changing enzyme relationships much more than by merely closing the stomata. If an ABA rise is lowering rbcS, rbcS may not be a good marker of O₃ fumigation except under highly controlled conditions.

AX9.2.7 Carbohydrate Transformations and Translocation

The question of whether translocation of the sugars out of the leaf is inhibited by O₃ exposure arises, because productivity is often dramatically inhibited by O₃ fumigation. Though nearly 35 years have passed since Dugger and Ting (1970) investigated the question of sugar transport within the leaf, the question has since been little studied. Translocation (Cooley and Manning, 1987) appears to be inhibited, because root functions are impaired by O₃ exposure. Many observed events suggest that while carbon assimilation within the leaf declines, translocation of carbon is inhibited even more so, because plant growth points are inhibited and root/shoot ratios are altered (Dugger and Ting, 1970; Gerant et al., 1996; Tjoelker et al., 1995).

Many of the experiments with O₃ fumigation indicate that O₃ exposure decreases the net growth or dry mass of the plant, but the mechanism is poorly understood. Generally the decrease in assimilation is much less than the decrease in growth, but not always. Under many conditions, the stomata will close partially, decreasing assimilation by a smaller factor. Only a long exposure, or high levels of exposure for a short time, generate enough decline in Rubisco to make the growth of the plant problematical. No convincing argument has linked the decrease in growth with a small decline in assimilation, either by a conductance- or Rubisco-limitation. Measures of assimilation with crops are frequently done on upper canopy leaves, which are the last leaves to exhibit O₃ injury, while leaves deeper in the canopy exhibit injury and early senescence. Crop root growth must be sensitive to these and other O₃ effects, because root biomass is often suppressed early by elevated O₃.
Volin et al. (1998) found O$_3$ exposures statistically decreased leaf area ratio, specific leaf area, leaf weight ratio, and root weight ratio in *Populus tremuloides* and two C3 grasses (*Agropyron smithii* and *Koeleria cristata*) but not in *Quercus rubra* and in the C4 grasses *Bouteloua curtipendula* and *Schizachyrium scoparium*. There was no statistically significant change in any species in leaf conductance (4% level decline in *K. cristata*) nor in assimilation (although there was a decline in assimilation at the 6% level for *P. tremuloides* and a decline at the 1% level in *B. curtipendula*). They also reported a correlation between growth decline and decreased stomatal conductance among all species.

Tradeoffs are made by plants. Birch grown in highly fertilized conditions exhibited a greater leaf turnover when exposed to O$_3$, in that leaves not only formed faster but abscised faster, presumably due to early senescence; whereas birch grown under poorer fertilized conditions retained their leaves longer and had a greater respiration rate within those leaves (Maurer and Matyssek, 1997). Again, one must be careful in comparing short-term versus long-term exposures. Grulke et al. (2001) observed that maximum concentrations of carbohydrates in 1-year-old needles that had not abscissed due to early senescence declined when subjected to year-long exposures along an increasing pollution gradient. Furthermore, the monosaccharide concentrations (along with starch) in fine roots were largely decreased, suggesting that needle sugars were limiting, leading to root-sugar limitations. However, determination of the total productivity and detailed balance of carbohydrate was impossible, because these were older, larger trees and the data were taken over a full growing season. For a shorter-term exposure of 9 days, Smeulders et al. (1995) observed that O$_3$ appeared to increase the retention of labeled photosynthates within the needles of Douglas Fir, and, at higher exposures (400 versus 200 or 0 µg/m$^3$), the total starch within the needles decreased, suggesting that less carbohydrate was produced within the cell or perhaps that it was in compounds not measured.

Studies with Pima cotton (*Gossypium barbadense*), aspen (*Populus* spp.) and bean seedlings (*Phaseolus vulgaris*) indicate that acute O$_3$ exposures inhibit export of the current assimilate that provides carbohydrates to the roots from source leaves of cotton as well as recent assimilate from the older leaves of aspen and bean (Grantz and Yang, 2000). Grantz and Yang (2000) attempted to distinguish between potential mechanisms of O$_3$ phytotoxicity operating at the level of the whole plant. Four hypotheses were tested by fumigating cotton: (1) O$_3$ exposure reduces leaf pools of soluble sugars; (2) pruning leaf area and reducing source strength to match
that of O₃-treated plants reproduces O₃ effects; (3) pruning lower leaf area more closely reproduces O₃ effects than pruning the upper leaf area; and (4) manipulating plant age and, thereby, plant size to match O₃-treated plants reproduces O₃ effects. All were shown to be incorrect. Under each of the above conditions, Grantz and Yang (2000) reduced the amount of foliage to match that caused by O₃ injury. While the treatments reduced the biomass and leaf area, they did not alter biomass allocation nor root function. They concluded that a simple loss of foliage does not induce the changes in translocation to the roots to the same extent as does O₃ injury.

This finding by Grantz and Yang (2000) is important in that it suggests that O₃ can trigger a plant-wide response that may be linked to alterations in signal transduction and the generation of whole plant signals. Stitt (1996) suggested that “…allocation is regulated by long-distance signals that act to influence growth of selected sinks and to modify the delivery of resources to these sinks in parallel.” Cooley and Manning (1987), citing McLaughlin and McConathy (1983), suggested three possible ways that O₃ fumigation might alter translocation: (1) malfunction of the phloem loading process, (2) increased translocation to leaf injury repair, and (3) an altered balance between the leaf and sinks caused by reduced carbon fixation and a greater demand for assimilate in the leaf.

Ethylene has been shown to reverse this sugar inhibition of development and to be antagonistic to the ABA effect (Finkelstein and Gibson, 2002). However, these effects depend greatly upon the developmental stage of the plant. Thus, the balance of the effectors (sugars, ABA, and ET) may interact to generate the variation observed in the O₃-induced productivity decline. For example, O₃ fumigation can induce a shift in the carbon transfer between roots and shoot, and this shift can be amplified by mild drought (Gerant et al., 1996). Furthermore a regulation of source-sink relations with the defense responses induced by elicitors was observed by wounding the leaves of Chenopodium rubrum (Roitsch, 1999). Ethylene appears to be able to repress the expression of extracellular invertase, which is critical for control and downloading of sucrose derived from the translocational stream (Roitsch, 1999). In addition, the development of Arabidopsis at high concentrations of glucose or sucrose is arrested by increasing the ABA level (Coruzzi and Zhou, 2001).
Clearly more work is needed on the interactions between assimilation, translocation, and source/sink relations with O$_3$ exposure. In these interactions, one must be aware of the developmental age of the plants and their phytohormonal status.

**AX9.2.7.1 Lipid Synthesis**

Heath (1984) summarized several early reports of O$_3$-exposure induced lipid alterations. Most concerned the production of MDA (malonyldialdehyde) as a measure of lipid oxidation as well as the loss of unsaturated fatty acids. However, a series of experiments by Sakaki and coworkers (Sakaki et al., 1983, 1985) concentrated on one type of fumigation system and one metabolic pathway. This literature provides the best, most complete story with regard to lipid metabolism and O$_3$ fumigation and suggests that O$_3$ injures cellular membrane systems via lipid destruction.

Sakaki and coworkers (Sakaki et al., 1983, 1985) used spinach, which is a sensitive plant but which has not been much evaluated with respect to O$_3$ fumigation. While the O$_3$ level was high (0.5 ppm), enough work has been done to be able to discern what is happening. The first paper showed that chlorophyll bleaching does not begin until the plants have been exposed to O$_3$ for more than 10 h, whereas some MDA production begins with as little as 6 h exposure (Sakaki et al., 1983). Consistent production of MDA, indicative of gross disruptions, occurred only after 8 h exposure (Sakaki et al., 1985), within the timescale when chlorophyll and carotenoid levels begin to decline. Concurrently, the total fatty acid (FA) level decreased from ~481 to 358 nmol/cm$^2$ as the MDA level increased from 0.6 to 2.4 nmol/cm$^2$, indicating FA peroxidation (Sakaki et al., 1985).

Sakaki et al. (1983) also studied the development of changes by cutting disks from exposed leaves and floating them on water solutions for varied time periods (up to 24 h). This permitted feeding experiments to be done easily, whereas the cutting gives rise to an additional wound response and eliminates metabolite movement to and from other portions of the plant. The floating experiments indicated that, after exposure, scavengers of singlet oxygen ($^{1}$O$_2$), such as D$_2$O, and of hydroxyl radicals, such as benzoate and formate, have no effect on development of the MDA response after 8 h of in vivo fumigation, while scavengers of (O$_2^{-}$), such as tiron and ascorbate, lowered the amount of MDA formed. By measuring metabolites immediately after cessation of fumigation, they were able to show that ascorbate loss began with the onset of

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fumigation, as did SOD loss. A lag time associated with the production of DHA suggested that the reaction of ascorbate with fumigation did not immediately produce the oxidation product. The first 4 h of exposure yielded 30 nmole/cm$^2$ of ascorbate loss with 5 nmole/cm$^2$ of DHA production, whereas the second 4 h of exposure yielded 20 nmole/cm$^2$ of ascorbate loss with 20 nmole/cm$^2$ of DHA production.

Nouchi and Toyama (1988) exposed Japanese morning glory (*Ipomea nil*) and kidney bean (*Phaseolus vulgaris*) to 0.15 ppm O$_3$ for 8 h. Under these conditions, little visible injury was found with up to 4 h of exposure, while injury increased by ~50% after 8 h of exposure. Morning glory produced more MDA than kidney bean, which produced the same as the zero-time control. Morning glory also demonstrated a slight (5%) drop in MDGD (monogalactosyldiacylglycerol), with increases in PC (phosphatidylcholine), PG (phosphatidylglycerol), PI (phosphatidylinositol), and PE (phosphatidylethanolamine) after 4 h. Twenty-four hours later, the drop in MGDG (monogalactosyldiacylglycerol) was much larger and was thought to be related to an inhibition of UDP-galactose galactotransferase due to a rise in free fatty acids (FFAs) in the chloroplast. Note that the two distinct timescales involved in O$_3$ fumigation, immediately postfumigation and a day or so later, allows for comparison after the plant metabolism responds to the fumigation event.

The pathway for the formation of MGDG and DGDG (digalactosyldiacylglycerol) is located on the chloroplast envelope. Diacylglycerol (DG) arrives from either the endomembrane system or the stroma and the enzyme UDP galactose-1,2-diacylglycerol galactosyltransferase (UDGT) forms MGDG with galactose from UDP-galactose. Sakaki et al. (1990) suggested that the O$_3$-induced inhibition of UDGT was due to a release of FFAs from within the chloroplast. These FFAs are inhibitory to UDGT, but not to GGGT, which is stimulated by high concentrations of Mg$^{2+}$ (Sakaki et al., 1990). The Sakaki et al. (1990) data indicate that the in vivo measured activities of both enzymes isolated after fumigation are not affected by O$_3$ fumigation. Both enzymes have sensitivity sulfhydryls, and both are located on the envelope. Ozone, if it reaches those sulfhydryls, should inhibit these enzymes; yet inhibition was not seen.

It has been thought for years that tocopherols functioned as antioxidants in biological systems (Tappel, 1972). Hausladen et al. (1990) examined the role of antioxidants in red spruce (*Picea rubens*) by following seasonal changes. They fit the level of tocopherol within the needles (/g-FW) to the time of the year and found little change (fit as level = A + Bt + Ct$^2$).
From this empirical fit, they found that the constant A was lower with higher levels of O₃. The seasonal variation coefficients, B and C, were also lower, suggesting year-long low tocopherol levels. Variation with the season is not particularly surprising, given that phytochrome action may be linked to tocopherol biosynthesis (Lichtenthaler, 1977). Hausladen et al. (1990) reported a significant (p < 0.05) trend in the difference between the high and low level of treatment; although there was no discussion of why it occurred or what it meant in relation to metabolism. Their major conclusion was that the antioxidant changes due to O₃ exposure may decrease cold hardiness.

Sterols, believed to act as membrane stabilizers, have been investigated by several groups with mixed results. Tomlinson and Rich (1971), who exposed common bean at 0.25 ppm for 3 h, and Grunwald and Endress (1985), who exposed soybean at 0.07 ppm for 6 h for 48 days, reported an increase in free sterols and a decline in esterified sterols. However, Trevathen et al. (1979) exposed tobacco at 0.3 ppm for 6 h and reported opposite results. None of these investigators believed that O₃ had attacked the sterols directly, instead, they believed that these changes involved metabolism and membrane stability. If O₃ induced a metabolic shift that disturbed the polar lipid to sterol balance, membrane reactions to other stressors, such as cold tolerance, would certainly also be affected, perhaps detrimentally.

AX9.2.8 Role of Age and Size Influencing Response to Ozone

Clearly many changes occur with O₃ exposure can be observed within hours, or perhaps days, of the exposure. This document has argued that many of those events are connected with wounding and elicitor-induced changes in gene expression, but those are relatively fast acting changes (a timescale of tens of hours). Two other effects due to O₃ take longer to occur and tend to become most obvious under long periods of low-O₃ concentrations. These have been linked to senescence or some other physiological response very closely linked to senescence. These two responses, separated by a time sequence, are shown diagrammatically in Figure AX9-15.

The understanding of how O₃ affects long-term growth and resistance to other biotic and abiotic insults in long-lived trees is unclear. Often, the conditions to which a tree is subjected to in one year will affect the response of that tree in the next year. This has been called “memory effect”, although the term “carry-over” is preferred. In other words, a condition in an earlier year sets the stage for a reaction in the next year; thereby giving a “cause-effect” scenario.
In perennial plant species, growth affected by a reduction in storage carbohydrates may result in the limitation of growth the following year (carry-over effects) (Andersen et al., 1997). Carry-over effects have been documented in the growth of tree seedlings (Hogsett et al., 1989; Sasek et al., 1991; Temple et al., 1993; U.S. Environmental Protection Agency, 1996) and in roots (Andersen et al., 1991; U.S. Environmental Protection Agency, 1996). Accumulation of the carry-over effects over time will affect survival and reproduction. Data on the cumulative effects of multiple years of O$_3$ exposures have been, for the most part, the result of 2- to 3-year seedling studies. The difficulty of experimentally exposing large trees to O$_3$ has lead to the tacit assumption that seedling response to O$_3$ is a good predictor of large-tree response to O$_3$ (U.S. Environmental Protection Agency, 1996).

The carry-over effects of O$_3$ exposures as observed in tree seedlings cited above by Hogsett et al. (1989) have been termed “memory effects” by Langebartels et al. (1997) and proposed by Schmieden and Wild (1995) to explain the sensitivity of spruce seedlings to frost in the winter after having been exposed to O$_3$ during the previous summer. Norway spruce (*Picea abies* L.) exposed to 80 ppb O$_3$ for a whole growing season, demonstrated visible injury symptoms the following year when the new needle flush appeared (Langebartels et al., 1997). Additional studies using Norway spruce and Scots pine (*Pinus sylvestris* L.) seedlings have shown similarly delayed responses following O$_3$ exposures. Carry-over symptoms were noted to develop at
different times of the year, depending on the species of seedling exposed: in early spring for Norway spruce, and in early autumn for Scots pine (Lange et al., 1989). Visible effects of O$_3$ exposures on spruce and pine may develop after a substantial delay during the “sensitive” periods of the year when chlorophyll and needle loss normally occur. Norway spruce and Scots pine differ in their sensitive periods because of the different needle classes normally remaining on the tree (Langebartels et al., 1997).

Nutrient status of the tree during the overwintering phase of its life (Schmieden and Wild, 1995) and chronic exposure to ambient O$_3$ (less severe with fewer peaks of very high levels) induce (1) mineral nutrient deficiency; (2) alterations of normal metabolism, including allocation of carbohydrates and probably nitrogen; and (3) disturbance of normal transpiration and diurnal cycling, leading to water stress. This condition, termed “Montane yellowing”, appears to be related to nutrient deficiencies rather than senescence (although early loss of leaves and needles occurs). While generalized low nutrient concentrations may not occur within the foliage, localized deficiencies may. However, they are hard to observe or prove without a great deal of work involving all portions of a tree and without a general hypothesis of what is occurring.

**AX9.2.9 Summary**

As the understanding of wounding responses of plants and more genome details and varied plant mutants become available, the cellular and physiological responses of plants to O$_3$ exposures are slowly becoming clearer. However, more studies are needed on a larger variety of species. Nevertheless, several key findings and conclusions can be highlighted:

1. The entrance of O$_3$ into the leaf through the stomata remains the critical step in O$_3$ sensitivity. Not only does O$_3$ modify the opening of the stomata, usually closing it partially, but O$_3$ also appears to alter the response of stomata to other stressful situations, including a lowering of water potential and ABA responses. The concentration of O$_3$ within the leaf is not the same as the external concentration due to reactions within the leaf, but it is not “zero”.

2. The initial reactions of O$_3$ within the leaf are still unclear, but the involvement of H$_2$O$_2$ is clearly indicated. The detection of possible products by EPR spectroscopy has progressed, but has not reached the point where any products can be identified. Nonetheless, reaction of O$_3$ (or its product) with ascorbate and possibly other antioxidants present in the apoplastic space of the mesophyll cells is clear and serves to lower the amount of O$_3$ or product available to alter the plasma membrane of the cells.
(3) The initial sites of membrane reactions seem to involve transport properties and, possibly, the external signal transducer molecules. The alteration and mechanism of the alteration of the varied carriers of $K^+$ and $Ca^{2+}$ is far from clear, but it would seem that one of the primary triggers of $O_3$-induced cell responses is a change in internal $Ca^{2+}$ levels.

(4) The primary set of metabolic reactions that $O_3$ triggers now clearly includes those typical of “wounding” responses generated by cutting of the leaf or by pathogen/insect attack. Again, this seems to be due to a rise in cytoplasmic $Ca^{2+}$ levels. Ethylene release and alteration of peroxidases and PAL activities, as well as activation of many wound-derived genes, seem to be linked to some of the primary reactions.

(5) The alteration of normal metabolism due to wounding has effects outside of the cytoplasm. What effects are due to the “spreading of the problem” to other cellular organelles is less clear. One of the secondary reactions is linked to an activation of a senescence response. The loss of Rubisco and its messenger RNA is linked to an early senescence or a speeding up of normal development leading to senescence. The loss of photosynthetic capacity is linked to the lowered productivity of plants, and problems with efficient translocation are indicated, although photosynthesis and translocation still occur at a reasonable rate. The loss of productivity is not yet clearly explained.

It is important to note that the dramatic strides in understanding the genetic makeup of plants, gene control, and signal transduction/control over the last few years will likely accelerate in the future. That understanding will translate into better models of the hypotheses listed above and into more detailed schemes of how $O_3$ alters much of basic plant metabolism. Thus, while understanding of how $O_3$ interacts with the plant at a cellular level has dramatically improved, the translation of those mechanisms into how $O_3$ is involved with altered cell metabolism, with whole plant productivity, and with other physiological facts remain to be more fully elucidated.

**AX9.3 MODIFICATION OF FUNCTIONAL AND GROWTH RESPONSES**

**AX9.3.1 Introduction**

The responses of plants to any air pollutant may be significantly influenced by a wide range of biological, chemical, and physical factors. A plant’s genetic makeup is an important inherent biological determinant of its response, but response can also be modified by other biological agents such as disease-causing organisms, insects and other pests, and by other higher
plant species with which it may be competing for resources. Chemical factors that may influence response to an air pollutant range from mineral nutrients obtained from the soil to other air pollutants and agricultural chemicals. Physical factors that may influence response include light, temperature and the availability of moisture, which are components of climate and climate change.

Some environmental factors can be controlled, to some degree, by man, while others cannot. The biological factors (e.g., pests, diseases, symbioses, competition) are partly controllable in agriculture but much less so (if at all) in natural ecosystems. It is possible to control agricultural soil fertility and the use of agricultural chemicals, as well as to exercise some control over the supply of water and airborne chemical factors. In contrast, the physical factors (e.g., light and temperature) are uncontrolled in the field even though they may be controllable in specialized situations such as greenhouses or shade houses. Although light and temperature are components of climate, they are initially reviewed as individual physical factors, even though temperature effects are revisited to some extent in the discussion of interactions with climate change.

The impacts of these various factors on plant response to O$_3$ and other oxidants were extensively reviewed in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996). It was noted in that document that, since any combination of these factors may come into play at some time in a plant’s life history, “response will be dictated by the plant’s present and past environmental milieu, which also includes the temporal pattern of exposure and the plant’s stage of development.” That document also stressed that both the impact of environmental factors on response to oxidants and the corollary effects of oxidants on responses to environmental factors have to be considered in determining the impact of oxidants on vegetation in the field. The variability observed in plant responses to defined exposures to O$_3$, particularly under field conditions, is a consequence of the influences of genetics and the range of environmental variables.

In view of the large number of factors to be considered, this section focuses mainly on situations in which there is clear evidence that environmental factors truly interact with oxidant effects, i.e., they magnify or diminish the impact of O$_3$ and are not merely additive to it. Conversely, it will cover situations where O$_3$ acts synergistically or antagonistically, but not additively, with effects induced by other factors. It will also emphasize those interactions as a
result of which overall plant growth, development, and yield are adversely affected, rather than
the details of interactions at the mechanistic level, unless the latter are deemed to be essential to
an understanding of larger-scale effects.

Few studies reported since the 1996 document have systematically investigated
quantitative responses to \(O_3\) exposure concurrent with other variables. Although the 1996
document cited almost 300 references pertaining to environmental interactions, and the present
review cites more than 350 new references, the bulk of the recently published work has
continued to be specific and frequently narrowly focused. Hence, the new findings are scattered
and far from uniformly distributed among the various subtopics. In some instances, little or no
new research has been published that adds to our understanding since the 1996 document. In
such cases, the present review is, therefore, restricted to summarizing the understanding that was
current in 1996.

A few reviews have appeared since the early 1990s dealing with various environmental
interactions, and these are cited in relevant sections below. More general recent reviews are
those of Wellburn (1994); multi-authored volumes edited by Alscher and Wellburn, 1994; Yunus
and Iqbal, 1996; De Kok and Stulen, 1998; and Bell and Treshow, 2002); and reports by the
United Nations Environment Programme (UNEP, 1999) and the Intergovernmental Panel on
Climate Change (IPCC, 2001). Several biotic and abiotic interactions involving forest trees are
discussed in the review by Johnson et al. (1996b).

Although many reports have provided quantitative information on interactive effects,
in most cases the information simply describes a specific situation involving only two or three
levels of a variable. While this may be adequate to provide statistical information about the
existence of interactions with environmental factors, it does not permit the development of
response surfaces or models to show the form that any influence of such factors might take
on \(O_3\) exposure-response relationships or how \(O_3\) might quantitatively influence responses to the
factors in question. This, together with the fragmented information available on the effects of
most factors, has contributed to the relative lack of development of simulation models of
oxidant-environmental factor interactions. Yet, as noted by Taylor et al. (1994), the large
number of variables constrains the assessment of pollution effects by experimentation alone.
The only alternative is to use mathematical models to attempt to predict the outcome of
different \(O_3\) and environmental factor scenarios, building up their complexity in stages.

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The few models thus far used to investigate O$_3$ stress have been adapted from existing process models of crop or tree growth that include limited numbers of physical or chemical variables such as temperature, soil water stress, or nutrient deficiency. Taylor et al. (1994) provided a listing of several simulation models developed for trees at the individual, stand, and regional levels; these and many other models have been critically reviewed by Kickert and Krupa (1991) and Kickert et al. (1999). However, regardless of whether such models are descriptive/empirical or process/mechanistic, their outputs will always be associated with varying degrees of uncertainty and require validation against observable responses wherever possible. Kickert et al. (1999) also noted that very few of the models that have been described provide risk assessments that address likelihood, in contrast to consequence assessments that address the magnitudes of effects. Thus, even though capable simulation models of plant response to O$_3$ involving complex mixes of many biological, physical, and chemical factors may be out of reach at the present time, the use of newer mathematical approaches such as artificial neural networks (ANNs) has enabled insightful analyses to be performed in several field studies involving numerous micrometeorological and other environmental variables (e.g., Balls et al., 1996; Mills et al., 2000).

Because the ensuing subsections deal with studies of O$_3$ interactions involving an extremely wide array of biological, physical, and chemical factors in the plant’s environment, it is inevitable that many different exposure facilities and regimes have been used in these studies. To provide specific information regarding the O$_3$ exposure concentrations, profiles, hours and days of exposure (as well as the types of systems and facilities used for the exposures) would add a wealth of detail that would do little to assist our understanding of the roles of environmental factors in modifying the impact of O$_3$ on vegetation or to facilitate our ability to estimate the magnitudes of any such modifications. Thus, only experiments in which the exposure levels and regimes were within the bounds of ambient experience in North America are discussed in the ensuing subsections, regardless of the type of exposure profile used. The cutoffs used have been ~200 ppb for peak hourly concentrations or for short-term exposures, ~100 ppb for daytime means involving prolonged exposures for several hours, or a doubling of ambient levels in cases in which enriched exposure levels were a function of ambient levels. Actual details of the exposure regimes and conditions can, of course, be obtained from the original references but are only stated here when any distinction is required between the effects of
different exposure levels. Hence, it should be understood that ensuing statements such as “. . .it was found that O$_3$ caused. . .” should always be read as “. . .it was found that exposures to O$_3$ [within the range of those that have been measured in ambient air] caused.”

**AX9.3.2 Genetics**

The response of individual plants to O$_3$ is affected by several factors, including the environment in which it is growing, competition with neighboring plants, ontogeny, and genetics. This section examines the role of genetics in plant response to O$_3$. In addition, major knowledge gaps in the understanding of genetic aspects of O$_3$ response are pointed out.

It is well known that species vary greatly in their responsiveness to O$_3$ (U.S. Environmental Protection Agency, 1996). This again has been recently demonstrated for grassland species (Bungener et al., 1999b; Bungener et al., 1999a; Franzaring et al., 2002; Nussbaum et al., 2000a; Pleijel and Danielsson, 1997; Warwick and Taylor, 1995), wild herbaceous plants (Bergmann et al., 1999; Danielsson et al., 1999), agricultural crops (Benton et al., 2000; Elagöz and Manning, 2002; Fumagalli et al., 2001; Heagle and Stefanski, 2000; Köllner and Krause, 2003; Nali et al., 2002; Nussbaum et al., 2000b; Ollerenshaw et al., 1999; Postiglione et al., 2000; Renaud et al., 1997), horticultural shrubs and trees (Findley et al., 1997; Hormaza et al., 1996), and forest trees (Bortier et al., 2000a; Guidi et al., 2001; Landolt et al., 2000; Matsumura, 2001; Momen et al., 2002; Nali et al., 2002; Oksanen and Rousi, 2001; Pääkkönen et al., 1997; Pell et al., 1999; Saitanis and Karandinos, 2001; Volin et al., 1998; Zhang et al., 2001). These studies have shown a wide range of responses to O$_3$, from growth stimulation by a few species such as *Festuca ovina* L. (Pleijel and Danielsson, 1997) and *Silene dioica* and *Chrysanthemum leucanthemum* (Bungener et al., 1999b; Bungener et al., 1999a) to significant growth reduction, depending on environmental conditions and exposure dose.

While determining the explanation for differences in species sensitivity to O$_3$ remains one of the challenges facing plant biologists (Pell et al., 1999), a number of hypotheses have been suggested. Reich (1987) proposed that variation in O$_3$ sensitivity could be explained by variation in total uptake of the gas. Others have suggested that (1) fast-growing species are more sensitive than slower-growing ones (Bortier et al., 2000b), (2) overall O$_3$ sensitivity may be closely linked to root responses to O$_3$ (Warwick and Taylor, 1995), or (3) the relative ability of species to detoxify O$_3$-generated reactive oxygen free radicals may determine O$_3$ sensitivity (Alscher et al.,
Volin et al. (1998) suggested that the relative rate of stomatal conductance and the photosynthesis rate at a given conductance both contribute strongly to determining a species sensitivity to $O_3$. Likely, there is more than one mechanism determining sensitivity, even in a single species.

Within a given species, individual genotypes or populations can also vary significantly in $O_3$ sensitivity (U.S. Environmental Protection Agency, 1996). For example, the intraspecific variation in $O_3$ sensitivity was a factor of two for *Phleum pratense* (Danielsson et al., 1999) and *Trifolium repens* L. (Postiglione et al., 2000). A similar range of intraspecific variations in $O_3$ responses was demonstrated for clonal differences in *Betula pendula* by Pääkkönen et al. (1997) and *Prunus serotina* (Lee et al., 2002). These examples of wide ranges within species responses suffice to show that caution should be taken when ranking species categorically as having an absolute degree of $O_3$ sensitivity (Davison and Barnes, 1998).

**AX9.3.2.1 Genetic Basis of Ozone Sensitivity**

Plant response to $O_3$ is determined by genes that are directly related to oxidant stress and to an unknown number of genes that are not specifically related to oxidants. The latter includes genes that control leaf and cell wall thickness, stomatal conductance, and the internal architecture of the air spaces. Although there is currently a great emphasis on individual antioxidants that can be manipulated by molecular methods, the challenge is to determine the relative contributions of all of the components to plant response and to understand the interplay between them. Recent studies using molecular biological tools are beginning to increase the understanding of $O_3$ toxicity and differences in $O_3$ sensitivity.

While much of the research in developing the understanding of $O_3$ responses has been correlative in nature, recent studies with transgenic plants have begun to positively verify the role of various genes and gene products in $O_3$ tolerance. The finding that the overexpression of MnSOD (manganese superoxide dismutase) in transgenic tobacco plant chloroplasts increased $O_3$ tolerance (Van Camp et al., 1994) provided the first definitive proof of antioxidants key role in $O_3$ tolerance. Subsequently, Broadbent et al. (1995) showed that the simultaneous overexpression of pea glutathione reductase in both chloroplasts and mitochondria enhanced $O_3$ tolerance in transgenic tobacco. Similarly, increased $O_3$ tolerance to $O_3$-induced foliar necrosis was shown for transgenic tobacco plants overexpressing the cytosolic Cu/Zn-SOD gene (Pitcher...
Transgenic tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase, which reduces ascorbate peroxidase production, showed increased susceptibility to $O_3$ injury, suggesting a key role in $O_3$ tolerance for the antioxidant ascorbate peroxidase (Örvar and Ellis, 1997).

The consensus among molecular studies of $O_3$ sensitivity is pointing to $O_3$ as triggering salicylic acid, ethylene, and jasmonic acid production and that the signaling of these molecules determines, in some cases, the $O_3$ susceptibility of plants (DeCaria et al., 2000; Moeder et al., 2002; Nunn et al., 2002; Overmyer et al., 2000; Rao and Davis, 1999; Tamaoki et al., 2003; Vahala et al., 2003a,b). Increased levels of jasmonic acid production in $O_3$-tolerant compared to $O_3$-sensitive plants has been shown for Arabidopsis (Overmyer et al., 2000) and Populus (Koch et al., 1998, 2000). Blockage of ethylene production by using antisense methods with 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase suggest strongly that ethylene synthesis and perception are required for $H_2O_2$ production and cell death following $O_3$ exposure of Lycopersicon esculentum (Moeder et al., 2002). Ethylene signaling may have multiple roles in $O_3$ tolerance determination as was demonstrated recently by Vahala et al. (2003a,b) who found that, in Populus tremula × P. tremuloides hybrid clones differing in $O_3$ sensitivity, ethylene accelerated leaf senescence in sensitive plants under low $O_3$, but under acute $O_3$, ethylene seemed to be required for protection from cell death.

While changing the expression of single antioxidant genes has proven very useful in identifying possible mechanisms of $O_3$ sensitivity and tolerance (Kuzniak, 2002), it should be noted that increased $O_3$ tolerance has not been shown in some studies of transgenic plants with enhanced antioxidant production (Strohm et al., 1999; Strohm et al., 2002; Torsethaugen et al., 1997). Clearly, ethylene production plays a role in $O_3$ sensitivity, but the roles of various antioxidants in $O_3$ tolerance regulation are yet to be fully elucidated (Wellburn and Wellburn, 1996). It is unlikely that single genes are responsible for $O_3$ tolerance responses, except in rare exceptions (Engle and Gabelman, 1966). Regulation of stomatal opening and leaf structure (Elagöz and Manning, 2002; Fujita et al., 1992) are likely to play key roles in $O_3$ tolerance in plants. Newly developing opportunities to examine simultaneous regulation of larger numbers of genes are also likely to yield more clarification of the genes controlling $O_3$ tolerance (Desikan et al., 2001; Matsuyama et al., 2002).
Attempts to demonstrate conclusive changes in antioxidant and protective pigments for O$_3$ sensitive and tolerant mature trees growing in the field have largely been unsuccessful (Tausz et al., 1999a,b). However, evidence for antioxidant expression differences contributing to differences in O$_3$ sensitivity of 4-year-old Populus tremuloides trees has been found (Wustman et al., 2001).

**AX9.3.3 Environmental Biological Factors**

The biological factors within the plant’s environment that may directly or indirectly influence its response to O$_3$ in a positive or negative manner encompass insects and other animal pests, diseases, weeds, and other competing plant species. Although such interactions are ecological in nature, those involving individual pests, plant pathogens, or weeds, or agricultural crop or forest tree species are considered in this section. More complex ecological interspecies interactions are dealt with in Section AX9.5.

The different types of biological factors are dealt with separately, as in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996). Still, it is important to recognize certain general features of relationships of plants with the biological components in their environments:

- Successful infestation or infection involves complex interactions among the target or host species, the causal organism and environmental factors.
- Infestations and infections may co-occur.
- The successful development and spread of a pest, pathogen, or weed require favorable environmental factors.
- Significant losses to crops and forest trees result from pests and pathogens.
- Significant losses to crops and seedling trees result from weed competition.

Ozone and other photochemical oxidants may influence the severity of a disease or infestation by a pest or weed, either by direct effects on the causal species, or indirectly by affecting the host, or both. In addition, the interaction between O$_3$, a plant, and a pest, pathogen, or weed may influence the response of the target host species to O$_3$. A perceptive overview of the possible interactions of O$_3$-exposure with insect pests and fungal diseases has been provided by Jones et al. (1994), based on a model system involving two insects and two pathogens affecting cottonwood (Populus deltoides). Their study also included effects on the decomposition of leaf litter.
In contrast to detrimental biological interactions, there are mutually beneficial relationships or symbioses involving higher plants and bacteria or fungi. These include (1) the nitrogen-fixing species *Rhizobium* and *Frankia* that nodulate the roots of legumes and alder and (2) the mycorrhizae that infect the roots of many crop and tree species, all of which may be affected by exposure of the host plants to O$_3$.

In addition to the interactions involving animal pests, O$_3$ may also have indirect effects on higher herbivorous animals, e.g., livestock, due to O$_3$-induced changes in feed quality.

**AX9.3.3.1 Oxidant-Plant-Insect Interactions**

The 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) stressed the variability in the reported effects of O$_3$ on host plant-insect interactions. Since relatively few plant-insect systems have been studied, few consistent patterns of response have emerged, as noted in other reviews such as those of Colls and Unsworth (1992), Heliövaara and Väisänen (1993), Whittaker (1994), Docherty et al. (1997), and, most recently, Flückiger et al. (2002).

None of the studies reported in the past decade have clarified the situation in terms of clearly consistent effects. A 1997 review by Docherty et al. (1997), for example, examined 17 reports of studies of aphid species on a range of hosts and classed the O$_3$ effects on aphid performance as 35% positive, 41% negative, and 24% showing no significant effect. A tabulation of 19 studies by Flückiger et al. (2002) gave the corresponding figures: 42%, 21%, and 37%.

Other recent studies with the aphids *Schizolachnus pineti* and *Cinara pinea* on Scots pine (*Pinus sylvestris*) and *Cinara pilicornis* on Norway spruce (*Picea abies*) have also yielded variable results, but suggested that O$_3$ enhances aphid density on pine and aphid performance on spruce (Holopainen et al., 1997; Kainulainen et al., 2000a). In an earlier study with *Schizolachnus pineti* on Scots pine, Kainulainen et al. (1994) had observed no significant effects of O$_3$-treatment on aphid performance. However, more recent observations of long-term effects on aphid populations on aspen (*Populus tremuloides*) exposed to O$_3$ in a FACE system revealed that O$_3$ significantly increased aphid populations while decreasing the populations of predatory insects (Percy et al., 2002).
The observations of Brown et al. (1993) and Jackson (1995) led Whittaker (1994) and Brown (1995) to suggest that aphid response was dependent on ambient temperature as well as the dynamics of O$_3$ exposure and that growth tended to be stimulated with maximum temperatures below ~20 °C but was reduced at higher temperatures. The present situation with plant-aphid responses, therefore, remains confused and, although numerous suggestions have been offered to explain specific findings, they are difficult to assemble into a coherent picture.

Variability has also been found with the interactions involving chewing insects. For example, Lindroth et al. (1993) reported a small negative O$_3$ effect (8% reduction) on the growth of gypsy moth larvae (*Limantra dispar*) on hybrid poplar (*Populus tristis × P. balsamifera*) but no effect when growing on sugar maple (*Acer saccharum*). Ozone exposure reduced the growth rate of the larvae of the bug *Lygus rugulipennis* on Scots pine, but enhanced the growth of larvae of the sawfly *Gilpinia pallida* (Manninen et al., 2000). Costa et al. (2001) observed no significant O$_3$ effects on the growth and fecundity of the Colorado potato beetle (*Leptinotarsa decemlinata*) on potato (*Solanum tuberosum* L.) in greenhouse and field experiments.

Fortin et al. (1997), in a 2-year study of the forest tent caterpillar (*Malacosoma disstria*) on sugar maple, observed that O$_3$ exposure increased the growth rate of female larvae in only one year; fourth- and fifth-instar larvae also showed a feeding preference for treated foliage in that year. However, studies based on open-air exposures of aspen indicated O$_3$-enhanced growth of *M. disstria* in terms of pupal weight (Percy et al., 2002) and larval performance (Kopper and Lindroth, 2003b). Jackson et al. (2000) observed inconsistency in studies on the larva of the tobacco hornworm (*Manduca sexta*) on tobacco (*Nicotiana tabacum*). In one year, feeding on O$_3$-treated foliage resulted in significantly greater larval weight, whereas the increase was not statistically significant in a second year although survival was increased. Also, oviposition by hornworm moths was increased if ambient O$_3$ levels were increased by 70% and returned to normal in ambient O$_3$ levels (Jackson et al., 1999).

Studies of the two-spotted spider mite (*Tetranychus urticae*) on white clover (*Trifolium repens*) and peanut (*Arachis hypogea*) by Heagle et al. (1994a) and Hummel et al. (1998) showed that O$_3$-exposure stimulated mite populations on an O$_3$-sensitive clover clone and on peanut. The lack of significant effects on mites on the O$_3$-resistant clover clone suggests that the responses were host-mediated.
There, therefore, appears to be a clearer indication of the likelihood that increased chewing insect and mite performance will result from O₃-induced changes in the host plant. However, negative effects continue to be reported, indicating that the response is probably also being determined, in part, by other environmental, genetic, or temporal variables.

Reported O₃-induced enhancement of attack by bark beetles (Dendroctonus brevicomis) on Ponderosa pine (Pinus ponderosa) has been suggested by Dahlsten et al. (1997) to be due to greater brood development on injured trees, possibly related to decreased numbers of predators and parasitoids. This view gains some support from the observation that O₃ exposure adversely affected the searching behavior of the parasitoid Asobara tabida for larvae of Drosophila subobscura which led to fewer parasitized fly larvae (Gate et al., 1995). Such observations reveal another level of complexity in the O₃-plant-insect interrelationship: O₃ may reduce the effectiveness of the natural control of insect pests. The phenomenon is probably related to effects on olfactory cues, as it was shown by Arndt (1995) that O₃ can affect fly behavior by modifying the pheromones involved in fly aggregation.

These reports focus on the direct or indirect effects on the insect or mite feeding on foliage previously or currently exposed to O₃. They provide little, if any, information on the host plant effects other than qualitative references to the injury caused by the O₃ exposure. Enhanced pest development will ultimately lead to increased adverse effects on the hosts in the long term, but the only report of an O₃-plant-insect interaction directly affecting the host plant in the short term still appears to be that of Rosen and Runeckles (1976). They found that infestation by the greenhouse whitefly (Trialeurodes vaporariorum) sensitized bean plants (Phaseolus vulgaris) to injury by otherwise noninjurious low levels of O₃, leading to premature senescence of the leaves.

The overall picture regarding possible O₃ effects on plant-insect relations, therefore, continues to be far from clear. Only a few of the very large number of such interactions that may affect crops, forest trees, and other natural vegetation have been studied. The trend suggested in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) that O₃ may enhance insect attack has received some support from a few recent studies. However, the variability noted in most of the studies makes it clear that we are still far from being able to predict the nature of any particular O₃-plant-insect interaction or its magnitude or severity.
AX9.3.3.2 Oxidant-Plant-Pathogen Interactions

Plant diseases are caused by pathogenic organisms, e.g., fungi, bacteria, mycoplasmas, viruses, and nematodes. Ozone impacts on disease are briefly discussed in earlier reviews by Ayres (1991) and Colls and Unsworth (1992) and, more recently, by Flückiger et al. (2002). Biotic interactions with forest trees have been reviewed by Chappelka and Samuelson (1998); Sandermann (1996) and Schraudner et al. (1996) have summarized molecular similarities and interrelationships between necrotic O\textsubscript{3} injury to leaves and pathogen attack. A few recent publications have added to our fragmented knowledge of O\textsubscript{3}-plant-disease interactions and the mechanisms involved, but there appear to have been no reports to date of studies involving mycoplasmal diseases.

The 1996 O\textsubscript{3} AQCD (U.S. Environmental Protection Agency, 1996) noted the concept put forward by Dowding, (1988) “that pathogens and pests which can benefit from damaged host cells and from disordered transport mechanisms are enhanced by pollution insult to their hosts, whereas those pathogens and other symbionts which require a healthy mature host for successful invasion are depressed by pollutant stress to their host.” The pathogens of the first type are mostly facultative necrotrophic fungal parasites, whereas the second type are largely obligate biotrophic fungi, bacteria, and viruses. Based on this distinction, the majority of the cases cited in the 1996 document supported Dowding’s (1988) view, as have several more recent studies summarized in Table AX9-11. However, there are also some contradictions.

Most investigations have focused on the incidence and development of disease on plants previously or concurrently exposed to O\textsubscript{3}, rather than on the corollary effect of disease on the response to O\textsubscript{3}. In all of the studies of facultative pathogens and the nematode studies, exposure to O\textsubscript{3} tended to result in increased disease severity through increased spore germination or increased fungal growth and development; although in the case of grey mold (*Botrytis cinerea*) on kidney bean (*Phaseolus vulgaris*), this was only observed on an O\textsubscript{3}-sensitive cultivar inoculated with conidia (Tonneijck, 1994). After mycelial inoculation, O\textsubscript{3} exposure reduced disease development in the O\textsubscript{3}-sensitive cultivar, but no satisfactory explanation was offered to account for the difference in response. With poplar leaf spot, *Marssonina tremulae*, on hybrid poplar (*Populus trichocarpa × balsamifera*), low level exposures to O\textsubscript{3} increased disease (in agreement with theory) but higher levels (200 ppb, 8 h per day for 15 days) reduced conidial germination (Beare et al., 1999b).
<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Pathogen</th>
<th>Effect of O₃ on Disease</th>
<th>Effect of Disease on O₃ Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Obligate Biotrophs</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bottle gourd (<em>Lagenaria siceraria</em>)</td>
<td>Powdery mildew (<em>Sphaerotheca fulginea</em>)</td>
<td>Increased in 50ppb O₃; decreased in 100+ppb</td>
<td>Decreased; partial protection</td>
<td>Khan and Khan (1998a)</td>
</tr>
<tr>
<td>Cucumber (<em>Cucumis sativa</em>)</td>
<td>Powdery mildew (<em>Sphaerotheca fulginea</em>)</td>
<td>Increased in 50ppb O₃; decreased in 100+ppb</td>
<td>Synergistic increase in 50ppb O₃; antagonistic decrease in 100+ppb; partial protection</td>
<td>Khan and Khan (1999)</td>
</tr>
<tr>
<td>Pea (<em>Pisum sativum</em>)</td>
<td>Powdery mildew (<em>Erysiphe polygoni</em>)</td>
<td>Decreased infection</td>
<td>Decreased; partial protection</td>
<td>Rusch and Laurence (1993)</td>
</tr>
<tr>
<td>Aspen (<em>Populus tremuloides</em>)</td>
<td>Leaf rust (<em>Melampsora medusae f. sp. tremuloidae</em>)</td>
<td>Increased severity</td>
<td>Not reported</td>
<td>Karnosky et al. (2002)</td>
</tr>
<tr>
<td>Hybrid poplar (<em>Populus trichocarpa × balsamifera</em>)</td>
<td>Leaf rust (<em>Melampsora larici-populina or M. allii-populina</em>)</td>
<td>Increased infection and severity</td>
<td>Increased sensitivity (synergistic)</td>
<td>Beare et al. (1999a)</td>
</tr>
<tr>
<td>Broad bean (<em>Vicia faba</em>)</td>
<td>Bean rust (<em>Uromyces viciae-fabae</em>)</td>
<td>Not reported</td>
<td>Decreased; partial protection</td>
<td>Lorenzini et al. (1994)</td>
</tr>
<tr>
<td><strong>Facultative Necrotrophs</strong></td>
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<tr>
<td>Kidney bean (<em>Phaseolus vulgaris</em>)</td>
<td>Grey mold (<em>Botrytis cinerea</em>)</td>
<td>Increased from conidia on O₃ - sensitive cultivar; decreased from mycelium</td>
<td>Not reported</td>
<td>Tonneijck (1994)</td>
</tr>
<tr>
<td></td>
<td>Grey mold (<em>Botrytis cinerea</em>)</td>
<td>Increased infection</td>
<td>Not reported</td>
<td>Tonneijck and Leone (1993)</td>
</tr>
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<td></td>
<td>White mold (<em>Sclerotinia sclerotiorum</em>)</td>
<td>Increased infection</td>
<td>Not reported</td>
<td>Tonneijck and Leone (1993)</td>
</tr>
<tr>
<td>Scots pine (<em>Pinus sylvestris</em>)</td>
<td>Annosus root and butt rot (<em>Heterobasidion annosum</em>)</td>
<td>Increased development*</td>
<td>Not reported</td>
<td>Bonello et al. (1993)</td>
</tr>
</tbody>
</table>
Table AX9-11 (cont’d). Interactions Involving O₃ and Plant Pathogens

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Pathogen</th>
<th>Effect of O₃ on Disease</th>
<th>Effect of Disease on O₃ Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loblolly pine (<em>Pinus taeda</em>)</td>
<td>Pitch canker (<em>Fusarium subglutinans</em>)</td>
<td>Increased development</td>
<td>Increased sensitivity</td>
<td>Carey and Kelley (1994)</td>
</tr>
<tr>
<td>Hybrid poplar (<em>Populus deltoides × nigra</em>)</td>
<td>Canker (<em>Septoria musiva [=Mycosphaerella populinum]</em>)</td>
<td>Increased incidence</td>
<td>Not reported</td>
<td>Woodbury et al. (1994)</td>
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<tr>
<td>Hybrid poplar (<em>Populus trichocarpa × balsamifera</em>)</td>
<td>Leaf spot (<em>Marssonina tremulae</em>)</td>
<td>Increased spore germination and lesion growth after 100ppb O₃ (30 days); decreased germination after 200ppb (15 days)</td>
<td>Not reported</td>
<td>Beare et al. (1999b)</td>
</tr>
<tr>
<td>Wheat (<em>Triticum aestivum</em>)</td>
<td>Blotch (<em>Septoria nodorum</em>)</td>
<td>Increased infection</td>
<td>Not reported</td>
<td>Tiedemann and Firsching (1993)</td>
</tr>
<tr>
<td></td>
<td>Tan spot (<em>Pyrenophora tritici-repentis</em>)</td>
<td>Increased infection of disease-susceptible genotypes</td>
<td>Not reported</td>
<td>Sah et al. (1993)</td>
</tr>
</tbody>
</table>

**Nematodes**

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Pathogen</th>
<th>Effect of O₃ on Disease</th>
<th>Effect of Disease on O₃ Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato (<em>Lycopersicon esculentum</em>)</td>
<td>Root-knot nematode (<em>Meloidogyne incognita</em>)</td>
<td>Increased development</td>
<td>Increased foliar injury; reduced plant growth (synergistic)</td>
<td>Khan and Khan (1997); Khan and Khan (1998a)</td>
</tr>
</tbody>
</table>

* Increase completely countered by mycorrhizae (*Hebeloma crustuliniforme*).
The situation with obligate biotrophic pathogens is less consistent. The effects on powdery mildew \((Sphaerotheca fulginea)\) on both bottle gourd \((Lagenaria siceraria)\) and cucumber \((Cucumis sativa)\) resembled the situation with the necrotrophic poplar leaf spot disease, since low \(O_3\) exposures increased disease severity (in disagreement with theory), although higher levels decreased it. The decreased infection in the pea-powdery mildew \((Erysiphe polygoni)\) situation agrees with theory, but the situations with leaf rust \((Melampsora sp.)\) on hybrid poplar or aspen do not. However, these reports are in contrast to earlier reports included in the 1996 \(O_3\) AQCD (U.S. Environmental Protection Agency, 1996) of observations with other species of \(Erysiphe\) (Tiedemann et al., 1991) and \(Melampsora\) (Coleman et al., 1987). In contrast to the recent report of a synergism with \(Melampsora\) on poplar, infections caused by the other biotrophs \((Sphaerotheca, Erysiphe, Uromyces spp.)\) reduced the severity of injury caused by \(O_3\) (in agreement with numerous earlier reports), but only at high \(O_3\) exposures in the case of \(Sphaerotheca\) on cucumber. At low exposure levels, the disease and \(O_3\) exposure acted synergistically. The only other recent observations of such disease-related synergisms are the tomato-nematode reports of Khan and Khan (Khan and Khan, 1997, 1998b).

It is, therefore, clear that the type and magnitude of exposure to \(O_3\) plays an important role in determining both the responses of both the disease organism and the host.

No recent studies involving interactions between \(O_3\) and bacterial diseases appear to have been reported since 1996. With regard to viruses, a laboratory study by Yalpani et al. (1994) added to several reports of \(O_3\) decreasing the severity of tobacco mosaic virus infection of tobacco; and Jimenez et al. (2001) reported that previous \(O_3\) exposure resulted in increased adverse effects on tomato yield attributed to several viral diseases.

Similarities between the sensitivities of different cultivars or clones to \(O_3\) and to specific diseases have been noted. For example, Sah et al. (1993) found that the severity of injury caused by tar spot and \(O_3\) exposures of 12 wheat \((Triticum aestivum L.)\) cultivars were closely correlated \((R^2 = 0.986)\). Such similarities appear to have a mechanistic basis, as several studies have noted similarities in the molecular and biochemical changes that occur in plants infected with pathogens and in \(O_3\) exposed plants. Schraudner et al. (1992), Ernst et al. (1992), Eckey-Kaltenbach et al. (1994a,b), Yalpani et al. (1994), and Bahl et al. (1995) have presented evidence that \(O_3\) exposures result in responses such as increased levels of salicylic acid, the signaling agent for increased induced resistance to pathogens. This, in turn, leads to the activation of the
genes that encode defense proteins, including the so-called pathogenesis-related proteins. The induction of such proteins might account for the decreased infection with *Sphaerotheca* and *Melampsora* at higher O₃ exposures but does not account for increased infections seen at lower exposure levels. The issue is discussed more fully by Sandermann (1996) and Schraudner et al. (1996). More recently Sandermann (2000) has extended the theory relating O₃ exposure and disease by suggesting that, because of O₃ “memory effects” in affected host plants that may persist over weeks or months, analysis for various induced biomarkers of gene activation may provide a useful tool for improving our ability to predict the outcome of O₃-plant-pathogen interactions.

There have been no reports of O₃ studies with mixed infections by pathogens, but the complete suppression of *Heterobasidion* butt and root rot of Scots pine by the mycorrhizal symbiont *Hebeloma crustuliniforme* indicates the possibility of interactions involving more than one fungus (see Section AX9.3.4.3.3 below).

In summary, our understanding of oxidant-plant-disease interactions is far from complete. However, a combined tabulation of the evidence presented in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) and that noted in Table AX9-11 leads to the following summary of O₃ effects on plant diseases and corollary effects of infection on plant response to O₃, as indicated by the number of studies showing increases or decreases in disease or susceptibility.

For obligate biotrophic fungi, bacteria, nematodes:

- O₃ increased disease: 9
- Increased susceptibility to O₃: 3
- O₃ decreased disease: 15
- Decreased susceptibility to O₃: 9

For facultative necrotrophic fungi:

- O₃ increased disease: 25
- Increased susceptibility to O₃: 2
- O₃ decreased disease: 3
- Decreased susceptibility to O₃: 4

Thus, although O₃ may reduce the severity, but not the incidence, of some of the diseases caused by the obligate pathogens, the evidence overall indicates that with most diseases, their severity is more likely to be increased by O₃ than not. However, the actual consequences will be specific to the disease and level of exposure, and, most importantly, will be determined by environmental suitability and epidemiological requirements for disease to develop. Conversely, some evidence
suggests that infection by obligate pathogens may confer some degree of “protection” against O₃, a dubious benefit from the plant’s point of view.

AX9.3.3.3 Oxidant-Plant-Symbiont Interactions

No further studies have appeared regarding O₃ effects on the important bacterial symbiont of legumes, *Rhizobium*, since those summarized in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996). Hence, our present understanding is that, although relatively high levels of exposure (>200 ppb) can result in severe (>40%) reductions in nodulation (and therefore nitrogen-fixation) on soybean roots, lower O₃ exposures may cause lesser reductions in nitrogen fixation. However, the data are inadequate to attempt to define any quantitative exposure-response relationships.

There have been a few recent reports on O₃-plant-mycorrhizae interrelationships. These have mostly involved seedlings of coniferous tree species. A transient O₃-induced stimulation of mycorrhiza on Scots pine roots reported by Kasurinen et al. (1999) was not observed in a later study by Kainulainen et al. (2000b). Studies of the mycorrhiza *Paxillus involutus* on birch (*Betula pendula*) seedlings showed that, although O₃ reduced mycorrhizal growth rate, it led to greater extension growth which in turn resulted in greater mycorrhizal infection of neighboring Aleppo pine (*Pinus halepensis*) seedlings (Kytöviita et al., 1999). However, O₃ reduced nitrogen acquisition by *P. halepensis* from its mycorrhizal symbiont (Kytöviita et al., 2001). The complex interrelationships that may occur in the rhizosphere were revealed by the observation by Bonello et al. (1993) that the mycorrhiza *Hebeloma crustuliniforme* could overcome the O₃-stimulated severity of root rot on Scots pine caused by the fungus *Heterobasidion annosum* (noted in Section AX9.3.4.3.2).

In summary, the available evidence is far too fragmented and contradictory to permit drawing any general conclusions about mycorrhizal impacts. The negative effects of O₃ on mycorrhizae and their functioning that have been reported have not necessarily been found to lead to deleterious effects on the growth of host plants. Thus, little has changed from 1991 when Dighton and Jansen (1991) asked: “Atmospheric Pollutants and Ectomycorrhizae: More Questions than Answers?” Because of their important roles in ecosystems, mycorrhizae are further discussed in Section AX9.6.
**AX9.3.3.4 Oxidant-Plant-Plant Interactions: Competition**

Plant competition involves the ability of individual plants to acquire the environmental resources needed for growth and development: light, water, nutrients, and space. Intraspecific competition involves individuals of the same species, typically in monocultural crop situations, while interspecific competition refers to the interference exerted by individuals of different species on each other when they are in a mixed culture.

In cropping situations, optimal cultural practices for row spacing and plant density/row tend to balance the negative effects of intraspecific competition with the goal of maximum yield. Although interspecific competition is agriculturally undesirable when it involves weak infestations, the use of mixed plantings may be agriculturally deliberate, e.g., grass-legume mixtures used for pasture or forage. In natural plant communities, monocultures are rare, and complex interspecific competition is the norm.

Although weak competition is the largest global cause of crop losses, little is known about the impact of O$_3$ on crop-weed interactions. The topic does not appear to have been investigated in recent years. We can only speculate as to the possible consequences of O$_3$ exposure on weed competition based on our limited understanding of the effects on a few, mostly two-component mixtures of cultivated species.

The tendency for O$_3$-exposure to shift the biomass of grass-legume mixtures in favor of grass species, reported in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) has been confirmed by recent studies. In a ryegrass (*Lolium perenne*) + clover (*Trifolium repens*) mixture grown in an open-air fumigation system, clover growth was impaired by extended exposures to above-ambient O$_3$, leaving patches for weed invasion (Wilbourn et al., 1995). An open-top chamber study (OTC) by Nussbaum et al. (1995b) using the same species confirmed the greater effect on clover but observed that the magnitude of the effect depended highly on the pattern of O$_3$-exposures over extended growing periods. Low-level exposures shifted species composition in favor of *Lolium*, but exposures to higher peak O$_3$ levels depressed total mixture yield. With an alfalfa (*Medicago sativa*) + timothy (*Phleum pratense*) mixture, Johnson et al. (1996a) noted that O$_3$ decreased alfalfa root growth and increased timothy shoot growth and height. Nussbaum et al. (2000a) reported that, with increased exposure to O$_3$, well-watered red clover (*Trifolium pratense*) plants suffered from increased competition from the grass *Trisetum flavescens*, but the O$_3$ exposure
also negatively affected grass growth, depressing overall total yield. However, a greater adverse effect on Trisetum resulted from O₃-induced increased competition when grown with brown knapweed (Centaurea jacea), a weed species.

Andersen et al. (2001) demonstrated the potential for competition and O₃ exposure to work together to affect the growth of tree seedlings. Ozone had no direct adverse effect on pine growth in a 3-year study of ponderosa pine (Andersen et al., 2001) seedlings grown in mesocosms with three densities of blue wild-rye grass (Elymus glaucus), but O₃ exposure increased the competitive pressure of the grass which caused a major reduction in pine growth.

Three studies have been reported on more complex plant associations. Ashmore and Ainsworth (1995) studied mixed plantings of two grasses, Agrostis capilaris and Festuca rubra, with two forbs, Trifolium repens (a legume) and Veronica chamaedrys, exposed to O₃ in OTCs. The proportion of forbs, Trifolium in particular, declined, especially when cut at biweekly intervals. In a related study, Ashmore et al. (1995) used artificial mixtures of grasses and forbs and transplanted swards of native calcareous grassland species and found that, regardless of whether total biomass was adversely affected by exposures to O₃, higher exposures progressively shifted species composition, usually at the expense of the forb species. The observed shifts in competitive balance in favor of grasses is consistent with observations that many grass species are less sensitive to O₃ than forbs. However, as previously shown by Evans and Ashmore (1992), knowledge of the relative sensitivities to O₃ of the component species grown in isolation or in monoculture does not always predict the impact of O₃ on the components in a mixed culture.

Barbo et al. (1998) exposed an early successional plant community to O₃ in OTCs for two growing seasons. Ozone decreased community structure features such as height of canopy, vertical canopy density (layers of foliage), and species diversity and evenness. Surprisingly, blackberry (Rubus cuneifolius), a species considered to be O₃-sensitive, replaced sumac (Rhus copallina) canopy dominance. Barbo et al. (2002) also demonstrated the role of competition in determining the impact of O₃ on loblolly pine (Pinus taeda). They reported that the increased growth of natural competitors in OTCs using charcoal-filtered air to reduce the ambient O₃ concentrations resulted in decreased pine growth. They noted that this is contrary to the

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15 Forb: any non-grassy herbaceous species on which animals feed.
frequently reported increased growth observed in reduced O_3 levels in the absence of interspecific competition.

McDonald et al. (2002) classified four clones of aspen (Populus tremuloides) as either competitively advantaged or disadvantaged, based on their height relative to the height of neighboring trees, and exposed them to 1.5× ambient O_3 in a FACE facility over a 4-year period. Competitively disadvantaged trees were proportionately more adversely affected by O_3 than competitively advantaged or neutral trees (McDonald et al., 2002). However, one clone of the disadvantaged trees demonstrated enhanced growth.

In summary, our present knowledge of how O_3 may affect the competitive interspecific plant-plant relationships typifying the agricultural and natural worlds is very limited. However, as noted in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), “the development and use of field exposure systems have permitted many recent studies of crop species to be conducted at normal planting densities and hence have incorporated intraspecific competition as an environmental factor.” Such facilities were used in most of the studies of interspecific competition discussed above. But we are still far from being able to use small model competing systems to extrapolate to the realities of natural ecosystem complexity.

**AX9.3.4 Physical Factors**

The physical features of a plant’s aerial and edaphic environments exercise numerous controls over its growth and development. Thus, many of their effects may be modified by exposure to atmospheric oxidants and, alternatively, plants may modify responses to such exposures. As in the 1996 O_3 AQCD (U.S. Environmental Protection Agency, 1996), this section focuses on the defining features of plant microclimate: light, temperature, relative humidity (HR, or saturation vapor pressure deficit), and the presence and availability of water, especially in the soil. Monteith and Elston (1993) suggested that light energy and mass of water should be viewed as climatic resources and that the other two elements (temperature and saturation vapor pressure deficit) be viewed as rate modifiers that determine how fast the resources are used. The modifications of plant response by physical environmental factors has recently been reviewed by Mills (2002).

Another physical feature of the microclimate, wind and air turbulence, which affects the thicknesses of the boundary layers over leaves and canopies and, hence, affects gas exchange
rates (including the fluxes of O$_3$ and other oxidants into the leaves) is discussed elsewhere (Section AX9.4).

Physical features of the environment are also important components of larger-scale regional and global climates. However, the following discussions are confined to issues related to individual factors at the plant level; meso-scale effects are reviewed in Section AX9.3.4.8, which addresses the issues of climate change interactions.

**AX9.3.4.1 Light**

Plants are the primary producers of biomass on the planet through their ability to capture light energy (by the process of photosynthesis) and convert it to the many forms of chemical energy that sustain their own growth and that of secondary consumers and decomposers. Light intensity is critical because the availability of light energy (a resource, *sensu* Monteith and Elston (1993) governs the rate at which photosynthesis can occur, while light duration (i.e., photoperiod) profoundly effects development in many species. Although light quality (i.e., the distribution of incident wavelengths) may also affect some physiological plant processes, there is no evidence to indicate that such effects are of relevance to concerns over oxidant pollution, except at the short wavelengths of UV-B. This topic is discussed in the context of climate change in Section AX9.3.8.2, and as a stress factor per se affected by atmospheric O$_3$ in Chapter 10. However, as noted above and in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996), none of these features is controllable in natural field situations. A brief discussion of light intensity-O$_3$ interactions is included in the review by Chappelka and Samuelson (1998).

The conclusion in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) that low light intensities and short photoperiods tended to increase susceptibility to foliar O$_3$-injury may still be valid, but this may or may not translate into adverse effects on growth. For example, Tjoelker et al. (1993) found that, when seedlings of sugar maple, a shade-tolerant species, were grown in 7% full sunlight, O$_3$ reduced shoot and root growth, but had no significant effect in 45% sunlight (a 6-fold increase). In contrast, the reverse was observed with a shade-intolerant hybrid poplar, with the greater impact of O$_3$ occurring in the higher light intensity treatment.
The greater sensitivity of maple in low light has also been confirmed in other studies. Tjoelker et al. (1995) noted a greater $O_3$-induced inhibition of photosynthetic $CO_2$ assimilation in shaded leaves than in leaves in full sunlight. However, in the absence of differences in stomatal conductance, the effect was considered to be independent of $O_3$ flux; it appeared to be a consequence of reduced chlorophyll contents and quantum efficiencies induced by $O_3$. In contrast, Bäck et al. (1999), who also observed a greater inhibition of net photosynthesis by $O_3$ in shaded leaves, reported decreased stomatal conductance. Although reduced conductance might suggest reduced $O_3$ flux and, therefore, decreased adverse effects, the authors concluded that the effects of reduced conductance were offset by long-term changes in leaf structure, leading to less densely packed mesophyll cells and greater internal air space within the leaves. Morphological differences between lower and upper crown leaves of black cherry (Prunus serotina) have been suggested as the basis for the greater $O_3$-susceptibility of the lower crown leaves (Fredericksen et al., 1995). Bäck et al. (1999) also observed accelerated foliar senescence induced by $O_3$ on shaded leaves, a response also noted by Topa et al. (2001). Sensitivity to $O_3$ was found to be increased in shade- but not sun-leaves of shade-tolerant red oak (Quercus rubra) (Samuelson and Edwards, 1993). Similarly, Mortensen (1999) observed that seedlings of mountain birch (Betula pubescens) grown in 50% shade suffered greater foliar injury from $O_3$ than those grown in full sunlight.

Not all shade-intolerant species exhibit greater reductions in photosynthesis and growth due to $O_3$ when grown in full sunlight. Higher than ambient levels of $O_3$ failed to inhibit photosynthesis in leaves of shade-intolerant yellow poplar (Liriodendron tulipifera) grown in nearly full sunlight (Tjoelker and Luxmoore, 1991). Greater foliar injury in the lower, shaded leaves of shade-intolerant black cherry trees and saplings, was attributed to higher stomatal conductance and greater $O_3$ uptake relative to net photosynthetic rate (Fredericksen et al., 1996a). However, in a 3-year study of Norway spruce seedlings in OTCs, Wallin et al. (1992) observed that photosynthetic efficiency was more adversely affected by $O_3$ in high than in low light.

The suggestion of greater sensitivity to $O_3$ of shade-tolerant species in low-light conditions and the greater sensitivity of shade-intolerant species in high light is somewhat of an oversimplification when dealing with mature trees, for which light intensity varies considerably within the canopy because of shading. Chappelka and Samuelson (1998) noted that the
interaction between sensitivity to O\textsubscript{3} and the light environment in forest trees is further complicated by developmental stage, with seedlings, saplings, and mature trees frequently giving different results. Topa et al. (2001) also cautioned that O\textsubscript{3} effects on leaf-level photosynthesis may be poor predictors of the growth responses of sugar maple in different light environments.

In high-light intensities, many species exhibit some degree of photoinhibition of the photosynthetic process through the overloading of the mechanisms that protect the photosynthetic reaction centers in the chloroplasts. Guidi et al. (2000) reported complex interactions between high-light intensities (inducing photoinhibition) and O\textsubscript{3} exposures in kidney bean with high intensities tending to enhance the detrimental effect of O\textsubscript{3} on photosynthesis. One of the studies in the extensive European Stress Physiology and Climate Experiment-wheat (ESPACE-wheat) program (Bender et al., 1999), conducted in 1994 and 1995, included an investigation of the effects of climatic variables on yield response to O\textsubscript{3} using two simulation models, AFRCWHEAT2-O\textsubscript{3} and LINTULCC (Ewart et al., 1999; Van Oijen and Ewart, 1999). Among the observed trends, it was noted that relative yield loss of wheat due to elevated O\textsubscript{3} tended to increase with light intensity. In contrast, Balls et al. (1996) used ANNS to investigate microclimatic influences on injury caused by O\textsubscript{3} to clover (\textit{Trifolium subterraneum}) and found that, especially at mid-range cumulative O\textsubscript{3} exposures (350 to 500 ppb-h), injury tended to decrease with increasing light intensity. Similar observations by Davison et al. (2003) of foliar injury to wild populations of cutleaf cone flower (\textit{Rudbeckia laciniata}) exhibiting a range of PAR levels within their canopies led the authors to conclude that the variation in injury symptoms observed was “unlikely to be due to differences in ozone flux and more likely to be due to variation in light.” Antonielli et al. (1997) found evidence indicating that the high sensitivity of the bioindicator tobacco cultivar \textit{Nicotiana tabacum cv. Bel-W3} is partly determined by its high photosynthetic electron transport rates at high-light intensities, which exceed the capabilities of the plant to dissipate energy and oxyradicals.

The 1996 O\textsubscript{3} AQCD referred to the important role of light in controlling stomatal opening and suggested that light duration (i.e., photoperiod) might dictate the actual uptake of O\textsubscript{3} to some degree. However, it should also be noted that Sild et al. (1999) found that clover plants could suffer foliar injury even if they were exposed to O\textsubscript{3} during the dark period of the day-night cycle, when stomatal conductance is at its lowest.
A possible indirect effect of light intensity was noted by Reiling and Davison (1992c) in their study of the O$_3$-tolerance of common plantain (*Plantago major* L.) plants grown from seeds collected from populations at 28 different sites in Britain. Ozone-tolerance, defined in terms of plant growth, was found to be a function of both previous O$_3$-exposure history and hours of bright sunshine during the year before the seeds were collected. However, the authors cautioned that, since tropospheric O$_3$-formation is itself dependent upon irradiation, the observation does not necessarily imply a direct effect of light intensity on the plants’ response to O$_3$.

The only recent studies concerning interactions with light quality appear to be those involving O$_3$ and UV-B as a component of climate change. These are dealt with in Section AX9.3.4.8.2. The effects of photoperiod on response to O$_3$ or the converse do not appear to have received any recent attention.

Although the intensity, quality, and duration of light are not controllable in the natural world, the interactions of O$_3$ with light intensity, in particular, clearly have relevance to the growth of shade-tolerant and shade-intolerant species in mixed forest stands. It appears that the nature of light intensity–O$_3$ interactions may depend upon the type of light environment to which the species are best adapted, with increased light intensity increasing the sensitivity of light-tolerant and decreasing the sensitivity of shade-tolerant species to O$_3$. Although there is certainly some evidence to the contrary, this hypothesis is a reasonable summation of current understanding with regard to O$_3$-light intensity interactions.

**AX9.3.4.2 Temperature**

“Temperature determines the start and finish and rate and duration of organ growth and development” (Lawlor, 1998). Such processes depend on fundamental physiological activities that are mostly enzyme-mediated and whose kinetics are directly affected by temperature. Since the processes of enzyme deactivation and protein denaturation also increase as temperatures rise, each enzymatic process has a unique optimum temperature range for maximal function. However, the optima for different processes within the plant vary appreciably and, hence, the optimum temperature range for overall plant growth is one within which all of the individual reactions and vital processes are collectively functioning optimally, not necessarily maximally. Furthermore, individual features of plant development (e.g., shoot and root growth, flowering, pollen tube growth, fruit set, seed development) have different specific optima, so that
differential responses to temperature occur, leading to temperature-induced developmental changes. For example, despite increased assimilation, increased temperatures may result in decreased grain yields of crops such as wheat, because the growing season is effectively shortened by a more rapid onset of senescence (Van Oijen and Ewart, 1999).

Rowland-Bamford (2000) noted that a plant’s response to temperature changes will depend upon whether it is growing at its near optimum temperature for growth or its near maximum temperature and whether any increase in mean temperature results in temperatures rising above the threshold for beneficial responses. Impairment by O$_3$ of any process may be thought of as being analogous to a downward shift below and away from the temperature optimum or an upward shift above and away from the optimum. Since a temperature rise toward the optimum would result in a rate increase, the combined effects of O$_3$ and such an increase might neutralize each other, while the effects of O$_3$ and a decrease in temperature would likely be additively negative. Above the optimum temperature, the situations would be reversed with the effects of increased temperatures and O$_3$ being additively negative, and decreasing temperatures counteracting any negative effect of O$_3$. Thus, it is difficult to generalize about the interactions of temperature and O$_3$ on overall plant responses such as growth in which the different temperature-rate relationships of different growth components are merged, because they depend upon the relationship of any temperature changes to the optimum for a species.

Studies of the effects of temperature on the impact of O$_3$ have increased recently because of an increased need to understand the consequences of global warming as a component of climate change. Direct interactions of temperature with O$_3$ are reviewed here, but the issues are addressed again in Section AX9.3.8.1 in relation to changes in atmospheric CO$_2$ levels.

The 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) stressed the interdependence of the temperature within the tissues of the leaf (where the various temperature-sensitive processes occur) on three distinct components: the ambient air temperature, the heating effect of incident infrared radiation during the photoperiod, and the evaporative cooling effect caused by transpirational loss of water. It also cautioned that, especially in experiments using controlled environment chambers, the effects of temperature could well be confounded with those of humidity/vapor pressure deficit (VPD). Temperature and VPD are strongly interrelated, and VPD plays an important role in regulating stomatal transpiration. Because of the role that evaporative cooling plays in determining internal leaf temperatures, any factor that causes
stomatal closure and reduced conductance inevitably leads to increased leaf temperatures. Such interactions add to the difficulties in distinguishing the effects of temperature from those of other factors, as actual leaf temperatures are rarely measured and reported.

Despite these caveats, there is some evidence that temperature per se influences plant response to O₃. For example, in rapid-cycling Brassica (Brassica rapa) and radish (Raphanus sativus), marked O₃-inhibitions of growth were observed at low root temperatures (13 °C) but not at 18 °C (Kleier et al., 1998, 2001). With regard to air temperature, this was included in the range of micrometeorological variables studied in several recent extensive field studies and was found to have a significant effect on response to O₃ in most cases. Balls et al. (1996) used ANNs in an analysis of the growth of clover (Trifolium subterraneum) and concluded that light and VPD had greater influences than temperature on the visible injury response to O₃. However, in three studies with different cultivars of white clover (Trifolium repens), temperature was found to be important to the growth response. Ball et al. (1998) exposed T. repens cv. Menna to ambient O₃ in OTCs at 12 European sites at a range of latitudes and altitudes from 1994 to 1996. The impact of O₃ on growth was determined as the ratio of growth with and without treatment with the O₃-protectant, EDU (see Section AX9.2). Artificial neural network analysis showed that O₃ exposure (measured as the AOT40 index, see Section AX9.3.6), VPD, and temperature were consistently the three most important variables governing response to O₃ over a range of different ANN models. However, the authors did not describe the form of the O₃-response relationship with temperature. Similar observations were reported by Ball et al. (2000) and Mills et al. (2000) for O₃-sensitive and -tolerant clones of T. repens cv. Regal, grown at 14 to 18 European locations from 1995 to 1998. In both studies, the impact of O₃ was measured as the sensitive/tolerant growth (biomass) ratio. Although Ball et al. (2000) found temperature to be less important than O₃ exposure and VPD, Mills et al. (2000) found temperature to be the most important input variable after O₃ exposure (AOT40). In both cases, the adverse effect of O₃ increased with increasing temperature.

A study of black cherry seedlings and mature trees in Pennsylvania, using micrometeorological variables aimed to predict O₃ uptake, found temperature to be unimportant (Fredericksen et al., 1996b), but in the study of populations of common plantain referred to in Section AX9.3.4.1, Reiling and Davison (1992c) noted a weak, positive correlation between mean temperature at the collection site and O₃ tolerance (based on growth rate) of the different
populations. In contrast, Danielsson et al. (1999) collected genotypes of *Phleum arvense* from a wide range of Nordic locations and found a positive effect of temperature on the growth of genotypes from locations with higher summer temperatures, but sensitivity to O₃ did not vary systematically with geographic location.

Van Oijen and Ewart (1999) studied the effects of climatic variables on the response to O₃ in the ESPACE-wheat program, based on two distinctive simulation models (AFRCWHEAT2-O₃ and LINTULCC [Ewart et al., 1999]) and noted that although the relative yield loss of wheat due to elevated O₃ tended to increase with temperature, the effect was of minor significance.

In contrast to the variable results obtained in studies of the effects of temperature on response to O₃, the corollary effect of O₃ exposure on subsequent sensitivity to low temperature stress, noted in the 1996 criteria document, is well recognized. In reviewing low temperature-O₃ interactions, Colls and Unsworth (1992) noted that winter conditions produce three kinds of stress: desiccation, chilling or freezing temperatures, and photooxidation of pigments. Of these, they suggested that while the first two were important, the last may play a particularly significant role because the “combination of high irradiance and low temperatures permits a buildup of free radicals in leaf tissue, and these free radicals then attack chlorophyll.” Chappelka and Freer-Smith (1995) suggested that the injury and losses to trees caused by this delayed impact of O₃ may be equally or more important than the direct impacts of O₃ on foliage of visible injury and necrosis, or the disruption of key physiological processes such as photosynthesis. In this context, the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) referred to the conceptual framework of Eamus and Murray (1991), which is still valid: brief periods of mild temperatures in the severest winters result in dehardening; O₃ decreases frost hardiness per se, but also increases the predisposition to dehardening; dehardening places O₃-exposed trees at greater risk from subsequent low temperatures. However, no quantified models of these effects have yet appeared.

The 1996 O₃ AQCD also noted that O₃ adversely affects cold hardiness of herbaceous species. More recently, Foot et al. (1996, 1997) observed winter injury and decreased growth in low-growing perennial heather *Calluna vulgaris* exposed to O₃ (70 ppb, 8 h/day, 5 days/week for 6 months) during the winter (6.8 °C mean), but found no significant effects from the same exposures during the summer (12.3 °C mean). Although Potter et al. (1996) observed a similar
situation with the moss *Polytrichum commune*, the reverse was found with the moss *Sphagnum recurvum*.

In summary, unequivocal evidence exists that O$_3$ causes sensitization to the adverse effects of low temperatures, but there is no clear pattern in the evidence regarding the effects of temperature on O$_3$ response. The many contradictory responses to temperature and O$_3$ probably reflect our lack of detailed knowledge of the temperature optima for the different growth components of the studied species. The topic of temperature-oxidant interactions is revisited later in Section AX9.3.4.8 in the context of global warming as a feature of climate change.

**AX9.3.4.3 Humidity and Surface Wetness**

The moisture content of the ambient air (or its VPD) is a rate modifier (*sensu* Monteith and Elston (1993)) and an environmental regulator of stomatal conductance. Both of the previous O$_3$ AQCDs (U.S. Environmental Protection Agency, 1986, 1996) concluded that the weight of evidence indicated that high RH (=low VPD) tended to increase the adverse effects of O$_3$, principally because the stomatal closure induced in most situations by O$_3$ is inhibited by high RH, leading to increased O$_3$ flux into the leaves.

Recent reports have confirmed this role of RH. The studies by Balls et al. (1995, 1996) and Ball et al. (1998) showed that VPD was an important determinant of O$_3$-induced injury and reduced growth in two species of clover, *Trifolium repens* cv. Menna and *T. subterraneum*. However, Mills et al. (2000) found it to be unimportant in the case of *T. repens* cv. Regal. Such difference between cultivars is not unexpected, because considerable differences also occur among species and genera. For example, Bungener et al. (1999a) studied 26 Swiss grassland species and found clear evidence that O$_3$ injury increased with decreased VPD (i.e., increased RH) in only eight species. However, the 1995 data from the European cooperative study of O$_3$ injury, which involved 28 sites in 15 countries and six crop species, led to the development of two 5-day critical-level scenarios involving O$_3$-exposure (calculated as the AOT40 index) and mean VPD (0930-1630h): 200 ppb-h at >1.5 kPa, and 500 ppb-h at < 0.6 kPa (Benton et al., 2000).

With forest tree species, Fredericksen et al. (1996b) found significant correlations between stomatal conductance of black cherry leaves and RH (+ve) and VPD (−ve), and studies on free-standing Norway spruce and larch (*Larix decidua*) showed that although ambient VPD was
highly positively correlated with ambient O₃ concentration, increased VPD caused stomatal closure, reducing O₃ uptake and impact (Wieser and Havranek, 1993, 1995).

Surface wetness may affect O₃ response through its direct effects on deposition to the surface and through changes in RH. Effects on the deposition of O₃ have been reviewed by Cape (1996). A surface film of water on leaves was found to increase O₃ deposition in four studies involving field-grown grape (*Vitis vinifera*) (Grantz et al., 1995), red maple (*Acer rubrum*) (Fuentes and Gillespie, 1992), deciduous forest dominated by largetooth aspen (*Populus grandidentata*) and red maple (Fuentes et al., 1992), and clover-grass mixed pasture (*Trifolium pratense*, *Phleum pratense*, and *Festuca pratensis*) (Pleijel et al., 1995a). In each case, the increased deposition could be attributed partly to an increased stomatal conductance through the abaxial (lower) surface and partly to uptake into the aqueous film on the adaxial (upper) surface. In contrast, decreased deposition was noted by Grantz et al. (1997) with field-grown cotton (*Gossypium hirsutum*). Since cotton is amphistomatous, with functional stomata on both leaf surfaces, it was suggested that, in this case, the water layer effectively sealed the adaxial surface stomata, more than offsetting any increase in conductivity of the stomata in the abaxial surface. However, none of the studies investigated the consequences of the differences in deposition.

Although it could be inferred that, with part of any increased deposition being the result of increased O₃ flux into the leaves, there would be the likelihood of increased O₃ adverse effects, as suggested by earlier studies (Elkiey and Ormrod, 1981) that, by misting bluegrass (*Poa pratensis*) during exposure to O₃, injury was significantly increased.

To conclude, the effects of high RH (low VPD) and surface wetness have much in common, as they both tend to enhance the uptake of O₃, largely through effects on stomata leading to increased impact.

**AX9.3.4.4  Drought and Salinity**

The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) concluded that the available evidence clearly indicated that exposure to drought conditions could reduce the adverse effects of O₃ on the growth of herbaceous and woody plants, but it also noted that no quantitative models of the O₃-soil moisture deficit (SMD) interaction had yet appeared in print. Nevertheless, the “protective” effect was inconsistent, and only appeared when SMD was accompanied by high evaporative demand. Since that time, further studies have confirmed the
interaction, and simulation models have begun to appear. Mills (2002) has recently provided a brief review of the topic.

With regard to herbaceous species, Vozzo et al. (1995) observed less O₃-induced injury and suppression of net photosynthesis and growth in water-deficient soybean (*Glycine max*) than in well-watered plants. In several studies with wheat (*Triticum aestivum*), on the other hand, although adverse effects of both O₃ and SMD were noted, they were consistently additive (Bender et al., 1999; Fangmeier et al., 1994a,b; Ommen et al., 1999).

In attempting to model the stomatal conductance of wheat in relation to O₃ and soil moisture, Grüters et al. (1995) found that although O₃-induced stomatal closure was enhanced by SMD, reducing O₃ uptake, the R² of the overall model was only 0.40, indicating that other significant factors or relationships were involved.

With regard to native vegetation, Bungener et al. (1999a) used mixed plantings of 24 Swiss grasses, herbs, and legumes and observed that, although O₃-drought interactions were species-specific, they tended to reflect stomatal functioning. They found that SMD reduced O₃ injury in two clovers (*Trifolium repens* and *T. pratensis*) and two grasses (*Trisetum flavescens* and *Bromus erectus*), but noted no interactions in the other 20 species. With relative growth rate as the measure of response to O₃, interactions with SMD were noted in only three species: *Trifolium repens* and two weedy herbs, *Knaoutia arvense* and *Plantago lanceolata* (Bungener et al., 1999b). Although this variability in response among species was noted in the review by Davison and Barnes (1998), they also pointed out that in severely droughted regions of Europe, notably in Greece and Spain, O₃-induced injury and growth reductions were common on many (usually irrigated) crops, but there were virtually no records of injury symptoms in wild species.

Thus, the situation with herbaceous species is essentially unchanged from 1988 when Heagle et al. (1988) summarized the extensive NCLAN experiments that incorporated water stress as a variable: “SMD can reduce the response of crops to O₃ under some conditions but not under other conditions. Probably the occurrence of O₃ by SMD interactions was dependent on the degree of SMD-induced plant moisture stress.”

With regard to trees, O₃ interactions with soil water availability have been discussed in several recent reviews: Chappelka and Freer-Smith (1995), who focused on O₃-induced predisposition to drought stress (Johnson et al., 1996b; Chappelka and Samuelson, 1998; and Skärby et al., 1998).
Several recent studies with conifers have yielded mixed results. No interactions with drought were observed by Broadmeadow and Jackson (2000) on Scots pine, by Karlsson et al. (2002) on Norway spruce, or by Pelloux et al. (2001) on Aleppo pine. More recently, Le Thiec and Manninen (2003) reported that drought reduced O₃-induced growth suppression of Aleppo pine seedlings. Panek and Goldstein (2001) inferred less impact of O₃ on droughted Ponderosa pine, and Van Den Driessche and Langebartels (1994) reported that drought reduced injury and O₃-induced ethylene release by Norway spruce. But Karlsson et al. (1997), in a comparative study of fast- and slow-growing clones of *P. abies*, only observed a drought-induced reduction of O₃-inhibited root growth in the fast-growing clone. In contrast, Grulke et al. (2002b) noted a synergistic interaction between O₃ and drought stress on gross photosynthesis of *Pinus ponderosa*, and Wallin et al. (2002) reported a synergistic growth response of Norway spruce in the third year of a 4-year study. A similar response was noted by Dixon et al. (1998) with the Istebna strain of Norway spruce.

With broad-leaved trees, studies of Durmast oak (*Quercus petraea*) (Broadmeadow et al., 1999; Broadmeadow and Jackson, 2000) and European ash (*Fraxinus excelsior*) (Broadmeadow and Jackson, 2000; Reiner et al., 1996) showed that drought provided partial protection against O₃-induced growth reduction. Although European beech (*Fagus sylvatica*) is reportedly an O₃- and drought-sensitive species, neither Pearson and Mansfield (1994) nor Broadmeadow et al. (1999) observed any interactions between these stresses, while Dixon et al. (1998) observed partial protection. Pääkönen et al. (1998) observed only additive effects in a sensitive clone of birch (*Betula pendula*). However, the experiments of Schaub et al. (2003) and the survey by Vollenweider et al. (2003a) on black cherry clearly indicate antagonism between drought and O₃ stresses on this species.

With regard to the converse effect, in a critical review of the evidence for predisposition to drought stress being caused by O₃, Maier-Maercker (1998) supported the hypothesis and suggested that the effects were caused by the direct effects of O₃ on the walls of the stomatal guard and subsidiary cells in the leaf epidermis, leading to stomatal dysfunction.

The Plant Growth Stress Model (PGSW) developed by Chen et al. (1994) is a physiology-based process model which includes drought among several environmental variables. Simulations for Ponderosa pine incorporated antagonistic effects between O₃ and drought stresses, i.e., partial protection, although Karlsson et al. (2000) have since emphasized that
drought-induced “memory effects” should be considered when developing simulation models incorporating stomatal conductance.

Retzlaff et al. (2000) used the single-tree model, TREEGRO, to simulate the combined effects of $O_3$ and drought on white fir ($Abies concolor$). Although simulated reductions in precipitation $\geq 25\%$ reduced growth, they also reduced $O_3$ uptake (and impact). But lesser reductions in precipitation combined synergistically with $O_3$ stress to reduce growth, leading the authors to conclude that moderate drought may not ameliorate the response of white fir to $O_3$.

On a much larger scale with a modified forest ecosystem model (PnEt-II) incorporating $O_3$-response relationships for hardwood species, Ollinger et al. (1997) showed how predicted changes in net primary production and mean wood production in the northeastern U.S. hardwood forests due to $O_3$ would be reduced (but not countered or reversed) by drought stress, particularly in the southern part of the region. This geographic distribution of the effect was substantiated by the work of Lefohn et al. (1997) on the risk to forest trees in the southern Appalachian Mountains, based on localized estimates of $O_3$ levels and SMD. The TREEGRO and ZELIG models were combined by Laurence et al. (2001) to predict the impacts of $O_3$ and moisture (as precipitation) on the growth of loblolly pine and yellow poplar. Based on $O_3$ and precipitation data from three sites in the eastern United States, the six model regressions developed for the two species included both positive and negative coefficients for $O_3$ exposure and precipitation as determinants of growth.

As noted in the 1996 $O_3$ AQCD (U.S. Environmental Protection Agency, 1996), the effects of soil salinity are similar to those of SMD. In a study of rice ($Oryza sativa$) cultivars of differing sensitivity to salinity, Welfare et al. (1996) noted that although both $O_3$ and salinity reduced many features of growth additively, antagonistic interactions were only seen for leaf length and potassium accumulation. Similarly, a recent study on chickpea ($Cicer arietinum$) found no interactions with regard to most components of biomass accumulation (the effects of $O_3$ and salinity were additive), but with root growth, salinity suppressed the adverse effects of $O_3$.

In summary, the recently described interactions of $O_3$ and drought/salinity stresses are consistent with the view that, in many species, drought/salinity reduces the impact of $O_3$, but $O_3$ increases sensitivity to drought stress, i.e., the type of response is determined by the sequence of stresses. However, synergisms have also been observed and any antagonisms are species-
specific and unpredictable in the absence of experimental evidence. In no case has an antagonism been found to provide complete protection.

**AX9.3.5 Nutritional Factors**

The 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) noted that the large number of macro- and micronutrients and the wide range of species had limited the number of experimental investigation to all but a few cases of nutrient-O$_3$ interactions and most of these concerned nitrogen (N) and crops or forest tree species. The document also provided a comprehensive tabulation of the results of the relevant studies up to 1992.

The suboptimal supply of mineral nutrients to plants leads to various types of growth reductions. The consequences of suboptimal nutrition might, therefore, be expected to have some similarities to those of O$_3$ exposure. One might expect nutritional levels below the optimum either to amplify any effects of O$_3$ or at least lead to additive responses. The difficulty with this suggestion is that the available information has mostly been obtained from experimentation conducted using two or more arbitrarily selected levels of fertility with little or no regard to optima. Hence, it is not surprising that there have been contradictory reports, even among studies with the same species or cultivars conducted by different workers at different locations using different soils or soil mixes.

There appear to have been no recent studies on O$_3$ interactions with specific mineral nutrients other than N. Hence, the previous conclusions are still valid, viz. that increasing levels of the major elements potassium (K) and sulfur (S) usually reduce the impact of O$_3$, or, deficiency increases susceptibility, whereas increased phosphorus (P) usually increases injury, or, deficiency decreases susceptibility.

However, with N, a relationship to the optimum is usually demonstrable. Several earlier studies of O$_3$ × N interactions reported that the adverse effects of O$_3$ on growth were greatest at the optimum and decreased with increasing N-deficiency, a finding supported by the work on aspen of Pell et al. (1995), who also confirmed that excess N decreased O$_3$ impact on growth. Similarly, the adverse effects of O$_3$ on growth rate in wheat diminished with decreased N supply (Cardoso-Vilhena and Barnes, 2001). However, the effects of N are far from consistent. For example, Greitner et al. (1994) reported that O$_3$ and N-deficiency acted additively in aspen in reducing leaf surface area and rate of photosynthesis, Bielenberg et al. (2001) reported that the
rate of O$_3$-induced senescence was increased by N-deficiency in hybrid poplar (*Populus trichocarpa* × *P. maximovizii*), and Pääkkönen and Holopainen (1995) observed the least adverse effects of O$_3$ on European white birch (*Betula pendula*) at optimum N-fertility levels. With cotton, increased N-levels more than overcame the adverse effect of O$_3$ on growth and boll yield (Heagle et al., 1999b). In view of these contradictions, one may conclude that other, unrecorded factors may have contributed to the various findings. Thus, much remains unclear about O$_3$ × N-fertility interactions.

There have been two recent studies on the effects of overall soil fertility. Whitfield et al. (1998) observed that low general fertility increased O$_3$ sensitivity in selections of common plantain. At the biochemical level, well-fertilized European white birch saplings were found to be less adversely affected by O$_3$ than nutrient-stressed plants (Landolt et al., 1997).

TREEGRO model simulations of the growth of red spruce (*Picea rubra*) in conditions of nutrient deficiency and O$_3$ stress showed that, in combination, the two stresses acted less than additively (Weinstein and Yanai, 1994). Minimal amelioration by nutrient deficiency was predicted with Ponderosa pine.

Plants may also obtain N and S from airborne sources such as NO$_x$, HNO$_3$, NO$_3^-$, SO$_2$, and SO$_4^{2-}$, although, depending upon their concentration, these may also be phytotoxic. In various parts of the world, the deposition of N and S in these forms contributes significantly to the levels of nutritionally available N and S in soils. Such depositions may, in turn, influence the impact of O$_3$ on sensitive species through their roles as nutrients independent of any interactions that may occur because of their acidic properties (see Section AX9.3.6.5). For example, Takemoto et al. (2001) recently reviewed the situation in southern California’s mixed conifer forests and noted that, where N-deposition is appreciable, its combination with O$_3$ is causing a shift in Ponderosa pine biomass allocation toward that of deciduous trees, with increased needle drop so that only 1- and 2-year needle classes overwinter. Such changes are having significant consequences on the balance of the forest ecosystem and are discussed more fully in Section AX9.5.

Of the micronutrient elements, only manganese (Mn) appears to have been studied recently. In beans (*Phaseolus vulgaris*) Mn-deficiency increased O$_3$ toxicity, despite causing reduced O$_3$ uptake (through decreased stomatal conductance) and inducing increased levels of Mn-SOD (Mehlhorn and Wenzel, 1996).
In view of the foregoing, it is impossible to generalize about the interactions of soil fertility with O$_3$. While this is especially true of the interactions involving soil nitrogen, for which there is much conflicting evidence, the interactions with other nutrients need much more thorough investigation than has occurred to date, before any clear patterns become apparent.

AX9.3.6 Interactions with Other Pollutants

The ambient air may have pollutant gases other than O$_3$ and its photochemical oxidant relatives. In particular, industrial, domestic, and automobile emissions and accidents can lead to significant atmospheric concentrations of gases such as sulfur dioxide (SO$_2$) and nitric oxide (NO) and nitrogen dioxide (NO$_2$), collectively referred to as NO$_x$, both locally and regionally. Local releases of gases such as hydrogen fluoride (HF), hydrogen chloride (HCl), and chlorine (Cl$_2$) may result from industrial emissions and accidents. Agricultural fertilizer and manure usage can lead to significant increases in ambient ammonia (NH$_3$) and ammonium sulfate ((NH$_4$)$_2$SO$_4$). The sulfur and nitrogen oxides may undergo reactions in the atmosphere leading to the formation of sulphate (SO$_4^{2-}$) and nitrate (NO$_3^-$) ions and resultant acid deposition.

The 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) discounted much of the early research on pollutant combinations, because of its lack of resemblance to the ambient experience: the concentrations used were unrealistically high or the exposure regimes employed almost invariably used gas mixtures, whereas Lefohn et al. (1987) showed that the co-occurrence patterns of significant levels of O$_3$ with SO$_2$ or NO$_2$ in the United States were most frequently sequential or partially sequential with overlap; only rarely were they entirely concurrent. On the other hand, O$_3$ and peroxyacetyl nitrate (PAN) frequently co-occur, as both form photochemically under similar conditions.

To the list of reviews mentioned in the 1996 O$_3$ AQCD should be added the more recent ones by Barnes and Wellburn (1998), Robinson et al. (1998), and Fangmeier et al. (2002), which also explore some of the potential mechanisms underlying pollutant-pollutant interactions.

AX9.3.6.1 Oxidant Mixtures

In 1998, Barnes and Wellburn noted that virtually no information existed on the effects on plants of concurrent exposures to O$_3$ and other components of photochemical oxidant other than PAN. The situation has not changed since their review appeared, and the topic appears to have
attracted no research interest since before the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996). The continuing conclusion must, therefore, be that, from the limited information available, the two gases appear to act antagonistically, with O₃ raising the threshold for the visible injury response to PAN and PAN reducing the harmful effects of O₃.

**AX9.3.6.2 Sulfur Dioxide**

In reviewing O₃ × SO₂ interactions, Barnes and Wellburn (1998) remarked: “The outcome of exposure to this combination of pollutants has probably been the most studied, yet is one of the least understood.” More recent studies have only added to the conflicts referred to in the 1996 criteria document (U.S. Environmental Protection Agency, 1996), rather than resolve them. For example, Diaz et al. (1996) reported that, after a year of daily exposures of Aleppo pine seedlings to 50 ppb O₃ and/or 40 ppb SO₂, the combination of pollutants synergistically reduced shoot and root growth and impaired mycorrhizal colonization of the roots. With tomato (*Lycopersicon esculentum*), on the other hand, effects on growth ranged from synergistic at low exposures (50 ppb) to antagonistic at exposures of 200 ppb of each gas (Khan and Khan, 1994). Although various physiological measurements were made in these and earlier studies, it has not been possible to determine any consistent mechanism or mechanisms that might account for the conflicting results.

Since the information available about O₃ × SO₂ interactions appears to be highly dependent upon species, the type of response measured, and the experimental protocol used, it would still appear prudent to heed the statement of Heagle et al. (1988) in their summary of the studies undertaken in 12 field experiments over several years within the NCLAN program: “There were no cases where O₃ and SO₂ interactions significantly affected yield.” (emphasis added.)

**AX9.3.6.3 Nitrogen Oxides, Nitric Acid Vapor, and Ammonia**

The major oxides of nitrogen that occur in ambient air are nitrous oxide (N₂O), NO, and NO₂, of which the latter two (conveniently symbolized as NOₓ) are particularly important in connection with O₃, because they are components of the reaction mix that leads to photochemical O₃ formation and because they can interact with O₃-responses. Their reactions in the atmosphere can also lead to the occurrence of nitric acid vapor (HNO₃) in ambient air. The other major
N-containing contaminant of ambient air in many parts of the world is NH₃, largely released through agricultural practices.

Despite various combinations of O₃ and NOₓ being probably the most common air pollutant combinations found in the field, Barnes and Wellburn (1998) noted that they have been little studied. Much early work with O₃ and NOₓ focused on O₃ × NO₂ interactions and can be discounted, because of the unrealistic concentrations employed and their use as mixtures rather than in types of sequences. The 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) concluded that evidence from studies involving concurrent exposures to both O₃ and NOₓ at realistic concentrations was so fragmented and varied that no firm conclusions could be drawn as to the likelihood and nature such interactions. However, the few recent investigations taken together with the earlier data are now beginning to reveal a pattern of response.

With regard to NO, Nussbaum et al. (1995a, 2000b) reported their findings with concurrent exposures to NO and O₃ and observed that, at low O₃ levels, NO tended to act similarly to O₃ by increasing the scale of responses such as growth reductions. However, in ambient air in which O₃ is a dominant factor, the effects of NO were usually found to be negligible due to low levels, although the authors admitted that the effects observed were confounded by the inevitable O₃-induced oxidation of NO to NO₂.

Two possible mechanisms whereby NO may influence plant response to O₃ are suggested by recent biochemical studies. First, there is growing evidence for the role of NO as a signaling agent in plants that can induce defense responses to a range of biotic and abiotic stressors (Beligni and Lamattina, 2001; Neill et al., 2002). Second, a role for NO as an antioxidant scavenger of reactive oxygen species has been demonstrated by Beligni and Lamattina (2002) in potato leaves and chloroplasts. However, both of these cases concern endogenously synthesized NO, and it must be noted that in none of these or other reports of studies of NO signaling have the authors considered the potential significance of exogenous NO in ambient air.

An independent case for O₃ × NO interactions comes from Mills et al. (2000). The ANN model developed to predict the O₃ effects on white clover biomass based on experiments at 18 locations throughout Europe suggested that the minimum daily NO concentration (at 5 p.m.) may have been a contributor to adverse effects.

Turning to NO₂, Maggs and Ashmore (1998) found that, although concurrent but intermittent exposures of Bismati rice (Oryza sativa) revealed no significant growth
interactions, NO₂ reduced the rate of O₃-induced senescence, an antagonistic response possibly related to enhanced N-metabolism.

With regard to sequential exposures, two studies on gene activation in tobacco revealed that NO₂ counteracted the effect of O₃ in reducing mRNA levels for three genes encoding photosynthetic proteins (Bahl and Kahl, 1995) and tended to counteract the O₃-induced enhancement of defense-protein gene activation (Bahl et al., 1995). However, despite compelling evidence for significant interactive effects provided by earlier studies (Bender et al., 1991; Goodyear and Ormrod, 1988; Runeckles and Palmer, 1987), the only recent investigation of growth effects seems to have been that of Mazarura (1997) using sine-wave exposure profiles. He found that although 4 weeks of twice daily 3-h exposures to NO₂ (120 ppb peak concentrations) slightly stimulated growth of radish (Raphanus sativa) and while daily 6-h exposures to O₃ (120 ppb peak concentration) did not significantly reduce growth, the daily sequence, NO₂ - O₃ - NO₂, led to a 13% drop in dry matter production.

The combined evidence to date, therefore, suggests that, in leguminous species, the effects of these sequences are antagonistic with NO₂ tending to reduce (or reverse) the negative effects of O₃ on growth, while the effects are increased in other species. These conclusions differ from those of Barnes and Wellburn (1998) who suggested that sequential exposures tended to result in antagonistic effects (largely based on the summary by Bender and Weigel (1992), whereas simultaneous exposures were likely to lead to synergistic responses. With disagreements both among the data and their interpretation, it is not possible to determine the circumstances under which specific interactions of O₃ and NO₂ may occur, but there is no reason to doubt the validity of the individual findings of each study. Far more systematic investigation is needed to clarify the situation.

There appear to have been no studies of O₃ interacting with HNO₃ in the vapor phase. However, in the southern California montane forests (Takemoto et al., 2001), in Sweden (Janson and Granat, 1999), and elsewhere, significant amounts of N are deposited in this form because of the vapor’s high deposition velocity. As a consequence, although much of it ultimately reaches the ground through leaching and leaf fall and enters the soil as NO₃⁻, it may also be used as a N source by the foliage itself (Garten and Hanson, 1990; Hanson and Garten, 1992; Norby et al., 1989). This nutritional role is independent of any contribution that HNO₃ vapor may make to acidic deposition. Indirect interactions with the effects of O₃ through N-deposition of NOₓ.
HNO$_3$, and NH$_3$ are related to the interactions of O$_3$ with N as a nutrient, and have recently been examined in the review by Takemoto et al. (2001). The 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) stated that the evidence available at that time led to estimates of total forest dry deposition, including HNO$_3$, ranging from 5.7 to 19.1 kg N ha$^{-1}$ year$^{-1}$ (Taylor et al., 1988a). However, Takemoto et al. (2001) pointed out that in parts of the mid-elevation forests of southern California, dry deposition rates may reach more than 40 kg N ha$^{-1}$ year$^{-1}$. As a result, some locations have seen the conversion from N-limited to N-saturated forests. The concern for California’s forests is well stated by Takemoto et al.: “As potential modifiers of long-term forest health, O$_3$ is a stressor and N deposition is an enhancer of ponderosa/Jeffrey pine physiology and growth (Grulke and Balduman, 1999). The progression toward a deciduous growth habit, higher shoot:root biomass ratios, increasing depths of litter, tree densification, and elevated NO$_3^-$ levels in soil and soil solution, all point to the replacement of pine species with nitrophilous, shade- and O$_3$-tolerant tree species, such as fir and cedar (Minnich, 1999; Minnich et al., 1995).”

Few studies have been reported of interactions of O$_3$ with NH$_3$. The 1996 criteria document made reference to the work on kidney bean by Tonneijck and Van Dijk (1994, 1998). Although NH$_3$ alone tended to increase growth and O$_3$ alone to inhibit it, one interaction was noted (Tonneijck and Van Dijk, 1994) on the number of injured leaves. Dueck et al. (1998) studied the effects of O$_3$ and NH$_3$ on the growth and drought resistance of Scots pine. Significant interactions were found for some growth features, but there were no consistent patterns of the effects of NH$_3$ on O$_3$ response or vice versa. However, O$_3$ was found to ameliorate the enhancement of drought stress caused by NH$_3$ on Scots pine.

At this time there is insufficient information to offer any general conclusions about the interactive effects of O$_3$ and NH$_3$.

**AX9.3.6.4 Hydrogen Fluoride and Other Gaseous Pollutants**

Although HF and other fluorides are important local air pollutants associated with aluminum smelting and superphosphate fertilizer manufacture, no studies of possible interactions with oxidants appear to have been reported since that of MacLean (1990). He found that HF retarded the accelerated senescence and loss of chlorophyll resulting from O$_3$ exposure in corn seedlings. However, such an isolated observation cannot be taken to indicate that HF can reduce
the impact of $O_3$ on other species or even that the effect would ultimately have led to an effect on mature plants.

**AX9.3.6.5 Acidic Deposition**

The deposition of acidic species onto vegetation may elicit direct effects on the foliage or indirect effects via changes induced in the soil. The 1996 $O_3$ AQCD (U.S. Environmental Protection Agency, 1996) included an extensive listing of investigations into the effects of $O_3$ and acidic deposition (usually in the form of simulated acid rain, SAR) on plant growth and physiology. The majority of studies found no effects of SAR or acidic mists or fogs at pH values greater than about 3.0 and no interactive effects with $O_3$. (In ambient air, pH values less than 3.0 have rarely been reported.) In the few reports in which significant interactions were found, most were antagonistic and were explained as probably being the result of increased fertility due to $NO_3^-$ and $SO_4^{2-}$ supplied in the SAR.

Although numerous reviews have recently appeared (e.g., Bussotti and Ferretti, 1998; Flückiger et al., 2002; Fox and Mickler, 1996; Nussbaum et al., 1999; and Sheppard and Cape, 1999), the shift in interest in air pollution effects away from acid deposition has resulted in little new research having been reported over the past 10 or so years. In most of the reported studies, no effects due to the $O_3$ exposures, the SAR treatments used, or their combinations were observed, e.g., Baker et al. (1994) on loblolly pine; Laurence et al. (1997), and Vann et al. (1995) on red spruce; and Laurence et al. (1996) on sugar maple. Branch chamber studies of 12-year-old Ponderosa pine trees by Momen et al. (1997, 1999) revealed no $O_3$ effects or interactions. With red spruce, Sayre and Fahey (1999) noted no effects of $O_3$ on the foliar leaching of Ca or Mg, which only became significant with SAR at pH 3.1. Izuta (1998) observed no interactions with Nikko fir (*Abies homolepis*), although SAR at pH 4.0 reduced dry matter. Shan et al. (1996) reported adverse effects of $O_3$ but none attributable to SAR on the growth of *Pinus armandi*.

With herbaceous species, Ashenden et al. (1995) noted significant antagonistic interactions of $O_3$ and acid mist in white clover, in which the adverse effect of low pH was countered by $O_3$. In contrast, Ashenden et al. (1996) found that, although pH 2.5 mist caused a significant stimulation of the growth of ryegrass attributed to a fertilizer effect, and $O_3$ caused reduced growth, there was no interaction. Bentgrass (*Agrostis capillaris*) behaved similarly.
A study by Bosley et al. (1998) on the germination of spores of the moss, *Polytrichum commune*, and the ferns, *Athyrium felix-femina* and *Onoclea sensibilis*, revealed no effect of O$_3$ on moss spores, while SAR at pH < 4.0 was completely inhibitory. With the ferns, germination was progressively reduced by both increased O$_3$ and acidity.

In summary, the few findings of interactions in these recent studies are consistent with the previous conclusion regarding the likelihood of such interactions being antagonistic. However, the interactions observed were in each case largely the result of the response to the lowest pH used, which, in several cases, was below 3.0, and hence may not be relevant to most field conditions.

**AX9.3.6.6 Heavy Metals**

As there appears to have been no further research into the interactions of oxidants with heavy metal pollutants, our understanding is unchanged from at the time of the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996). As noted therein, the limited data available from early studies indicates varying degrees of enhancement of any adverse effects of O$_3$ but precludes the development of any response relationships.

**AX9.3.6.7 Mixtures of Ozone with Two or More Pollutants**

In many airsheds, the mixtures that occur, both concurrently and over time, may involve three or more pollutants. Very little useful information exists on the effects of O$_3$ with multiple pollutants. As the 1996 criteria document and others have pointed out, most of the early studies on such combinations can be discounted, because of their use of (1) high and environmentally irrelevant exposure concentrations and (2) unrealistic, repetitive exposure profiles (Barnes and Wellburn, 1998; U.S. Environmental Protection Agency, 1996).

The large investment in experimental facilities required to study these complex interactions is a major deterrent. So, although the topic has been included in several reviews that have appeared in the last decade, there appear to have been only two studies that have provided new information on the effects of O$_3$ in combination with more than one other pollutant stress. Ashenden et al. (1996) studied the effects of O$_3$ and/or (SO$_2$ + NO$_2$) with four acidities of SAR applied to each gas treatment, on white clover and two pasture grasses (*Lolium perenne* and *Agrostis capillaris*). With each species, the antagonism reported for the O$_3$ × SAR interaction
(Section AX9.3.4.6.5) tended to be nullified by concurrent exposure to the other gases, while the combination of the three gaseous pollutants resulted in the most severe growth inhibition, regardless of the acidity of the SAR.

With such meager evidence, no clear conclusions can be drawn as to the ways in which the effects of multiple airborne stressors could influence or be influenced by O₃.

**AX9.3.7 Interactions with Agricultural Chemicals**

The review of interactions involving O₃, plants, and various agricultural chemicals presented in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) remains a valid assessment of our limited knowledge of these interrelationships. Our knowledge is largely based on the protection against O₃ afforded to a range of crop species by applications of various chemicals, particularly fungicides, such as benomyl (benlate; methyl-1-[butylcarbamoyl]-2-benzimidazolecarbamate) and several carbamates and triazoles. A recent report has added azoxystrobin (AZO) and epoxyconazole (EPO) to the list (Wu and Tiedemann, 2002). Foliar sprays of either AZO or EPO provided 50 to 60% protection against O₃ injury to barley (*Hordeum vulgare*) leaves. Both had similar modes of action involving stimulation of the levels of antioxidant enzymes such as SOD, ascorbate peroxidase, guaiacol peroxidase, and catalase.

In contrast, applications of herbicides have yielded variable results ranging from increased sensitivity to protection from O₃; the nature of the effect is usually species- or cultivar-dependent. Although of less wide application, some plant growth retardants have also been found to provide protection, but no insecticide appears to have been clearly shown to have similar properties.

Despite the attraction of the use of permitted chemicals to provide crop protection, the statement in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) is still valid: “It is premature to recommend their use specifically for protecting crops from the adverse effects of O₃, rather than for their primary purpose.”

**AX9.3.8 Factors Associated with Global Climate Change**

During the last decade, interest in the effects of climatic change on vegetation has replaced concerns over the purported causes of forest decline and the effects of acidic deposition. Two
Specific components of climate change have been singled out as the foci of most of the research activity:

- the effects of increasing mean global CO$_2$ concentrations in the lower atmosphere, and
- the effects of increasing levels of surface-level irradiation by UV-B (the result of stratospheric O$_3$ depletion).

In spite of the crucial role of temperature as a climatic determinant (Monteith and Elston, 1993), the effects of increasing mean global temperatures and their interactions with increasing CO$_2$ levels in particular have received less attention.

All of the biotic and chemical interactions with oxidants discussed in the preceding sections may be modified by these climatic changes. However, research activities have largely focused on the two-way O$_3$ × CO$_2$ interaction. Little if any experimental evidence exists related to three-way interactions, such as O$_3$ × CO$_2$ × disease or O$_3$ × CO$_2$ × nutrient availability, although such interactions are difficult to predict from the component two-way interactions.

Numerous reviews have appeared since the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) dealing with the issues involved. General reviews include publications of IPCC (1996, 2001); and UNEP (1993, 1999); the volume by Wellburn (1994); the volumes edited by Alscher and Wellburn (1994), De Kok and Stulen (1998), Singh (2000), and Yunus and Iqbal (1996); and papers by Idso and Idso (1994), Krupa and Groth (2000), Luo et al. (1999), Polle and Pell (1999), Poorter and Pérez-Soba (2001), Runeckles (2002), and Weubbles et al. (1999). Effects on agriculture and crop production, growth, and metabolism have been reviewed by Groth and Krupa (2000), Rötter and Van De Geijn (1999), and Schnug (1998); effects on forests have been reviewed by Bortier et al. (2000a); with focus on insect pests, Docherty et al. (1997), Karnosky et al. (2001a, c), McLaughlin and Percy (1999), and Saxe et al. (1998).

As background to the discussion of interactions with O$_3$, it should be noted that the increased levels of CO$_2$ experienced since the mid-18th century are such that, without abatement of the rates of increase, increased levels of from 540 to 970 ppm have been projected by the year 2100 (IPCC, 2001). Such increases in the concentration of CO$_2$, the principal GHG released into the atmosphere, will inevitably lead to increased global mean temperatures, evidence for which is already available from oceanic, icepack, and other records. The latest estimates of the global warming are for an increase in the range of 1.4 to 5.8 °C over this century, in contrast to the
0.6 °C rise experienced since 1900 (IPCC, 2001). However, considerable uncertainty is associated with such projections of future increases in global temperature.

The use of elevated CO\textsubscript{2} concentrations has been common practice for many years in the production of many greenhouse crops. Much of our early knowledge of the effects of higher than ambient CO\textsubscript{2} levels on plant growth derives from this application, coupled with research of plant physiologists on how CO\textsubscript{2} concentrations affect the process of photosynthesis. Information available about the effects of increased CO\textsubscript{2} levels on photosynthesis and stomatal function, in particular, has provided the underlying bases for numerous process models that simulate plant growth under stress and in changed climates.

Although simple O\textsubscript{3} × temperature interactions were discussed in Section AX9.3.4.2, the close linkage between global CO\textsubscript{2} levels and global mean temperatures in the context of climate change requires that an assessment of the interactive effects with O\textsubscript{3} should focus, as much as possible, on interactions involving all three factors.

**AX9.3.8.1 Ozone-Carbon Dioxide-Temperature Interactions**

Idso and Idso (1994) reviewed several hundred reports published between 1982 and 1994 on the effects of increased CO\textsubscript{2} on plant growth and net photosynthesis. Their survey covered a wide range of temperate and tropical, herbaceous and perennial species, including coniferous trees. They concluded that, for responses to a 300-ppm increase in CO\textsubscript{2}, somewhat less than a doubling of present-day levels, but somewhat greater than the 540 ppm lower limit suggested by the IPCC (IPCC, 2001), averaged across all species:

- light intensity had a negligible effect on net photosynthesis other than at limiting low intensities under which the CO\textsubscript{2}-driven enhancement was increased;
- increased temperature tended to increase the CO\textsubscript{2}-driven enhancement of dry matter accumulation (growth) and net photosynthesis;
- drought conditions tended to increase the CO\textsubscript{2}-driven enhancements of both growth and net photosynthesis, but increased salinity had little effect;
- mineral nutrient deficiency (especially of nitrogen) tended to increase the CO\textsubscript{2}-driven enhancement of growth; and
- in the presence of air pollutants (especially SO\textsubscript{2} and NO\textsubscript{x}), the CO\textsubscript{2}-driven enhancement of net photosynthesis tended to be increased.
It should be noted that the statement that CO\textsubscript{2}-enhanced growth increased with temperature referred to total dry matter accumulation by the whole plant and not to the yield of grain, fruit, or seed. Unfortunately, despite the existence of several reports at the time, the summary of interactions with air pollutants contained only a single reference to O\textsubscript{3}, i.e., Pfirrmann and Barnes (1993), who reported surprisingly that a doubling of CO\textsubscript{2} levels led to a 27% increase in dry weight of radish but that the combination with O\textsubscript{3} led to a 77% increase.

The more recent reviews by Rudorff et al. (2000) and Olszyk et al. (2000) have addressed CO\textsubscript{2} \times O\textsubscript{3} interactions in detail, with the latter focusing on the implications for ecosystems. They concluded that:

- the effects of both gases on stomatal closure were predominantly additive, with little evidence of interaction;
- increased photosynthesis resulting from elevated CO\textsubscript{2} may be canceled by exposures to high O\textsubscript{3} levels;
- foliar O\textsubscript{3} injury is reduced by elevated CO\textsubscript{2}; and
- interactions between CO\textsubscript{2} and O\textsubscript{3} can affect storage carbohydrates, leaf free-radical metabolism, and carbon allocation to shoots and roots.

Olszyk et al. (2000) also made specific note of the relative lack of information on below-ground effects.

Much of the recently published information on the effects of increased CO\textsubscript{2} and O\textsubscript{3} levels is summarized in Table AX9-12. Note that the table only lists the directions of O\textsubscript{3}-induced effects and any modifications of these effects resulting from elevated CO\textsubscript{2}, not their magnitudes. These directions are usually, but not necessarily, the same as the corollary effects of O\textsubscript{3} on CO\textsubscript{2}-induced responses.

The bulk of the available evidence clearly shows that, under the various experimental conditions used (which almost exclusively employed abrupt or “step” increases in CO\textsubscript{2} concentration, as discussed below), increased CO\textsubscript{2} levels may protect plants from the adverse effects of O\textsubscript{3} on growth. This protection may be afforded in part by CO\textsubscript{2} acting together with O\textsubscript{3} in inducing stomatal closure, thereby reducing O\textsubscript{3} uptake, and in part by CO\textsubscript{2} reducing the negative effects of O\textsubscript{3} on Rubisco and its activity in CO\textsubscript{2}-fixation. Although both CO\textsubscript{2}-induced and O\textsubscript{3}-induced decreases in stomatal conductance have been observed primarily in short-term
### Table AX9-12. Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

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<tr>
<th>Plant Response</th>
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<th>Facility</th>
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Table AX9-12 (cont’d). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

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<td>CECP</td>
<td>Volin et al. (1998)</td>
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<tr>
<td>Specific leaf area-SLA</td>
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<td>Radish (R. sativus)</td>
<td>CEC, P</td>
<td>Barnes and Pfirrmann (1992)</td>
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<td>OTC, G</td>
<td>Mulchi et al. (1992)</td>
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Table AX9-12 (cont’d). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

<table>
<thead>
<tr>
<th>Plant Response</th>
<th>O₃ Response</th>
<th>CO₂ Modification</th>
<th>Species</th>
<th>Facility</th>
<th>Reference</th>
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<td>Growth, Yield (cont’d)</td>
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<tr>
<td>Specific leaf area-SLA (cont’d)</td>
<td>▼</td>
<td>▲</td>
<td>White clover (<em>T. repens</em>) (O₃-sensitive)</td>
<td>CSTR, P</td>
<td>Heagle et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>△</td>
<td>▲</td>
<td>White clover (<em>T. repens</em>) (O₃-tolerant)</td>
<td>CSTR, P</td>
<td>Heagle et al. (1993)</td>
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<td><em>Agropyron smithii</em></td>
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<td>Volin et al. (1998)</td>
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<td>CEC, P</td>
<td>Volin et al. (1998)</td>
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<td>▲</td>
<td><em>Bouteloua curtipendula</em></td>
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<td>Volin et al. (1998)</td>
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<td><em>Schizachyrium scoparium</em></td>
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<td>Volin et al. (1998)</td>
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<td>Trembling aspen (<em>P. tremuloides</em>)</td>
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<td>Volin et al. (1998)</td>
</tr>
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<td>CEC, P</td>
<td>Barnes and Pfirrmann (1992)</td>
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<tr>
<td></td>
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<td>▲</td>
<td>Alfalfa (<em>M. sativa</em>)</td>
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<td>Johnson et al. (1996a)</td>
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<td></td>
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<td>▼</td>
<td>White clover (<em>T. repens</em>) (O₃-sensitive)</td>
<td>CSTR, P</td>
<td>Heagle et al. (1993)</td>
</tr>
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<td>White clover (<em>T. repens</em>) (O₃-tolerant)</td>
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<td>Heagle et al. (1993)</td>
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<td>▲</td>
<td><em>Wheat</em> (<em>T. aestivum</em>)</td>
<td>CEC, P</td>
<td>McKee et al. (1997a)</td>
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Table AX9-12 (cont’d). Effects of Increased Carbon Dioxide on Ozone-Induced Responses of Plants at the Metabolic, Physiological, and Whole-Plant Levels

<table>
<thead>
<tr>
<th>Plant Response</th>
<th>O₃ Response</th>
<th>CO₂ Modification</th>
<th>Species</th>
<th>Facility</th>
<th>Reference</th>
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<tr>
<td>Root/shoot ratio (cont’d)</td>
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<td>Timothy (P. pratense)</td>
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<td>Johnson et al. (1996a)</td>
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<td>Soybean (G. max)</td>
<td>OTC, G,P</td>
<td>Booker et al. (2005)</td>
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<tr>
<td></td>
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<td>Black cherry (P. serotina)</td>
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<td>○</td>
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<td>Green ash (F. pensylvanica)</td>
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<td>Loats and Rebbeck (1999)</td>
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<td>○</td>
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<td>Yellow poplar (L. tulipifera)</td>
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<td>Loats and Rebbeck (1999)</td>
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<td>Potato (S. tuberosum)</td>
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<td>Donnelly et al. (2001b)</td>
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<tr>
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<td>Bean (P. vulgaris)</td>
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<td>Heagle et al. (2002)</td>
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<td></td>
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<td>Heagle et al. (1998a)</td>
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<tr>
<td></td>
<td>✓</td>
<td>▼</td>
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<td>OTC, P</td>
<td>Heagle et al. (1999b)</td>
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<td>✓</td>
<td>▼</td>
<td>Wheat (T. aestivum)</td>
<td>CEC, P</td>
<td>Barnes et al. (1995)</td>
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<tr>
<td></td>
<td>✓</td>
<td>▼</td>
<td>Trembling aspen (P. tremuloides)</td>
<td>FACE, G</td>
<td>Karnosky et al. (1999)</td>
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<tr>
<td></td>
<td>✓</td>
<td>▼</td>
<td>European beech (F. sylvatica)</td>
<td>CEC, P</td>
<td>Grams et al. (1999)</td>
</tr>
</tbody>
</table>

O₃ m ≤ 0.15 ppm.

CO₂ modifications of O₃-effects resulting from ~2× present levels. (Trends are shown in brackets. Pronounced changes with ontogeny are, for example, indicated thus: ○ ▼.)

Exposure facilities used: CEC: controlled environment chambers; CSTR: continuously stirred tank reactors (Heck et al., 1978); FACE: free air CO₂ enrichment facilities; OTC: open-top chambers. G: plants rooted in the ground; P: plants grown in pots. All species are C₃ except corn, Bouteloua and Schizachyrium.
studies, recent data clearly show a long-term and sustained reduction in stomatal conductance under elevated CO\(_2\) for a number of species (Ainsworth and Long, 2005; Ellsworth et al., 2004; Gunderson et al., 2002). Instances of increased stomatal conductance have also been observed in response to O\(_3\) exposure, suggesting partial stomatal dysfunction after extended periods of exposure (Maier-Maercker, 1998).

At the mechanistic level, Rubisco plays a key role in CO\(_2\)-assimilation, and while both O\(_3\) and elevated CO\(_2\) per se can lead to reduced activity, CO\(_2\) can also reverse the O\(_3\)-induced inhibition of Rubisco activity and photosynthesis (Table AX9-12). However, in their review of the possible mechanisms involved, Polle and Pell (1999) cautioned that Rubisco should not be regarded “as a unique target for the interaction of the two gases.” But it is clear from the bulk of the evidence in Table AX9-12 that elevated CO\(_2\) levels can ameliorate the inhibition of growth caused by O\(_3\) in many species, although the precise balance among the mechanisms involved may well vary from species to species. Three important caveats must be raised with regard to the findings presented in Table AX9-12:

- the applicability of results from experiments with an abrupt (step) increase in CO\(_2\) level to understanding the consequences of the gradual increase in CO\(_2\) predicted for the troposphere over the next hundred years;

- the validity of the findings in several long-term studies (particularly with tree species) conducted using potted plants, because of possible added stressors imposed on their root systems relative to trees growing in the field; and

- the relevance to understanding the effects of climate change of studies focused solely on CO\(_2\) enrichment at current ambient conditions of temperature and precipitation patterns that provide no insights into possible interactive effects as these other climatic variables change concurrently with increasing CO\(_2\) (Intergovernmental Panel on Climate Change [IPCC], 2001).

The first caveat concerns the distinctly different natures of the exposures to O\(_3\) and CO\(_2\) experienced by plants in the field. Changes in the ambient concentrations of these gases have very different dynamics. In the context of climate change, CO\(_2\) levels increase relatively slowly and may change little over several seasons of growth. On the other hand, O\(_3\) presents a fluctuating stressor with considerable hour-to-hour and day-to-day variability (Polle and Pell, 1999). Almost all of the evidence presented in Table AX9-12 comes from experimentation involving plants grown from the outset in, or subjected to, an abrupt or step increase to a higher
more or less double), steady CO$_2$ concentration. In contrast, the O$_3$ exposure concentrations usually varied from day to day. Luo and Reynolds (1999), Hui et al. (2002), and Luo (2001) noted the difficulties in predicting the likely effects of a gradual CO$_2$ increase from experiments involving a step increase or those using a range of CO$_2$ concentrations. Indeed, although using the much accelerated timescale of an 80-day growing season, Hui et al. (2002) clearly showed significant differences between the rates and magnitudes of various physiological and growth responses of plantain (*Plantago lanceolata*) to CO$_2$ between gradual and step increase treatments. The authors concluded that, even though there were major differences in most of the parameters studied between the gradual and step treatments, “the convergence of the measured parameters at the end of the experiment provides some encouragement for the applicability of step-type experiments in the field; however, the study suggests caution in interpreting early results from short-term studies.”

In long-term studies, the matter of photosynthetic acclimation to elevated CO$_2$ levels has to be considered. Lawlor and Keys (1993) define acclimation in terms of long-term (days, weeks), irreversible physiological changes, in contrast to regulation, which relates to more rapid (minutes, hours), reversible changes. Each may be positive or negative, but many studies indicate that, while positive acclimation to elevated CO$_2$ levels initially led to enhanced photosynthesis and growth, negative acclimation ultimately ensued and reduced CO$_2$ assimilation and growth rates. However, the consensus from recent studies and reviews is that such negative acclimation is most likely to occur in situations in which plants are grown under some additional stress, induced, e.g., by limitations to growth posed by lack of resources such as water or nutrients. The meta-analysis by Curtis (1996) revealed that slow or little negative acclimation was noted in studies on unstressed tree species with unhindered opportunities for root growth and development, a view originally suggested by Arp and Drake (1991) and largely supported in the review by Eamus (1996). A nonwoody perennial, the rhizomatous wetland sedge, *Scirpus olneyi*, grown in its natural environment with no edaphic limitations showed no negative acclimation after 4 years; in fact, photosynthetic capacity increased by 31% (Arp and Drake, 1991). No negative acclimation of well-watered, field-grown Ponderosa pine trees was observed by Tissue et al. (1999) after 6 years of growth at 2×-ambient CO$_2$ levels. Gifford and Morison (1993) have summarized the situation thus: "Where the aerial or root environment for a plant is restricted (as with inter-plant competition, for example), positive feedback is limited and

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adjustments to the changed resource input balance under high CO$_2$ can include ‘down-regulation’ of leaf photosynthesis rate as an integral part of a positive growth response.”

The influence of other environmental stressors is borne out by several long-term tree studies. After 3 years in 565 ppm CO$_2$ in the Duke Forest FACE facility in North Carolina, maturing loblolly pine trees showed only a marginal CO$_2$-induced carbon gain if grown on a nutritionally moderate site, but zero gain if grown on a nutritionally poor site (Oren et al., 2001). This is in sharp contrast to the substantially increased initial growth rates in elevated CO$_2$ reported by DeLucia et al. (1999), but it is supported by the observations of Tognetti et al. (2000) on five Mediterranean tree species growing for many years adjacent to geothermal springs releasing CO$_2$ sufficient to provide ambient levels averaging 700 ppm. No significant differences in radial growth of the oaks (*Quercus cerris*, *Q. ilex*, and *Q. pubescens*), strawberry tree (*Arbutus unedo*), and flowering ash (*Fraxinus ornus*) could be detected between trees at the naturally enriched site and those at a nearby site exposed to normal ambient CO$_2$ (~350 ppm). The authors concluded that limited availability of water and nutrients may have counteracted any positive effects of CO$_2$ on growth at the enriched site or that the trees had acclimated to the higher CO$_2$ levels.

Because the ameliorative effects of CO$_2$ on responses to O$_3$ (Table AX9-14) were reported mostly in short-term studies involving an abrupt increase in CO$_2$ level, it is appropriate to ask whether this amelioration is likely to persist to a time when the ambient CO$_2$ concentration is relatively stable at such levels. Regardless of any negative acclimation due to resource limitations that may occur in the interim, steadily rising CO$_2$ levels may well lead to natural selection and genetic change. Nevertheless, it seems reasonable to expect that the amelioration of O$_3$ impact at elevated CO$_2$ levels will be maintained in many situations, but the negative acclimation that will probably occur in situations where other resources become limiting will reduce the degree of protection.

Another caveat regarding the validity of some of the observations in Table AX9-12 is related to the matter of stress-induced negative acclimation to elevated CO$_2$ and concerns related to using potted plants. Although much of the recent information on CO$_2$ effects has come from experiments with plants rooted in the ground, more than half of the studies listed in Table AX9-12 used potted plants, whether in controlled environment and greenhouse chambers or in OTCs. The degree to which pot-based studies resulted in similar patterns of response to
soil-grown plants appears to depend on the treatment conditions and plant growth conditions used in the study. The use of potted plants was a confounding factor in the studies of Taylor et al. (2001) of the differences in leaf growth of poplar (Populus) hybrids between plants exposed to elevated CO$_2$ in controlled environment chambers (potted plants), OTCs, or a FACE facility. Loats and Rebbeck (1999) suggested that their lack of CO$_2$ response in three broadleaf species may have resulted from their use of pot-grown plants. In contrast, Heagle et al. (1999a) found that the relative enhancement of soybean photosynthesis, growth, and yield by CO$_2$ enrichment was similar in pots and in the ground. These findings were supported by Booker et al. (2005). The recent meta-analysis of data on the effects of elevated CO$_2$ on soybean physiology and growth by Ainsworth et al. (2002) revealed a threefold smaller stimulation of seed yield in pot-grown than in field-grown plants, even when large (>9 L) pots were used. However, Ainsworth et al. (2002) included a wide range of treatment conditions (e.g., CO$_2$ treatments ranging from 500 to 1200 ppm) and plant growth conditions in the meta-analysis, so caution is needed when generalizing conclusions about the applicability of pot-based studies.

Although the majority of the cases cited in Table AX9-12 indicate that O$_3$ and CO$_2$ act additively or synergistically in causing stomatal closure, there are numerous exceptions. Any reduction in stomatal aperture has consequences other than merely restricting O$_3$ uptake and the exchange of other gases. In particular, stomatal closure initially reduces the rate of transpiration, although increased leaf temperature and VPD associated with stomatal closure can offset decreases in transpiration. In instances where transpiration is reduced, water-use efficiency may increase, however decreased transpirational flux may lead to decreased mineral uptake, which could adversely impact growth over extended periods.

Hence, the final caveat regarding Table AX9-12 concerns the interactions of O$_3$ and CO$_2$ with other climatic variables, especially mean temperature. In light of the key role played by temperature in regulating physiological processes and modifying plant response to increased CO$_2$ levels (Long, 1991; Morison and Lawlor, 1999) and the knowledge that relatively modest increases in temperature may lead to dramatic consequences in terms of plant development (Lawlor, 1998), it is unfortunate that much of the large investment in time and resources spent on recent studies of the effects of climate change on vegetation have gone into investigations limited to increasing our knowledge of the effects of higher levels of CO$_2$ at current ambient temperatures.
Some attention is now being paid to investigating the concurrent effects of CO₂ increases and warming (recently reviewed by Rowland-Bamford [2000] and Morison and Lawlor [1999]), but the observed interactive effects on plant growth are inconsistent. For example, a FACE study with ryegrass (Lolium perenne) showed that increased temperatures (provided by infrared heaters) reduced the dry matter gain resulting from increased CO₂ levels (Nijs et al., 1996). The field studies by Shaw et al. (2002) on a California annual grassland dominated by the grasses Avena barbata and Bromus hordeaceus and the forbs Geranium dissectum and Erodium botrys involved free-air increased CO₂ as well as increased temperature, precipitation, and N supply. Not only did increased temperature reduce CO₂-stimulated net primary productivity (NPP), but increased CO₂ itself, combined with other factors, was found to be able to reduce NPP.

There have been several investigations of effects on wheat. Batts et al. (1997) used plastic tunnels to create temperature gradients and maintain elevated CO₂ levels over field-grown wheat and found that, in each of 4 years of study, a temperature rise of ~1.5 °C consistently canceled the growth and yield increases caused by a doubling of the CO₂ level above ambient. Similar findings were reported by Van Oijen et al. (1999) and Van Oijen and Ewart (1999) in OTC field studies. Half of the chambers were cooled 1.6 to 2.4 °C below the uncooled chambers, to cancel out the normal temperature increase over ambient, due to the so-called “chamber effect” (usually a 1 to 3 °C increase above ambient temperature (Heagle et al., 1988). Although temperature had no effect on CO₂-enhanced assimilation rates, the CO₂-enhanced growth and grain yields observed in the cooled chambers were effectively canceled out in the warmer chambers. The authors attributed this effect to accelerated phenology, a shorter period for grain filling, and a lower leaf area index (LAI; total leaf area per unit ground area) in the warmer chambers. Wheeler et al. (1996) observed that the benefit to wheat of doubling the CO₂ level was offset by a mean seasonal increase of only 1 to 1.8 °C. With the continuing use of OTCs for field research, Runeckles (2002), has suggested that the temperature rise due to the chamber effect in OTCs should be exploited (and measured) as a means of exploring temperature × CO₂ as well as temperature × CO₂ × O₃ interactions.

An indirect affirmation of the importance of temperature as a component of climate change on wheat yield was provided by Van Oijen and Ewart (1999) using two simulation models, AFRCWHEAT2-O₃ and LINTULCC (Ewart et al., 1999). They analyzed data from the ESPACE-wheat program, which involved 25 OTC experiments in 1994, 1995, and 1996 at nine
European locations (Jäger et al., 1999). Both models were able to predict control-treatment grain yields closely (5.5 ± 1.2 and 5.8 ± 1.2 t·ha⁻¹, respectively, versus the observed 5.9 ± 1.9 t·ha⁻¹), and both indicated a predominantly negative effect of temperature on the yield response to increased CO₂ (a 3 °C rise reduced the gain in yield from 30 to 14%). However, neither model had an R² > 0.3, indicating that the models included other sources of variability among the sites than the climatic factors. The multiple linear regression developed by Bender et al. (1999) based on the same datasets also included temperature as a highly significant covariant. Both studies are discussed more fully below.

Other studies, however, have found positive temperature-related growth effects, as suggested by the early Idso and Idso (1994) analysis. In an OTC study using the perennial grass Festuca pratensis in which a temperature increase of 3 °C above ambient was combined with CO₂-enrichment to 700 ppm, both CO₂ and temperature caused increases in total aboveground biomass (Hakala and Mela, 1996). Studies with potato (Cao et al., 1994) and soybean (Ziska and Bunce, 1997) using potted plants in controlled environment chambers also showed temperature-enhanced increases in growth in enriched CO₂ atmospheres. Read and Morgan (1996) compared the effects of enriched CO₂ and temperature on two grasses: cool-season Pascopyrum smithii and warm-season Bouteloua gracilis. In the latter (a C₄ species), 750 ppm CO₂ resulted in increased dry matter production at daytime temperatures as high as 35 °C, but in P. smithii (a C₃ species), CO₂-stimulated growth was greatest at 20 °C. However, the stimulation was progressively attenuated by increased temperature, so that at 35 °C, growth in 750 ppm was only one third of that in 350 ppm CO₂ at 20 °C.

Although the picture we have of temperature × CO₂ interactions is inconsistent, Rowland-Bamford (2000) has provided persuasive evidence that the nature of the response to temperature in the grain yield of crops with as different temperature optima as rice and wheat will depend upon whether the change is above or below the temperature optimum.

But what if we add O₃ as another variable? Unfortunately, there have been very few studies of the three-way interaction. With the information available on CO₂ × O₃ interactions (Table AX9-12) and the limited information on temperature × O₃ interactions (discussed in Section AX9.3.4.2) simulation modeling can attempt to provide estimates of O₃ × CO₂ × temperature effects, but experimental observation is still required to validate the models. The questions that need to be answered are: if increased temperature can offset the gains in
productivity afforded by increased CO$_2$ in important species such as wheat, and increased CO$_2$ can offset the reductions in productivity caused by O$_3$, will increased temperature modify this protective effect? And if so, in what manner?

To date, the only information available appears to consist of the reports by Van Oijen and Ewart (1999) and Bender et al. (1999) referred to above. In the former’s simulation studies, the overall yield depression of wheat caused by O$_3$ was found to be 7 ± 4% for both AFRCWHEAT2-O$_3$ and LINTULCC models versus an observed 9 ± 11%. The enhancements due to CO$_2$ were predicted to be 24 ± 9% and 42 ± 11%, respectively, which straddled the observed 30 ± 22% gain. Based on the 13 experiments that included all four treatments (±O$_3$, ±CO$_2$), an actual 10% yield loss due to O$_3$ at ambient CO$_2$ levels was reduced to a 4% loss by the elevated CO$_2$. The AFRCWHEAT2-O$_3$ model predicted 7 and 4% losses, and LINTULCC model predicted 8 and 5% losses due to the O$_3$ and O$_3$ + CO$_2$ treatments. The actual and simulated yield increases in response to CO$_2$ increased further with increasing temperature, but although temperature had no discernible effect on the observed depression of yield caused by O$_3$ alone, both models suggested that the yield reduction was diminished both by higher temperatures and higher CO$_2$ levels.

The analysis of the ESPACE-wheat experiments by Bender et al. (Bender et al., 1999) led to the following multiple linear regression:

$$Y=1004.6^{***} + 0.588^{***}[\text{CO}_2] - 1.908^{**}[\text{O}_3] - 31.230^{***}[T] + 7.309[I] - 1448.423^{***}[\text{H}_2\text{O}], \,(9-5)$$

where $Y =$ grain yield, g · m$^{-2}$; [CO$_2$] = ppm CO$_2$; [O$_3$] = ppb O$_3$, 12-h mean; [T] = °C; [I] = light intensity, MJ · m$^{-2}$/day; and [H$_2$O] is a dummy variable: well watered = 1; limited water supply = 2. (***, p < 0.001; **, p < 0.01; the coefficient for $I$ was not significant). With $R^2$ = 0.3983, adjusted for 258 degrees of freedom, a large part of the variability was still unaccounted for by the five variables. However, this analysis suggests that CO$_2$, O$_3$, temperature, and water-status are important codeterminants of wheat yield, but assumes no interactions. Substitution in the model at summer light intensities and with well-watered plants indicates that, at 20°C, a doubling of CO$_2$ levels to 700 ppm alone would lead to a 29.5% increase in yield, while 50 ppb O$_3$ alone would decrease yield by 10.9%. With both gases at those levels, the yield
would only increase 20%, but with a concurrent temperature rise of 2 °C, it would shrink to a 9.6% increase.

Both studies, therefore, indicate an amelioration of the effects of O\textsubscript{3} by CO\textsubscript{2}, the magnitude of which would be reduced at warmer temperatures. However, they relate to a single crop whose response to CO\textsubscript{2} is temperature-sensitive. Information about other species in which the effects of CO\textsubscript{2} and temperature are additive are limited. However, Wolf and Van Oijen (2003) recently described a model (LPOTCO) simulating the effects of changes in climatic variables, CO\textsubscript{2} and O\textsubscript{3} on tuber yield potential of irrigated potato (\textit{S. tuberosum} cv. Bintje) over locations within the European Union ranging from Finland to Italy. They noted that although increased CO\textsubscript{2}, O\textsubscript{3}, and light intensity were predominant controlling factors, increased temperature also influenced potential yields substantially, with increases in northern latitudes (attributed to a longer growing season) but decreases in southern latitudes (attributed to decreased assimilate production).

A clear understanding of the complex interactions of increased CO\textsubscript{2} and temperature with O\textsubscript{3} must await further experimentation or simulations. However, it seems likely that any CO\textsubscript{2}-induced amelioration of the adverse effects of O\textsubscript{3} on aspects of growth other than seed or grain yield may be lessened or increased by increased temperature, depending upon the temperature optima for the species, along the lines suggested by Rowland-Bamford (2000).

Other crop simulation models which incorporate O\textsubscript{3} and some of the various environmental factors, including elements of climate change, have been reviewed by Kickert et al. (1999) and Rötter and Van De Geijn (1999). However, to date, the applications tend to have focused on interactions of O\textsubscript{3} with factors such as soil moisture or nutrient availability.

With forest trees, the situation has the added complexity of a perennial growth form and the inevitability, over time, of subjection to additional environmental stresses such as nutrient-limitation. Here, too, although numerous models of tree growth have been described, there appear to have been few applications to interactions of O\textsubscript{3} and factors of climate change. Constable et al. (1996) used TREGRO to model the growth of Ponderosa pine exposed to three O\textsubscript{3} levels (0.5×, 1.0×, and 2× ambient), two levels of CO\textsubscript{2} (ambient and ambient + 200 ppm CO\textsubscript{2}), and two temperature regimes (ambient and ambient + 4 °C). Plant growth was predicted to be decreased 1, 19, and 39% by the three levels of O\textsubscript{3}, respectively. Increased CO\textsubscript{2} reduced the loss at the highest O\textsubscript{3} level to 7%; however, the combination of elevated CO\textsubscript{2} with the higher temperature more than overcame the adverse effects of O\textsubscript{3}, leading to a 4% increase,
largely attributed to increased fine root mass. The authors suggested that, in relation to the baseline conditions used in the simulations (Corvallis, OR), higher concentrations of CO$_2$ and O$_3$ and a warmer climate will have little impact on total-tree growth, but they noted the importance of undertaking multiple stress studies in order to be able to make accurate forecasts of the impact of such changes on forest trees.

More recently, Constable and Friend (2000) compared the capabilities of six published process-based models (CARBON, ECOPHYS, PGSM, TRE-BGC, TREGRO, and W91) for simulating tree response to elevated CO$_2$, O$_3$, and temperature. They concluded that, although these models were capable integrators of the effects of various environmental factors on individual processes such as photosynthesis, they were less reliable when extrapolating to growth.

Although most of the research emphasis has been on simple CO$_2$ × O$_3$ interactions, a few isolated studies of interactions have involved O$_3$, CO$_2$, and biotic environmental factors. Heagle et al. (1994a) observed that both O$_3$ and CO$_2$ tended to be additive in encouraging the growth of spider mite (Tetranychus urticae) populations on clover. Infection of wheat with leaf rust (Puccinia recondita) sensitized the plants to O$_3$ injury, but its severity was significantly reduced in elevated CO$_2$ (Tiedemann and Firsching, 2000). The effects of O$_3$ and CO$_2$ on mycorrhizal symbioses was studied by Kytöviita et al. (1999) who found that CO$_2$ did not ameliorate the adverse effects of O$_3$ on the root growth of Aleppo pine and European white birch. In another study with Aleppo pine, Kytöviita et al. (2001) noted that both O$_3$ and elevated CO$_2$ reduced mycorrhiza-induced N-uptake by the roots. In Scots pine, Kasurinen et al. (1999) observed transient effects of elevated CO$_2$ and O$_3$ on root symbiosis, but none of the effects persisted over the 3 years of the study.

The soil water × O$_3$ × CO$_2$ interaction was experimentally investigated by Broadmeadow and Jackson (2000) in Durmast oak, European ash, and Scots pine. No interactions were noted with ash and pine; but with oak, elevated CO$_2$ ameliorated and irrigation exacerbated the effects of O$_3$, although the resultant effects were essentially additive.

Booker (2000) noted that soil nitrogen levels interacted only slightly with O$_3$ and CO$_2$ in determining the composition of cotton leaves and roots. Carbon dioxide reversed the inhibition of leaf growth caused by O$_3$, but increased N-fertility tended to reduce this reversal.
Because of the small number of studies of possibly significant interactions of three or more environmental factors, it is impossible to draw any sweeping conclusions as to how O$_3$, in the context of global climate change, may affect relationships among plants and insects, diseases, and symbionts or among plants and nutrients or other air pollutants. The only interaction that has some degree of general support is the amelioration of adverse O$_3$ effects by elevated CO$_2$.

**AX9.3.8.2 Ozone-UV-B Interactions**

As noted in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996), depletion of stratospheric O$_3$ by halofluorocarbons has resulted in increased intensities of UV-B radiation (280 to 320 nm wavelengths) at the Earth’s surface. The situation is discussed more fully in Chapter 10.

While stratospheric O$_3$ depletion may result in increased surface UV-B irradiation, absorption of UV-B is a property of the O$_3$ molecule regardless of its location; surface UV-B flux is, therefore, also reduced by O$_3$ in the troposphere. Although only about 10% of the total atmospheric O$_3$ column occurs in the troposphere (Fishman et al., 1990), it contributes a disproportionately greater absorption effect than stratospheric O$_3$, because the UV radiation penetrating the troposphere becomes increasingly diffuse as it reaches the surface, with a consequent increase in mean path length (Brühl and Crutzen, 1989). Any benefits to vegetation from reduced ambient O$_3$ stress must, therefore, also be viewed in the context of possible adverse effects due to increased UV-B irradiation. There are, thus, two distinct types of possible interactions between surface level O$_3$ and UV-B radiation:

- direct interactions involving simultaneous, sequential, or mixed exposures to O$_3$ and UV-B stresses; and
- effects on responses to UV-B itself resulting from changes in radiation intensity caused by changes in surface-level O$_3$ concentrations.

Only the first type of interaction is discussed here. The second type of interaction has broad implications for both health and welfare and focuses on the impacts of UV-B radiation per se. This topic is dealt with separately in Chapter 10.

The most recent reviews specifically addressing the combined effects of tropospheric O$_3$ and UV-B on plants are by Runeckles and Krupa (1994) and Krupa and Jäger (1996), although the topic has also been included in several more general reviews of O$_3$ effects and factors of
climate change, such as those by Unsworth and Hogsett (1996), Krupa et al. (1998), Posthumus (1998), and Krupa and Groth (2000).

However, little new information has become available since Runeckles and Krupa (1994) noted that the scanty knowledge of the effects of UV-B and O$_3$ combinations available at that time was derived solely from studies of soybean. Miller et al. (1994) observed no interaction and no effect of UV-B on yield, in contrast to a previous report by Teramura et al. (1990) using the same cultivar, Essex. More recently, in a study of the saltmarsh grass *Elymus athericus* subjected to reciprocal exposures to O$_3$ and UV-B, Van De Staaij et al. (1997) observed no interactive effects and no adverse effects of UV-B following 14-day exposures, even though an earlier report showed that longer exposures to UV-B (65 days) could cause a 35% loss of biomass (Van De Staaij et al., 1993). However, in a study in which ambient, high-altitude UV-B levels were compared with near-zero levels, at ambient or 2×-ambient levels of O$_3$, interactions involving the levels of antioxidants in Norway spruce and Scots pine were reported by Baumbusch et al. (1998). Schnitzler et al. (1999) subsequently reported that O$_3$-induced injury and adverse effects on photosynthesis were more pronounced with near-zero UV-B levels, indicating an amelioration of the O$_3$-response. A later study with Scots pine (Zinser et al., 2000) revealed O$_3$ × UV-B interactions at the gene expression and biochemical levels. In contrast, Ormrod et al. (1995) reported that UV-B predisposed *Arabidopsis thalliana* to injurious growth effects from O$_3$ exposure.

At various organizational levels, Runeckles and Krupa (1994) identified several similarities between plant response to O$_3$ and UV-B, and at the level of gene expression, there have recently been several reports of both similarities and distinctions. Willekens et al. (1994) reported similar effects of O$_3$, UV-B, and SO$_2$ on the expression of antioxidant genes in *Nicotiana plumbaginifolia*. For parsley (*Petroselinum crispum*), Eckey-Kaltenbach et al. (1994a) found that O$_3$ was a cross-inducer for both the UV-B-induced enhanced biosynthesis of flavonoids and the pathogen-induced furanocoumarin phytoalexins, in keeping with the previously observed O$_3$-induction of fungal and viral defense reactions. In this regard, Yalpani et al. (1994) provided evidence that O$_3$ and UV-B acted similarly in increasing disease-resistance via a salicylate-mediated enhancement of defense proteins in tobacco. However, subsequent work with tobacco led Thalmair et al. (1996) to conclude that exposure to UV-B did not lead to the accumulation of pathogenesis-related proteins. In Scots pine, although O$_3$ is known to induce
stilbene synthase and cinnamyl alcohol dehydrogenase, UV-B was found to enhance the former but suppress the latter, revealing an interaction at the level of gene expression (Zinser et al., 2000).

In summary, the present base of information about possible interactions between increased UV-B radiation and O₃ is insufficient to draw any firm conclusions in terms of gross effects, but there is some evidence of similarities in the effects of O₃ and UV-B individually and of the mechanisms involved at the molecular level.

**AX9.3.8.3 Interactions of Ozone with Multiple Climate Change Factors**

Despite the need for experimental investigations of three-way or more complex interactions among O₃, CO₂, UV-B, temperature, and other climate change factors, few studies have been reported, even without O₃ as a factor. In an isolated report, using tomato seedlings, Hao et al. (2000) employed preexposure to UV-B (±CO₂ enrichment) followed by exposure to O₃ (±CO₂ enrichment). They observed that CO₂ enrichment more than overcame the inhibition of photosynthesis caused by O₃, but pretreatment with UV-B reduced the resultant increase.

In view of the unexpected observations made in their grassland study of the combined effects of CO₂, temperature, precipitation, and N-supply, Shaw et al. (2002) affirmed that “Ecosystem responses to realistic combinations of global changes are not necessarily simple combinations of the individual factors.” The addition of O₃ to the list of variables results in further complexity.

Although computer simulation modeling may ultimately lead to improved understanding of these complex issues, to date, no such models appear to have been applied to these interactions, possibly because of the scarcity of experimental data for parameterization.

**AX9.3.9 Summary - Environmental Factors**

Although O₃ and other photochemical oxidants are phytotoxic, their actions on vegetation may be modified by a host of biotic and abiotic factors in the environment; conversely, they may modify plant response to these other factors. The extensive review of these biological, physical, and chemical factors conducted for the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) concluded with a statement that our understanding was too fragmented to permit drawing
many general conclusions. With today’s increased awareness of the need for more complete
information on interactions, it is unfortunate that, in the interval since the 1996 O₃ AQCD,
rigorous, systematic investigations of interactions have been rare, and most of the new
information is as fragmented as before. This is inevitable, partly in view of the vast scope of the
possible interactions between O₃ and other environmental variables and partly due to the overall
lack of funding for such research in these areas.

In the area of biotic interactions, new evidence with regard to insect pests and diseases has
done little to remove the uncertainties noted in the 1996 criteria document. Most of the large
number of such interactions that may affect crops, forest trees, and other natural vegetation have
yet to be studied. The trend suggested previously that O₃ increases the likelihood and success of
insect attack has received some support from recent studies, but only with respect to chewing
insects. With the economically important group of sucking insects such as the aphids, no clear
trends have been revealed by the latest studies. Hence, although it seems likely that some insect
problems could increase as a result of increased O₃ levels, we are still far from being able to
predict the nature of any particular O₃ plant insect interaction, its likelihood, or its severity.

The situation is a little clearer with respect to interactions involving facultative
necrotrophic plant pathogens with O₃, generally leading to increased disease. With obligate
biotrophic fungal, bacterial, and nematode diseases, there are twice as many reports
indicating O₃-induced inhibitions than enhancements. The frequent reports that infection
by obligate biotrophs reduces the severity of O₃-induced foliar injury should not be interpreted
as “protection”, because of the negative effects on the host plant of the disease per se.
With obligate biotrophs, the nature of any interaction with O₃ is probably dictated by the unique,
highly specific biochemical relationships between pathogen and host plant. At this time,
therefore, although some diseases may become more widespread or severe as a result of
exposure to O₃, it is still not possible to predict which diseases are likely to present the greatest
risks to crops and forests.

Several studies have indicated that the functioning of tree root symbioses with mycorrhizae
may be adversely affected by O₃, but there is also evidence that the presence of mycorrhizae may
overcome root diseases stimulated by O₃ and that O₃ may encourage the spread of mycorrhizae
to the roots of uninfected trees. The latest studies, therefore, present no clearer picture of the
likely nature of simple interactions of O₃ and root symbionts, but in view of the importance of
mycorrhizae as below-ground components of ecosystems, they are discussed more fully in Section AX9.5.

The few recent studies of the impact of O₃ on intraspecific plant competition have again confirmed that grasses frequently show greater resilience than other types of plants. In grass-legume pastures, the leguminous species suffer greater growth inhibition. And the suppression of Ponderosa pine seedling growth by blue wild-rye grass was markedly increased by O₃. However, we are far from being able to predict the outcome of the impact of O₃ on specific competitive situations, such as successional plant communities or crop-weed interactions.

Light, a component of the plant’s physical environment, is an essential “resource” whose energy content drives photosynthesis and CO₂ assimilation. It has been suggested that increased light intensity may increase the sensitivity to O₃ of light-tolerant species while decreasing that of shade-tolerant species, but this appears to be an oversimplification with many exceptions. Temperature affects the rates of all physiological processes based on enzyme-catalysis and diffusion, and each process and overall growth (the integral of all processes) has a distinct optimal temperature range. Although some recent field studies have indicated that O₃ impact significantly increases with increased ambient temperature, other studies have revealed little effect of temperature. But temperature is unquestionably an important variable affecting plant response to O₃ in the presence of the elevated CO₂ levels contributing to global climate change (see below). In contrast, evidence continues to accumulate to indicate that exposure to O₃ sensitizes plants to low temperature stress by reducing below-ground carbohydrate reserves, possibly leading to responses in perennial species ranging from rapid demise to impaired growth in subsequent seasons.

Although the relative humidity of the ambient air has generally been found to increase the adverse effects of O₃ by increasing stomatal conductance and thereby increasing O₃ flux, abundant evidence indicates that the ready availability of soil moisture results in greater sensitivity to O₃. The partial “protection” against the adverse effects of O₃ afforded by drought has been observed in field experiments and modeled in computer simulations. There is also compelling evidence that O₃ can predispose plants to drought stress. Hence, the response will depend to some extent upon the sequence in which the stresses occur, but, even though the nature of the response is largely species-specific, successful applications of model simulations will lead to larger-scale predictions of the consequences of O₃ × drought interactions. However,
it must be recognized that regardless of the interaction, the net result on growth in the short-term is negative, although in the case of tree species, other responses such as increased water use efficiency could be a benefit to long-term survival.

Mineral nutrients in the soil, other gaseous air pollutants, and agricultural chemicals constitute chemical factors in the environment. The evidence regarding interactions with specific nutrients is still contradictory. Some experimental evidence indicates that low general fertility increases sensitivity to \( \text{O}_3 \), while simulation modeling of trees suggests that nutrient deficiency and \( \text{O}_3 \) act less than additively; however there are too many example of contrary trends to permit any sweeping conclusions. Somewhat analogously with temperature, it appears that any shift away from the nutritional optimum may lead to greater sensitivity, but the shift would have to be substantial before a significant effect on response to \( \text{O}_3 \) was observed.

Interactions of \( \text{O}_3 \) with other air pollutants have received relatively little recent attention. The situation with \( \text{SO}_2 \) remains inconsistent, but seems unlikely to pose any additional risk to those related to the individual pollutants. With \( \text{NO} \) and \( \text{NO}_2 \), the situation is complicated by their nutritional value as N sources. In leguminous species, it appears that \( \text{NO}_2 \) may reduce the impact of \( \text{O}_3 \) on growth, with the reverse in other species, but the nature of the exposure pattern, i.e., sequential or concurrent, also determines the outcome. Much more investigation is needed before we will be able to predict the outcomes of different \( \text{O}_3\)-\( \text{NO}\)-\( \text{NO}_2 \) scenarios. The latest research into \( \text{O}_3 \times \) acid rain interactions has confirmed that, at realistic acidities, significant interactions are unlikely. A continuing lack of information precludes offering any generalizations about interactive effects of \( \text{O}_3 \) with \( \text{NH}_3 \), HF, or heavy metals. More evidence has been reported that the application of fungicides affords some protective effects against \( \text{O}_3 \).

Over the last decade, considerable emphasis has been placed on research into \( \text{O}_3 \) interactions with the components of global climate change: increased atmospheric \( \text{CO}_2 \), increased mean global temperatures, and increased surface-level UV-B radiation. However, many of these studies have tended to regard increased \( \text{CO}_2 \) levels and increased mean temperatures as unrelated phenomena. Experiments into the effects of doubled \( \text{CO}_2 \) levels at today’s mean ambient temperatures may not reveal the impact of climate change on responses to \( \text{O}_3 \). For example, the limited experimental evidence and evidence obtained by computer simulation suggest that in a 600+ ppm world, although the enriched \( \text{CO}_2 \) would more than offset the impact of \( \text{O}_3 \) on responses as varied as wheat yield or the growth of young Ponderosa pine
trees, the concurrent increase in temperature would reduce, but probably not eliminate, the net gain. A similar decrease in the net gain resulting from the complete reversal by CO$_2$ of the inhibition of photosynthesis caused by O$_3$ has been reported for increased UV-B irradiation. Clearly, additional research is needed in this important area.

In conclusion, although the increased use of computer simulations may be important in suggesting outcomes of the many complex interactions of O$_3$ and various combinations of environmental factors, the results obtained will only be as reliable as the input data used for their parameterization. The data needed for good simulations can only come from organized, systematic study. For predicting the future, ignorance is as good as dependence on poor simulations.

**AX9.4 EFFECTS-BASED AIR QUALITY EXPOSURE INDICES**

**AX9.4.1 Introduction**

Indices are metrics that relate measured plant damage (i.e., reduced growth) to monitored ambient O$_3$ concentration over time. An index is needed to provide a consistent metric for reviewing and comparing exposure-response effects obtained from various studies. Such indices may also provide a basis for developing a biologically-relevant air quality standard for protecting ecological resources. Effects on plant growth and/or yield has been a major focus of the characterization of O$_3$ impacts on plants for purposes of the air quality standard setting process (U.S. Environmental Protection Agency, 1986, 1996b). The quantifying function over some time frame has frequently been referred to as “dose-response” and “exposure-response.” The distinction is in how the pollutant concentration is measured: “dose” is the measured pollutant concentration absorbed by the leaf over some time period, whereas “exposure” is the ambient air concentration measured near the plant over some time period.

A measure of plant O$_3$ uptake from the ambient air (either rate of uptake or cumulative seasonal uptake) is the ideal measure, because without O$_3$ or its reactive product(s) reaching the target tissue there is no effect (Tingey and Taylor, 1982). Uptake is controlled in part by stomata (see Section AX9.2 for a detailed discussion). An uptake measure should integrate all those environmental factors that influence stomatal conductance, e.g., temperature, humidity, soil water status. A direct measure of the internal leaf concentration of O$_3$, however, is technically
difficult and thus uptake values are generally obtained with simulation models that require species- and site-specific variables. Because of this, a surrogate for uptake was sought early on using statistical summaries of monitored ambient pollutant concentration over some integral of time (Lee et al., 1988; Lefohn and Benedict, 1982; O’Gara, 1922; U.S. Environmental Protection Agency, 1986, 1992, 1996b).

An index of exposure then must consider those abiotic and biotic factors known to modify the plant response by altering $O_3$ uptake (Hogsett et al., 1988; U.S. Environmental Protection Agency, 1996b), including the temporal dynamics of exposure (e.g., concentration, frequency, duration), plant phenology (see Section AX9.3), plant defense mechanisms (e.g., antioxidants) (see Section AX9.2), and site climate and soil factors (e.g., temperature, VPD, soil moisture) (see Section AX9.3). The development of such indices continues to be a challenge (U.S. Environmental Protection Agency, 1996b).

**AX9.4.2 Summary of Conclusions from the Previous Criteria Document**

The 1996 $O_3$ AQCD (U.S. Environmental Protection Agency, 1996b) focused on the research developing exposure indices to quantify growth and yield effects in crops, perennials, and trees (primarily seedlings) and not foliar injury. The indices were various functional and statistical summaries of monitored hourly $O_3$ concentrations over designated time periods. The testing of the adequacy of these indices to order the measured responses of growth and/or yield in crops and tree species as seedlings was accomplished through regression analyses of earlier exposure studies. No direct experimental testing of the adequacy of these indices was accomplished. Their development focused on consideration and inclusion of some, but not all, factors that affect $O_3$ uptake and expression of effects (e.g., Lee et al., 1988). The 1996 document (U.S. Environmental Protection Agency, 1996b) drew a number of conclusions that built on even earlier conclusions (U.S. Environmental Protection Agency, 1992). Based on a review of the research published since 1996, those conclusions are still valid.

Studies prior to 1996 indicated that the components of exposure, including concentrations, temporal dynamics (e.g., time of day of peak events), frequency of occurrence, duration, and respite time, were integral to developing indices of exposure related to growth response. Evidence from the few direct experimental studies of varying exposure components indicate the importance of peak concentrations, temporal pattern of occurrence, respite time and the
importance of cumulating the concentrations over the exposure period (Hogsett et al., 1985a; Musselman et al., 1983, 1986, 1994; U.S. Environmental Protection Agency, 1996b).

Exposure duration influences the degree of plant response. Single season, year-long, or multiyear experimental results indicated that greater yield losses occurred when plants were exposed for the longer duration and that a cumulative-type index was able to better describe the exposure-yield relationship. Indices that do not consider duration, e.g., 7-h seasonal mean concentration index, single peak event index, or the index that cumulated all concentrations (i.e., SUM00), were unable to adequately describe the relationship. These single event or mean-type indices do not consider the role of duration of exposure and either focus only on the peak event or put too much focus on the lower hourly average concentrations (U.S. Environmental Protection Agency, 1996b).

Higher hourly average concentrations had a greater effect on plant response. It was concluded that cumulative indices that gave greater weight to higher concentrations related well with plant response (crops and tree seedlings) and ordered the treatment means in monotonically decreasing fashion with increasing exposure, based on studies that applied two or more types of exposure regimes with replicate studies of the same species. Examples of these indices, among others, were: AOT60 (the seasonal sum of the difference between an hourly concentration above the threshold value of 60 ppb, minus the threshold value of 60 ppb), SUM06 (the seasonal sum of hourly concentrations at or above the threshold value of 60 ppb), W126 (a sigmoid functional weighting of all hourly concentrations for the season), (U.S. Environmental Protection Agency, 1996b).

No studies before or after 1996, have enabled a discrimination among the various weighted, cumulative indices. Various functional weighting approaches have been used, including allometric, sigmoid, and threshold weighting, and compared for best statistical fit of the plant growth or yield data; however, no one functional weighting was favored.

An exposure index that incorporated either the daily or seasonal temporal patterns of higher concentration occurrence with the temporal pattern of individual species’ stomatal conductance was not reported in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996b). Based on available data, it was unresolved how to proceed with weighting time of day or temporal patterns of species conductance.
The relative importance of cumulative peak concentrations (>0.10 ppm) versus cumulative mid-range concentrations (0.05 to 0.099 ppm) was questioned. Although controlled experiments had provided important evidence that the higher hourly average concentrations should be given greater weight than the mid-level values in developing indices, there was concern that, under ambient conditions in the field, the higher concentrations did not occur at the time of maximum plant uptake. This coincidence was considered to be the critical factor in determining peak concentration impacts on plants. Based on the evidence at that time, it was not possible to conclude whether the cumulative effects of mid-range concentrations were of greater importance than those of peak hourly average concentrations in determining plant response (U.S. Environmental Protection Agency, 1996b). No direct experimental studies, however, had addressed this question prior to 1996, nor have any since.

The composite exposure-response functions for crops and tree seedlings were derived from single and multiyear exposure studies that used modified or simulated ambient exposure profiles. These profiles were typified by episodic occurrence of a large number of higher O₃ concentrations. This type of pattern is not atypical but is not found in all rural agricultural and some forested areas in the United States. Selecting a concentration value from these crop and seedling response models may result in an over- or underestimation of growth effects if applied to regions of the country where a different type of temporal pattern of occurrence is prevalent (U.S. Environmental Protection Agency, 1996b). A multicomponent index was suggested that combined the concentration-weighted, cumulative index with the number of occurrences of hourly averaged concentrations ≥0.10 ppm that might reduce the uncertainty associated with selecting the exposure value for protection based on NCLAN-type studies (Lefohn and Foley, 1992; Musselman et al., 1994; U.S. Environmental Protection Agency, 1996b). No direct experimental studies addressed this question prior to 1996, nor have any since.

Since 1996, additional research has focused on the time of day when the higher hourly average concentrations occur, the time of day of maximum plant uptake, the diurnal variability of plant defense mechanisms, and various suggestions as to including these factors in any one of the cumulative, concentration-weighted exposure indices. A much broader literature has focused since 1996 on relating O₃ flux to plant response and how to use this as an index relating ambient concentration to effects. These new developments are discussed in the sections that follow.
AX9.4.3 Evaluation of Various Exposure Indices for Describing Ambient Exposure-Response Relationships

Mathematical approaches for summarizing ambient air quality information in biologically meaningful forms that can serve as surrogates for dose for O₃ vegetation effects assessment purposes have been explored for more than 80 years (O'Gara, 1922; U.S. Environmental Protection Agency, 1996b). Several indices have attempted to incorporate some of the biological, environmental, and exposure factors (directly or indirectly) that influence the magnitude of the biological response and contribute to observed variability (Hogsett et al., 1988). In the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996b), the exposure indices were arranged into five categories; (1) One Event, (2) Mean, (3) Cumulative, (4) Concentration Weighted, and (5) Multicomponent, and were discussed in detail (Lee et al., 1989). Figure AX9-16 illustrates how several of the indices weighted concentration and accumulate exposure.

Various components of the exposure-response relationship, including concentration, time of day, respite time, frequency of peak occurrence, plant phenology, predisposition, etc., were weighted with various functions and evaluated on their ability in ordering the regression of exposure versus growth or yield response. The statistical evaluations for each of these indices were accomplished using growth/yield response data from many earlier exposure studies (e.g., NCLAN). This retrospective approach was necessary, because there were no studies specifically designed to test the goodness of fit of the various indices. The regression approach selected those indices that most properly ordered and spaced the treatment means to optimize the fit of a linear or curvilinear model. This approach provided evidence for the best indices, albeit not as defensible as that from studies with experimental designs and analyses that focus on specific components of exposure.

Most of the early retrospective studies reporting regression approaches used data from the NCLAN program or data from Corvallis, Oregon or California (Lee et al., 1987; Lee et al., 1988; Lefohn et al., 1988; Musselman et al., 1988; U.S. Environmental Protection Agency, 1992; U.S. Environmental Protection Agency, 1986). These studies were previously reviewed by the EPA (U.S. Environmental Protection Agency, 1992, 1996b) and were in general agreement that the best fit of the data were cumulative concentration-weighted exposure indices. Lee et al. (1987) suggested that exposure indices that included all the 24-h data performed better than those that
used only 7 h of data; this was consistent with the conclusions of Heagle et al. (1987) that plants receiving exposures for an additional 5-h/day showed 10% greater yield loss than those exposed for 7-h/day. In an earlier analysis using the NCLAN data, Lee et al. (1988) found the “best” exposure index was a phenologically weighted cumulative index, with sigmoid weighting on concentration and a gamma weighting function as a surrogate for plant growth stage. This index
was the best statistical fit, but it depended upon a greater knowledge of species and site conditions making specification of weighting functions difficult for general use.

The next best fits were the several indices which only cumulated and weighted higher concentrations (e.g., W126, SUM06, SUM08, AOT40). Amongst this group it was not possible to distinguish a single best fit (Heagle et al., 1994b; Lee et al., 1988; Musselman et al., 1988).

A statistical approach based on profile likelihoods was used to estimate parameters in generalized exposure indices similar to the SUM06 and AOT40 indices (Blankenship and Stefanski, 2001) using data from experiments conducted during 1993 at eight sites in the eastern United States in which O₃-sensitive and -tolerant white clover genotypes were grown using methods developed by Heagle et al. (Heagle et al., 1994b). The results showed that for the SUMX family of indices, where X is a cutoff value, hourly O₃ concentrations over ~71 ppb contribute the most to yield prediction. For the AOTX family of indices, the parameter was 54.4 ppb. These values are similar to those used in the SUM06 and AOT40 indices already in use. Furthermore, investigation of weighting for time of day confirmed the importance of the mid-afternoon hours for this data set, unlike the results found for wheat in Sweden (Danielsson et al., 2003; Pleijel et al., 2000a).

Other factors, including predisposition time (Hogsett et al., 1988; McCool et al., 1988) and crop development stage (Heagle et al., 1991; Tingey et al., 2002), contributed to variation in the biological response and suggested the need for weighting O₃ concentrations to account for predisposition time and phenology. However, the roles of predisposition and phenology in plant response vary considerably with species and environmental conditions, so specification of a weighting function for general use in characterizing plant exposure was not possible.

European scientists took a similar approach in developing indices describing growth and yield loss in crops and tree seedlings, using OTCs with modified ambient exposures, but many fewer species and study locations were employed in the European studies. There is evidence from some European studies that a lower (Pleijel et al., 1997) or higher (Finnan et al., 1996, 1997) cutoff value may provide a better statistical fit to the experimental data. Finnan et al. (1997) used seven exposure studies of spring wheat to confirm that cumulative exposure indices emphasizing higher O₃ concentrations were best related to plant response and that cumulative exposure indices using weighting functions, including cutoff concentrations, allometric and sigmoidal, provided a better fit and that the ordering of these indices differed with different
linear or Weibull dose-response models. Weighting those concentrations associated with sunshine hours in an attempt to incorporate a element of plant uptake did not improve the index performance (Finnan et al., 1997). A more recent study using data from several European studies of Norway spruce, analyzed the relationship between relative biomass accumulation and several cumulative, weighted indices, including the AOT40 and the SUM06 (Skärby et al., 2004). All the indices performed relatively well in regressing biomass and exposure index, with the AOT20 and AOT30 doing slightly better ($r^2 = 0.46-0.47$). In another comparative study of four independent data sets of potato yield and different cumulative uptake indices with different cutoff values, a similarly narrow range of $r^2$ was observed (0.3 -0.4) between the different cumulative uptake of O$_3$ indices (Pleijel et al., 2002).

In both the United States and Europe, the adequacy of these statistical summaries of exposure in relating biomass and yield changes have, for the most part, all been evaluated using data from studies not necessarily designed to compare one index to another (Lee et al., 1988, 1989; Skärby et al., 2004). But given the available data, the cumulative, concentration-weighted indices perform better than the peak or mean indices. It is not yet possible, however, to distinguish differences between the cumulative, concentration-weighted indices with direct experimental studies.

The main conclusions from the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996b) regarding an index based on ambient exposure are still valid. No information has come forth in the interim to alter those conclusions significantly, and in fact, some recent studies have further substantiated them. These key conclusions can be restated as follows:

- O$_3$ effects in plants are cumulative;
- higher O$_3$ concentrations appear to be more important than lower concentrations in eliciting a response;
- plant sensitivity to O$_3$ varies with time of day and crop development stage;
- exposure indices that accumulate the O$_3$ hourly concentrations and preferentially weight the higher concentrations have better statistical fits to growth/yield response than do the mean and peak indices.

Following the 1996 criteria review process (U.S. Environmental Protection Agency, 1996a,b), the EPA proposed an alternative form of the secondary NAAQS for O$_3$ using a cumulative, concentration-weighted exposure index to protect vegetation from damage (Federal
Register, 1997). The EPA considered three specific concentration-weighted indices: the cutoff concentration weighted SUM06 and AOT60 and the sigmoid-weighted W126 exposure index (U.S. Environmental Protection Agency, 1996a). All three indices performed equally well in predicting the exposure-response relationships observed in the crop and tree seedlings studies conducted during the previous 20 years (Lee et al., 1989). In a workshop convened to consider the science supporting these indices (Heck and Cowling, 1997), the participants agreed that all the cumulative concentration-weighted indices considered were equally capable of predicting plant response.

The cutoff concentration-weighted index AOT40 was selected for use in Europe in developing exposure-response relationships based on OTC studies of a limited number of crops and trees (Grünhage and Jäger, 2003). The United Nations Economic Commission for Europe (UNECE, 1988) adopted the critical levels approach for assessment of O₃ risk to vegetation across Europe. As used by the UNECE, the critical levels are not air quality regulatory standards in the U.S. sense, but rather planning targets for reductions in pollutant emissions to protect ecological resources. Critical levels for O₃ are intended to prevent long-term deleterious effects on the most sensitive plant species under the most sensitive environmental conditions, but not to quantify O₃ effects. A critical level was defined as “the concentration of pollutant in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems, or materials may occur according to present knowledge” (UNECE, 1988). The nature of the “adverse effects” was not specified in the original definition, which provided for different levels for different types of harmful effect (e.g., visible injury or loss of crop yield). There are also different levels for crops, forests, and seminatural vegetation. The caveat, “according to present knowledge,” is important because critical levels are not rigid; they are revised periodically as new scientific information becomes available. For example, the original critical level for O₃ specified concentrations for three averaging times, but further research and debate led to the current critical level being stated as the cumulative exposure (concentration × hours) over a cutoff concentration of 40 ppb (AOT40) (Fuhrer et al., 1997). The level of 3 ppm-h was selected, corresponding to a 5% yield loss in spring wheat as determined from 15 OTC studies. The critical level was defined for a 3-month period calculated for daylight hours. This value is currently used for all crops, because it is the best supported value and because the limited data from other crop species do not provide strong evidence that a more stringent value is required.
(Fuhrer et al., 1997). “Level I” critical levels are currently used to map and identify areas in Europe in which the levels are exceeded, and that information is then used to plan optimized and effects-based emissions abatement strategies. In the 1990s, areas of exceedance were mapped, but analyses of many exposure studies led to the conclusion that the simple, exposure-based approach led to the overestimation of effects in some regions and underestimation in others (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996). The “Level I” approach did not differentiate between plant species, and it did not include modifying site and such micrometeorological factors of $O_3$ uptake as VPD, water stress, temperature, and light and variation in canopy height.

A decision was made to work towards a flux-based approach for the critical levels (“Level II”), with the goal of modeling $O_3$ flux-effect relationships for three vegetation types: crops, forests, and seminatural vegetation (Grünhage and Jäger, 2003). Progress has been made in modeling flux (see Section AX9.4.5 (Ashmore et al., 2004a,b) and the Mapping Manual is being revised (Ashmore et al., 2004a,b; Grennfelt, 2004; Karlsson et al., 2003a). The revisions may include a flux-based approach for three crops: wheat, potatoes, and cotton. However, because of a lack of flux-response data, a cumulative, cutoff concentration-based (AOTx) exposure index will remain in use for the near future for most crops and for forests and seminatural herbaceous vegetation (Ashmore et al., 2004a).

### AX9.4.4 Identifying Exposure Components That Relate to Vegetation Effects

The efficacy of exposure indices in predicting biological responses requires that researchers identify a relationship between measured growth and/or yield effects and exposure components and those environmental and site factors that control pollutant uptake by the plant effects. A number of these relationships were identified and discussed in the 1996 $O_3$ AQCD (U.S. Environmental Protection Agency, 1996a). A significant, but in some instances, unquantified role was identified for (1) concentration; (2) duration of exposure; (3) the diurnal and seasonal patterns of exposure, e.g., time of day of peak event, season of higher exposures, seasons of high precipitation and humidity, the frequency of occurrence of peak events to respite time (peak to valley ratios); (4) plant phenology; (5) plant canopy structure; (6) meteorological and site factors, e.g., light, humidity; and (7) plant defense mechanisms.
AX9.4.4.1 Role of Concentration

A significant role of higher concentrations was established earlier, based on several experimental studies (U.S. Environmental Protection Agency, 1996b). Several studies since the last review (Nussbaum et al., 1995b; Oksanen and Holopainen, 2001; Yun and Laurence, 1999a) have added support for the important role that peak concentrations, as well as the pattern of occurrence, plays in plant response to O$_3$. Oksanen and Holopainen (2001) found that the peak concentrations and the shape of the O$_3$ exposure (i.e., duration of the event) were important determinants of foliar injury in European white birch saplings, but growth reductions were found to be more related to total cumulative exposure. Based on air quality data from 10 U.S. cities, three 4-week exposure treatments having the same SUM06 value were constructed by Yun and Laurence (1999a). They used the regimes to explore effects of treatments with variable versus uniform peak occurrence during the exposure period. The authors reported that the variable peak exposures were important in causing injury, and that the different exposure treatments, although having the same SUM06, resulted in very different patterns of foliar injury. Nussbaum et al. (1995b) also found peak concentrations and the pattern of occurrence to be critical in determining the measured response. The authors recommended that to describe the effect on total forage yield, peak concentrations $>0.11$ ppm must be emphasized by using an AOT with higher threshold concentrations.

A greater role for higher concentrations affecting plant growth might be inferred based on recent air quality analyses for the Southern California area (Lee et al., 2003; Tingey et al., 2004). In the late 1960s and 1970s, extremely high O$_3$ concentrations had impacted the San Bernardino NF. However, over the past 15 plus years, significant reductions in O$_3$ exposure have occurred in the San Bernardino National Forest (Davidson, 1993; Lee et al., 2003; Lefohn and Shadwick, 2000; Lloyd et al., 1989). An illustration of this improvement in air quality is shown by the 37-year history of O$_3$ air quality at a site in the San Bernardino Mountains (Figure AX9-17) (Lee et al., 2003). The O$_3$ exposure increased from 1963 to 1979 concurrent with increased population and vehicular miles, followed by a decline to the present mirroring decreases in precursor emissions. The pattern in exposure was evident in various exposure indices including the cumulative concentration weighted (SUM06), as well as maximum peak event (1-h peak), and the number of days having hourly averaged O$_3$ concentrations $\geq 95$ ppb (i.e., the California O$_3$ standard). The number of days having hourly averaged O$_3$ concentrations $\geq 95$ ppb
Figure AX9-17. Trends in May to September 12-h SUM06, peak 1-h O\textsubscript{3} concentration and number of daily exceedances of 95 ppb for Crestline in 1963 to 1999 in relation to trends in mean daily maximum temperature for Crestline and daily reactive organic gases (ROG) and oxides of nitrogen (NO\textsubscript{x}) for San Bernardino county. Annual ROG and NO\textsubscript{x} emissions data for San Bernardino county were obtained from Alexis et al. (2001) and the California Air Resource Board’s emission inventory available at http://www.arb.ca.gov/emisinv/emsmain/emsmain.htm.

Source: Lee et al. (2003).
declined significantly from 163 days in 1978 to 103 days in 1997. The changes in ambient O\textsubscript{3} air quality for the site were reflected in the changes in the frequency and magnitude of the peak hourly concentration and the duration of the exposure (Figure AX9-17). Considering the role of exposure patterns in determining response, the seasonal and diurnal patterns in hourly O\textsubscript{3} concentration did not vary appreciably from year to year over the 37-year period (Lee et al., 2003).

The inference for a role of higher concentrations comes both from results of ground measures of tree conditions on established plots and from results of model simulations. Across a broad area of the San Bernardino NF, the Forest Pest Management (FPM) method of injury assessment indicated an improvement in crown condition from 1974 to 1988; and the area of improvement in injury assessment is coincident with an improvement in O\textsubscript{3} air quality (Miller and Rechel, 1999). A more recent analysis of forest changes in the San Bernardino NF using an expanded network of monitoring sites has verified significant changes in growth, mortality rates, basal area, and species composition throughout the area since 1974 (Arbaugh et al., 2003).

A model simulation of Ponderosa pine growth over the 40-year period in the San Bernardino NF showed a significant impact of O\textsubscript{3} exposure on tree growth and indicates improved growth with improving O\textsubscript{3} air quality. This area has also experienced elevated N deposition and based on a number of environmental indicators, it appears that this area is experiencing N saturation (Fenn et al., 1996). To account for this potential interaction, the model simulations were conducted under conditions of unlimited soil N, which helps account for these effects. The actual interactions are not known. The improvement in growth was assigned to improved O\textsubscript{3} air quality, but no distinction was made regarding the relative role of mid-range and higher hourly concentrations, only that improved growth tracked decreasing SUM06, maximum peak concentration, and number of days of hourly O\textsubscript{3} ≥95 ppb (Tingey et al., 2004). A summary of air quality data from 1980 to 2000 for the San Bernardino NF area of the number of “mid-range” hourly concentrations indicated no dramatic changes over this 20-year period, ranging from about 1500 to 2000 hours per year (Figure AX9-18). There was a slow increase in the number of mid-range concentrations from 1980 to 1986, which corresponds to the period after implementation of the air quality standard. Another sharper increase was observed in the late 1990s. This pattern of occurrence of mid-range hourly concentrations suggests a lesser role for
these concentration ranges compared to the higher values in either of the ground-level tree injury observations of the model simulation of growth over the 40-year period.

**AX9.4.4.2 Role of Duration**

Recent studies have called into question the period of time over which concentrations are accumulated and the form of the exposure index. Heagle and Stefanski (2000) reported that the form of the exposure index was important only for 24-h indices for which SUM00 (cumulated all hourly concentrations with no concentration weighting) provided the poorest fit. The authors reported that the SUM00, SUM06, W95 (Lefohn and Runeckles, 1987), W126, and AOT40 produced similarly good fits of the foliage biomass data for 6-, 5-, and 4-h midday accumulating periods. The study pooled data from San Bernardino (CA) and Riverside (CA) with data from Amherst (MA), Corvallis (OR), Kennedy Space Center (FL), Raleigh (NC), and Blacksburg (VA). Ozone exposures were much higher at the two California sites (indicated by high W126,
SUM06, W95, and AOT40 values) compared to the other locations. Because of the pooling of the data, the large number of high hourly average O\textsubscript{3} concentrations that occurred at the California sites may have resulted in the exposure indices being highly correlated with one another and made it difficult to identify one optimal index.

In another study in California, Arbaugh et al. (1998) reported that the SUM00 exposure index performed better for describing visible injury than the SUM06, W126, number of hours ≥0.08 ppm, and the number of days between measurement periods (U.S. Environmental Protection Agency, 1996b). These exposure indices were originally developed and tested using only growth/yield data, not foliar injury (U.S. Environmental Protection Agency, 1996b). This distinction is critical in comparing the efficacy of one index to another. However, for many locations in California, a large number of higher hourly average concentrations occur; thus the SUM00 could be highly correlated with the frequency of elevated hourly average concentrations and could be a good predictor of vegetation effects.

**AX9.4.4.3 Patterns of Exposure**

A significant factor in developing exposure indices is the temporal patterns of O\textsubscript{3} occurrence over a day, a month, and a year, as well as seasonally overlaying the daily and seasonal temporal patterns of those influential climatic and site factors. The coincidence of peak O\textsubscript{3} with maximal stomatal conductance and detoxification processes is key to affecting plant growth response (Musselman and Minnick, 2000).

**Daily Patterns**

The diurnal patterns of coincidence of the maximal leaf/needle conductance and occurrence of higher ambient concentrations are relevant to the question of which hours during the day over a season that hourly concentrations should be cumulated for those indices that cumulate and weight concentration.

A 12-h daylight period for cumulating exposure was proposed following the 1996 O\textsubscript{3} AQCD based primarily on the assumption that most species probably do not have significant conductance at night (U.S. Environmental Protection Agency, 1996a). An extensive review of the literature, however, reported that a large number of species had varying degrees of nocturnal stomatal conductance (Musselman and Minnick, 2000). The role of nighttime stomatal
conductance and \(O_3\) exposure was demonstrated experimentally as well. Grulke et al. (2004) showed that the stomatal conductance at night for Ponderosa pine in the San Bernardino NF (CA) ranged from one tenth to one fourth that of maximum daytime gas exchange. In June, at the high-elevation site, 11\% of the total daily \(O_3\) uptake of pole-sized trees occurred at night. In late summer, however, \(O_3\) uptake at night was negligible. Birch seedlings exposed to \(O_3\) at night show greater reductions in growth than those exposed to \(O_3\) in daylight (Matyssek et al., 1995). Field mustard (\textit{Brassica rapa} L.) plants exposed to \(O_3\) during the day or night showed little significant difference in the amounts of injury or reduced growth response to \(O_3\) treatment, although the stomatal conductance was 70 to 80\% lower at night (Winner et al., 1989). Tissue biomass of Ponderosa pine seedlings was significantly reduced when seedlings were exposed to either daytime or nighttime episodic profiles (Lee and Hogsett, 1999). However, the biomass reductions were much greater with daytime peak concentrations than with nighttime peak concentrations.

Although stomatal conductance was lower at night than during the day for most plants, nocturnal conductance could result in some measurable \(O_3\) flux into the plants. In addition, plants might be more susceptible to \(O_3\) exposure at night than during the daytime, because of possibly lower plant defenses at night (Musselman and Minnick, 2000). Nocturnal \(O_3\) flux also depends on the level of turbulence that intermittently occurs at night. Massman (2004) suggested that nocturnal stomatal \(O_3\) uptake accounted for about 15\% of the cumulative daily effective \(O_3\) dose that was related to predicted injury. Based on a review of the literature regarding plant nocturnal stomatal conductance, Musselman and Minnick (2000) recommended that any \(O_3\) exposure index used to relate air quality to plant response should use the 24-h cumulative exposure period for both exposure-response and effective flux models. However, in an evaluation of a very large number of indices that described the \(O_3\) impact on spring wheat, Finnan et al. (1997) did not find any improvement in performance of the cumulative concentration-weighted indices by weighting those concentrations occurring during sunlight hours.

Stomatal conductances are species-dependent and linked to both diurnal and seasonal meteorological activity as well as to soil/site conditions (e.g., soil moisture). Daily patterns of leaf/needle conductance were often highest in midmorning, whereas higher ambient \(O_3\) concentrations generally occurred in early to late afternoon when stomata were often partially
closed and conductances were lower. Total O₃ flux depends on atmospheric and boundary layer resistances, both of which exhibit variability throughout the day. Recent experimental studies with tree species demonstrated the decoupling of ambient O₃ exposure, peak occurrence, and gas exchange, particularly in areas of drought (Panek, 2004). Several recent studies have suggested that Ponderosa pine trees in the southern and northern Sierra Nevada Mountains may not be as susceptible to high O₃ concentrations as to lower concentrations, due to reduced needle conductance and O₃ uptake during the period when the highest concentrations occur (Arbaugh et al., 1998; Bauer et al., 2000; Panek et al., 2002; Panek and Goldstein, 2001). Panek et al. (2002) compared direct O₃ flux measurements into a canopy of Ponderosa pine and demonstrated a lack of correlation of daily patterns of conductance and O₃ occurrence, especially in the late-season drought period; they concluded that a consideration of climate or season was essential, especially considering the role of soil moisture and conductance/uptake. In contrast, Grulke et al. (2002a) reported high conductance when O₃ concentrations were high in the same species, but under different growing site conditions. The decoupling of conductance and higher ambient O₃ concentration would hold true for more mesic environments as well as xeric landscapes. The longer-term biological responses reported by Miller and Rechel (1999) for Ponderosa pine in the same region, and the general reduction in recent years in ambient O₃ concentrations, suggest that stomatal conductance alone may not be a sufficient indicator of potential vegetation injury or damage.

The generalized models of stomatal conductance may provide a means to link patterns of O₃ occurrence with climatic and site factors that affect O₃ uptake, provided conductance is modeled by regions of similar seasonal moisture and by similar canopy structure (Emberson et al., 2000a, 2000b) (Grünhage et al., 2000) (Massman, 2004).

Seasonal Patterns

Several of the recent studies measuring O₃ flux to pine canopies also reported on the importance of seasonal patterns in relating exposure to response (Bauer et al., 2000). These seasonal patterns can be early- versus late-season occurrence of higher O₃ concentrations, reflecting climate and precursor availability. The patterns also reflected seasonal drought and the role soil moisture played in stomatal conductance and O₃ uptake. Recently, studies looked directly at this linkage. Panek et al. (2002) compared direct O₃ flux measurements into a canopy
of Ponderosa pine with a number of exposure indices and demonstrated a lack of correlation, especially in the late-season drought period; the authors concluded that a consideration of climate, especially soil moisture, was essential. They suggested that a better metric for seasonally drought-stressed forests would be one that incorporates forest physiological activity, through mechanistic modeling, by weighting ambient O\textsubscript{3} concentrations by stomatal conductance, or by weighting O\textsubscript{3} concentrations by site moisture conditions. Panek (2004) demonstrated a decoupling of O\textsubscript{3} exposure and uptake seasonally as well, via seasonal drought influence. Maximum O\textsubscript{3} uptake occurred at the beginning of the season and in the winter, whereas the pines were nearly dormant during August to September.

Using TREGRO, a process-based tree growth model, Tingey et al. (2004) simulated long-term growth of Ponderosa pine over a 37-year period. The simulation showed a high degree of association between O\textsubscript{3} exposure and O\textsubscript{3}-induced reductions in tree growth ($R^2 = 0.56$). The scatter about the line, however, indicated that other factors beside O\textsubscript{3} are required to describe the association between exposure and response. Incorporating annual temperature and precipitation increased the $R^2$ to 0.67. In keeping with the observations of Panek (2004) on the decoupling of peak O\textsubscript{3} occurrence and maximal conductance, the remaining unexplained variation is attributed to differences in timing of peak O\textsubscript{3} uptake and peak O\textsubscript{3} exposure over the years.

**AX9.4.4.4 Frequency of Occurrence of Peak Concentrations**

Several earlier studies demonstrated the greater effect of episodic occurrence of O\textsubscript{3} peaks compared to daily peak events (U.S. Environmental Protection Agency, 1996b). Since the 1996 O\textsubscript{3} AQCD, a few studies have corroborated the importance of this pattern in growth response (Köllner and Krause, 2003; Yun and Laurence, 1999a; Nussbaum et al., 1995b). Köllner and Krause (2003) reported that, under equal exposure conditions, the most pronounced effects on the yield of sugar beet (Beta vulgaris L.) and soybeans occurred with those regimes that emphasized the episodic occurrence of peak events. Similarly, Yun and Laurence (1999b) used exposure regimes constructed from 10 U.S. cities to demonstrate that variable peak occurrence versus uniform occurrence was important in causing injury in tree seedlings. Nussbaum et al. (1995b) compared the effects of different patterns of peak occurrences with similar AOT40 values and reported a stronger effect on total forage yield from the episodic treatment.
AX9.4.4.5 Canopy Structure

Another factor important in either O₃ exposure or uptake is how canopy structure affects O₃ concentration in and under forest canopies. There have been several investigations of O₃ concentrations under tree canopies (Enders, 1992; Fontan et al., 1992; Fredericksen et al., 1995; Joss and Graber, 1996; Kolb et al., 1997; Lorenzini and Nali, 1995; Neufeld et al., 1992; Samuelson and Kelly, 1997). In general, they indicated a reduction in O₃ of ~20 to 40% in the area below the canopy but above the shrub/herb layers. An essential component in the determination of the AOT40 as a critical level was the height at which the O₃ concentration was measured. The measurement heights are related to the O₃ concentration measured at the top of the canopy, i.e., upper surface boundary of the (quasi-) laminar layer (Grünhage and Jäger, 2003). This location is presumably more related to stomatal uptake. Weighting the O₃ concentration at this location takes into account stomatal opening and, if weighted with the Jarvis-Steward factors for radiation, temperature, and soil moisture, the “toxicologically” effective AOT40 is obtained (Grünhage and Jäger, 2003). A question exists however as to whether this “canopy” O₃ concentration is clearly connected to stomatal O₃ uptake. During site conditions that limit stomatal conductance (e.g., low soil moisture, high VPD) at the top of the canopy, high concentrations of O₃ can occur with minimal risk.

In a study that considered those factors important in O₃ uptake that are also spatially distributed as a result of canopy structure, Davison et al. (2003) reported that the variation in visible injury in coneflower (Rudbeckia laciniata var. digitata) populations in Great Smoky Mountains National Park was unlikely to be due to differences in O₃ flux and more likely due to variation in PAR. At a height of 50 cm above ground, PAR was reduced by almost 90%, whereas the O₃ varied from about 15 to 90% of ambient. Ozone injury was not solely related to O₃ flux. Although there have been studies of the effects of different light levels on O₃ response, there have been few at the very low levels that occur in canopies of tall herbaceous stands or in the ground layer of forests. Davison et al. (2003) reported that conductance was not related to diurnal changes in light. The O₃ levels were still about 90% of the O₃ concentration above the canopy when light was less than 5%. Light intensity dropped to 1.5% of open at 130 cm from the edge of the canopy, while O₃ dropped to only 42%. The study, although reporting on the adequacy of visible foliar injury as an indicator of O₃ effects, suggested that consideration of
other factors such as light were important in predicting response. How this might be included in developing exposure-response indices was not considered.

**AX9.4.4.6 Site and Climate Factors**

Soil moisture is a critical factor in controlling $O_3$ uptake through its effect on plant water status and stomatal conductance. In an attempt to relate uptake, soil moisture, and ambient air quality to identify areas of potential risk, available $O_3$ monitoring data for 1983 to 1990 were used along with literature-based seedling exposure-response data from regions within the southern Appalachian Mountains that might have experienced $O_3$ exposures sufficient to inhibit growth (Lefohn et al., 1997). In a small number of areas within the region, $O_3$ exposures and soil moisture availability were sufficient to possibly cause growth reductions in some $O_3$-sensitive species (e.g., black cherry). The conclusions were limited, however, because of the uncertainty in interpolating $O_3$ exposures in many of the areas and because the hydrologic index used might not reflect actual water stress.

**AX9.4.4.7 Plant Defense Mechanism - Detoxification**

The non-stomatal component of plant defenses are the most difficult to quantify, but some studies are available (Barnes et al., 2002; Chen et al., 1998; Massman and Grantz, 1995; Plöchl et al., 2000), and a larger discussion can be found in Section AX9.3. Massman et al. (2000) developed a conceptual model of a dose-based index to determine how plant injury response to $O_3$ relates to the traditional exposure-based parameters. The index used time-varying-weighted fluxes to account for the fact that flux was not necessarily correlated with plant injury or damage. Their model applied only to plant foliar injury and suggested that application of flux-based models for determining plant damage (yield or biomass) would require a better understanding and quantification of the injury and damage relationship.

**AX9.4.5 Ozone Uptake or Effective Dose as an Index**

Another approach in developing an index that relates growth response to ambient $O_3$ is based on determining the $O_3$ concentration going from the atmosphere into the leaf, or flux. Interest has been increasing in recent years, particularly in Europe, in using mathematically tractable flux models for $O_3$ assessments at the regional and national scale (Emberson et al.,
Reducing uncertainties in flux estimates for areas with diverse surface or terrain conditions to within ±50% requires “very careful application of dry deposition models, some model development, and support by experimental observations” (Wesely and Hicks, 2000). As an example, the annual average deposition velocity of O$_3$ among three nearby sites in similar vegetation was found to vary by ±10%, presumably due to terrain (Brook et al., 1997). Moreover, the authors stated that the actual variation was even greater, because stomatal uptake was unrealistically assumed to be the same among all sites, and flux is strongly influenced by stomatal conductance (Brook et al., 1997). This uptake-based approach to quantify the vegetation impact of O$_3$ requires inclusion of those factors that control the diurnal and seasonal O$_3$ flux to vegetation (e.g., climate patterns and species and/or vegetation-type factors and site-specific factors). The models have to distinguish between stomatal and non-stomatal components of O$_3$ deposition to adequately estimate actual concentration reaching the target tissue of a plant to elicit a response. Determining this O$_3$ uptake via canopy and stomatal conductance by necessity relies on models to predict flux and ultimately the “effective” flux (Grünhage et al., 2004; Massman et al., 2000; Massman, 2004). “Effective flux” has been defined as the balance between the O$_3$ flux and the detoxification process (Dämmgen et al., 1993; Grünhage and Haenel, 1997; Musselman and Massman, 1999). The time-integrated “effective flux” is termed “effective dose”. The uptake mechanisms and the resistances in this process, including stomatal conductance and biochemical defense mechanisms, are discussed in the previous Section AX9.3. The flux-based index is the goal for the “Level II” critical level for assessment of ozone risk to vegetation and ecosystems across Europe (Ashmore et al., 2004a).

**AX9.4.5.1 Models of Stomatal Conductance**

Only a limited number of studies have measured O$_3$ concentration or its reaction products within the leaf (e.g., Moldau and Bichele (2002); see Section AX9.4.3). Altimir et al. (2002) described an enclosure technique for measuring O$_3$ flux to foliage at the shoot level that allowed determination of partitioning and seasonality of the removal pathways on the foliage. The loss of O$_3$ to the wall material of the chamber was great and required a correction when the stomatal activity was low. Only a few instances of direct measures of O$_3$ flux to foliage in the field have been reported. Most measures of O$_3$ flux are from canopy measurements made with micrometeorological techniques, but a number of assumptions are necessary and there are
limitations due to landscapes (Grünhage et al., 2000; Wesely and Hicks, 2000). Comparison of simulated and measured O$_3$ flux densities show good agreement in the mean (Grünhage et al., 2000). Comparison, however, of continuous O$_3$ concentrations and fluxes measured over a 5-year period by the gradient method (Fowler et al., 1989) in a 30-year-old Norway spruce stand demonstrated a correlation over 5 years but were not correlated on a diurnal or seasonal basis. The correlation was based on two uncoupled processes inside and outside the stomata, i.e., the destruction of O$_3$ outside the stomata in the canopy was influenced by those same factors (temperature, light, humidity) that control the diurnal opening and closing of the stomata.

A similar lack of correlation of measured concentration and estimated flux, daily and seasonally, into Norway spruce and cembran pine (Pinus cembra) at six sites was due mostly to the control of stomatal conductance by those same microenvironmental factors (temperature, humidity, irradiance) (Emberson et al., 2000b). Seasonal variation of flux was attributed to the temperature course. During the growing season, the leaf-air VPD was the environmental factor controlling stomatal conductance and O$_3$ flux into the needles.

Given the limitations of actual measures of flux and the lack of correlation between measured concentrations and flux, a key goal is to develop an uptake or flux-based response index using models that consider site, climatic, meteorological, and species-specific (e.g., detoxification reactions) factors. Models of O$_3$ conductance into plant tissue are available (Grünhage et al., 1997; Massman, 1993; Wesely, 1989). The European Monitoring and Evaluation Program (EMEP) developed an O$_3$ deposition model for application across Europe in conjunction with the EMEP photochemical model as a tool for the critical levels program (Emberson et al., 2000a). The model was developed to estimate vegetation type-specific O$_3$ deposition and stomatal flux, calculated according to a standard three-resistance formulation incorporating atmospheric, boundary layer, and stomatal resistances (Emberson et al., 2000a). The model used a multiplicative algorithm of the stomatal conductance of O$_3$ (Jarvis, 1976) and has been parameterized for 10 European tree species, seven crop species, and one type of seminatural vegetation. The model calculates conductance as a function of leaf phenology, temperature, photosynthetic flux density (PFD), VPD, and soil moisture deficit (SMD). The environmental variables are site-specific (or regionally-specific). The most important factors limiting O$_3$ with this model were VPD, SMD, and phenology (Emberson et al., 2000a). These
factors demonstrate the critical linkage of high VPD and stomatal closure, which typically co­occur with high O₃ concentrations.

A number of recent model-based studies have investigated the relationship of flux and plant growth response in several crop and forest tree species (Karlsson et al., 2004a,b; Pleijel et al., 2004; Altimir et al., 2004; Bassin et al., 2004; Elvira et al., 2004; Emberson et al., 2000b; Gerosa et al., 2004; Matyssek et al., 2004; Mikkelsen et al., 2004; Soja et al., 2004; Tuovinen et al., 2004; Wieser and Emberson, 2004). The studies used earlier exposure experiments as well as explicitly designed field studies, but no clear associations emerged to provide a basis for a flux-based index. Grünhage and Jäger (2003) emphasized the need for chamber-less experiments to develop flux-effect relationship based on flux estimates at canopy height.

The complexity of using flux as an index of O₃ exposure for growth response is shown in field studies that measured O₃ flux into Norway spruce and cembran pine (Emberson et al., 2000b). They demonstrated that stomatal conductance was the main limiting factor for O₃ uptake and showed the dependence of that measure on crown position, needle age, and altitude. Consideration of the role of climate illustrates the importance of a flux measure. Pleijel et al. (2000b) reported the improved relationship of yield in spring and winter wheat grown in OTCs in many areas across Europe when it was related to the cumulative stomatal O₃ uptake during the grain-filling period. Compared to the AOT40, the cumulative uptake index estimated larger yield losses in the relatively humid parts of western and northern Europe, while smaller yield loss was estimated for the dry summer climates in southern and central Europe.

Danielsson et al. (2003) compared the ability of two different stomatal models to relate grain yield in field-grown spring wheat to cumulated O₃ uptake and an exposure index of AOT40 and found that the cumulated O₃ uptake determined with either model performed better in relating exposure to yield than did the cumulative exposure index of AOT40.

Cumulative O₃ uptake (CU) was modeled for three deciduous and two coniferous species growing at different sites and elevations and compared with the AOT40 exposure measure at these sites (Matyssek et al., 2004). A general linearity was demonstrated between the two measures of O₃ exposure, and, at any given AOT40, there was a 25 ± 11% variation in CU. Although no correlation of growth alterations was observed with either the exposure or the uptake measure, the modeled cumulative uptake was able to describe the variation in tree size and site location, making for a better measure in risk assessment of O₃ (Matyssek et al., 2004).
Karlsson et al. (2004b) compared the biomass-response relationship in young trees at seven experimental sites across Europe using modeled cumulative O\textsubscript{3} uptake and AOT40. A weaker dose-response relationship was reported for the cumulative uptake metric compared to the AOT40 (Karlsson et al., 2004b).

Concern about the complexity of the stomatal models and the data needed to model O\textsubscript{3} uptake has led some researchers to offer modified accumulated exposure indices that consider the meteorological factors controlling uptake (Gerosa et al., 2004; Karlsson et al., 2004a). In a study of subterranean clover in Austria, Belgium, and southern Sweden, Karlsson et al. (2004a) reported on the performance of a modified accumulated exposure over the threshold (mAOT) which was based on solar radiation and VPD. This index improved the relationship for observed visible injury. But when modeled uptake of O\textsubscript{3} was derived from a simple stomatal conductance model considering solar radiation, VPD, and air temperature, this index gave an even greater improvement in the relationship to visible injury than did the ambient exposure index of AOT40 (Karlsson et al., 2004a). The added value of the mAOT was worthwhile, as was its lower degree of complexity and data requirements compared to simulating O\textsubscript{3} uptake with stomatal models. Based on a study of O\textsubscript{3} fluxes over a barley (Hordeum vulgare L.) field in Italy, a similar modified exposure index was reported and referred to as “effective exposure” (Gerosa et al., 2004). Their approach was similar in its consideration of physiological aspects in conjunction with monitored O\textsubscript{3} concentrations. It also addressed the shortcomings of the data needs for modeled O\textsubscript{3} uptake.

Models that partition O\textsubscript{3} uptake into stomatal and non-stomatal components are also now available and predict a significant non-stomatal component in calculating O\textsubscript{3} flux (Altimir et al., 2004; Bassin et al., 2004; Mikkelsen et al., 2004; Nikolov and Zeller, 2003; Nussbaum et al., 2003; Zeller and Nikolov, 2000). Altimir et al. (2004) compared the relative contributions of stomatal and non-stomatal sinks at the shoot level for Scots pine. Using the EMEP model with a revised parameterization for Scots pine, they demonstrated that a major removal of O\textsubscript{3} was due to the non-stomatal component; when a non-stomatal term was introduced dependent on ambient relative humidity, the non-stomatal contribution to the total conductance was about 50%. Zeller and Nikolov (2000) demonstrated a large non-stomatal O\textsubscript{3} uptake (41% of the total annual flux) in subalpine fir at a site in southern Wyoming using the biophysical model FORFLUX. In a 5-year study of measured O\textsubscript{3} flux to a Norway spruce canopy, Mikkelsen et al. (2004) showed
monthly patterns of non-stomatal and stomatal deposition as part of total deposition to the canopy. Their study demonstrated that daily means of O$_3$ concentration and fluxes averaged over 5 years correlated well, but the correlation was based on two different noncoupled processes outside and inside the stomata. The destruction of O$_3$ in the canopy was influenced by temperature, light, and humidity, and these same factors influence stomatal opening, e.g., midday and night closure. Consequently, the diurnal O$_3$ concentration and O$_3$ flux do not correlate at all during the growing season. The study estimated yearly stomatal uptake to be a minimum of 21% of total deposition (i.e., non-stomatal uptake was as high as 80% of total). The stomatal uptake was highest May to August (30 to 33%) and lowest November to February (4 to 9%).

**AX9.4.5.2 Nonlinear Response and Developing Flux Indices**

If O$_3$ flux were used as the only metric to predict vegetation injury or damage, the prediction might be overestimated, because of nonlinear relationships between O$_3$ and plant response (Amiro et al., 1984; Amiro and Gillespie, 1985; Bennett, 1979, 1996b, 1986; U.S. Environmental Protection Agency, 1978). The nonlinearity in the response surface suggests the existence of a biochemical threshold. Musselman and Massman (1999) suggested that those species having high amounts of detoxification potential might show less of a relationship between O$_3$ stomatal uptake and plant response. More recently, nonlinear relationships between O$_3$ flux and yield were shown for potato (Pleijel et al., 2002) and spring wheat (Danielsson et al., 2003). The relationship between O$_3$ flux and potato yield led to the use of an instantaneous flux threshold to overcome the nonlinear relationship (Pleijel et al., 2002). However, the authors did not report a substantial improvement in the mathematical fitting of the model when applying the threshold. Most of the flux was accumulated below 0.06 ppm. However, Danielsson et al. (2003) showed an improved relationship between O$_3$ uptake and yield of spring wheat using a threshold of 5 nmoles m$^{-2}$ sec$^{-1}$ (0.24 mg m$^{-2}$ sec$^{-1}$). These results suggest not all O$_3$ entering the stomata contribute to a reduction in yield, which depends to some degree on the amount of internal detoxification occurring for each particular species (see Section AX9.2). The cellular detoxification reactions and repair processes which both detoxify oxidants as well as play central roles in the carbon economy of the plant are another level of resistance to O$_3$ reaching the target tissue (see Section AX9.2). The magnitude of the response is
determined by the amount of the pollutant reaching the target site and the ability of the plant to reestablish homeostatic equilibrium. Thus, one would expect to observe a decoupling of O$_3$ uptake with vegetation effects, which would manifest as a nonlinear relationship between O$_3$ flux and injury or damage.

Additional factors for inclusion in flux-based models to predict vegetation effects would be the defense and repair mechanisms. However, the fact that the defense and repair mechanisms vary diurnally as well as seasonally may make it extremely difficult to apply a mathematically determined threshold to instantaneous flux measurements to calculate cumulative flux. The threshold models do not allow for the temporal (i.e., daily and seasonal) variability of defense mechanisms. Specifically, the relationship between conductance, O$_3$ concentration, and defense/repair mechanisms needs to be included. Recently, Massman (2004) illustrated that the combination of stomatal conductance, O$_3$ concentration, and diurnal variation of defense mechanisms showed the daily maximum potential for plant injury (based on effective dose) coincided with the daily peak in O$_3$ mixing ratio. Massman et al. (2000) stressed that the product of the overlapping mathematical relationships of conductance, concentration, and defense mechanisms results in a much different picture of potential impact to vegetation than just the use of conductance and concentration in predicting vegetation effects.

**AX9.4.5.3 Simulation Models**

Another approach for determining O$_3$ uptake and relating growth response to ambient O$_3$ exposure may be the use of physiologically-based simulation models. Several of these have been used in various contexts, comparing O$_3$ response in a number of tree species with varying climate and site factors (e.g., soil moisture) (Hogsett et al., 1997; Laurence et al., 2001; Ollinger et al., 1997, 1998; Weinstein et al., 2001, 2002). These process-based models provide for an integration of species, climate, and site factors controlling O$_3$ uptake with long-term growth. One of the important considerations in applying simulation modeling is to carefully assess the uncertainties associated with the modeling predictions. Further efforts need to be made to exercise the models so that they predict past growth losses associated with changes in O$_3$ exposures that can be verified with on-the-ground surveys.
AX9.4.6 Summary

A large number of studies pertinent to the development of exposure indices have been published since 1996, and these are predominantly focused on the development of a flux-based index to relate ambient O$_3$ to effects. There were only a few such studies prior to 1996 and these were reviewed in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996b). The few studies published since 1996 on the role of O$_3$ exposure components (including concentration, duration, and exposure patterns) in describing growth response to O$_3$ exposures have substantiated earlier conclusions of the importance of higher concentration, shape of the peak, and the episodicity of peak occurrence in the plant response to O$_3$ exposure. An inferred role of peak concentrations is possible from consideration of improved O$_3$ air quality in regions such as the San Bernardino Mountains in southern California. Studies provide the basis for focusing on the higher O$_3$ concentrations, while including the lower levels, when estimating the effects of emission reductions on vegetation.

A few studies have demonstrated the potential disconnection of the temporal patterns of peak events and maximal stomatal conductance. In addition, a few other studies have demonstrated the uptake of O$_3$ during nighttime hours, suggesting the need to cumulate O$_3$ exposure 24 h per day and not just during daylight hours.

Several studies since 1996 have demonstrated another critical concern in developing an index for exposure. The concern is that peak O$_3$ events and maximum stomatal conductance may be temporally separate. This disconnection introduces uncertainty in assessing O$_3$ impact when using the current ambient exposure based cumulative, concentration-weighted indices. If stomatal conductance is relatively low, as in the late afternoon in arid climates, and that is the same time as the peak O$_3$ concentrations, then use of an exposure index that does not consider this disconnect will overestimate the effect of the exposure. This concern is especially apparent when assessing the impact of O$_3$ across all the varied climatic regions of the United States or Europe. Some studies use stomatal models to predict uptake (Ashmore et al., 2004a) or physiological process-based models (Laurence et al., 2001) to integrate those species, climate, and site factors that drive this temporal pattern of stomatal conductance and exposure, and thus reduce some of the uncertainty in regional and national assessments of effects. These approaches, however, are still limited by being species-dependent.
The results of these studies and reviews indicate the need to continue to develop indices that are more physiologically and meteorologically connected to the actual dose of O$_3$ the plant receives. The cumulative concentration-weighted exposure indices are acknowledged surrogates for effective dose and are simple conceptually and easy to measure. They do not fully characterize the potential for plant uptake and resulting effects associated with O$_3$, because the indices, being measures of ambient concentration, do not include the physical, biological, and meteorological processes controlling the transfer of O$_3$ from the atmosphere through the leaf and into the leaf interior (U.S. Environmental Protection Agency, 1996b). Use of such indices is especially limited in spatial risk characterizations, because of the lack of linkage between meteorology and species- and site-specific factors influencing O$_3$ uptake. The flux-based approach should provide an opportunity to improve upon the concentration-based (i.e., exposure indices) approach. A cautionary argument was advanced in a few publications centered around the nonlinear relationship between O$_3$ uptake and plant injury (not growth alteration) response. The concern was that not all O$_3$ stomatal uptake results in a reduction in yield, which depends to some degree on the amount of internal detoxification occurring with each particular species; species having high amounts of detoxification potential may show less of a relationship between O$_3$ stomatal uptake and plant response.

The European approach and acceptance of flux-based critical values is a recognition of this problem; a concerted research effort is needed to develop the necessary experimental data and modeling tools that will provide the scientific basis for such critical levels for O$_3$ (Dämmgen et al., 1994; Fuhrer et al., 1997; Grünhage et al., 2004).

At this time, based on the current state of knowledge, exposure indices that differentially weight the higher hourly average O$_3$ concentrations but include the mid-level values represent the best approach for relating vegetation effects to O$_3$ exposure in the United States. A large database exists that has been used for establishing exposure-response relationships. Such a database does not yet exist for relating O$_3$ flux to growth response. The pattern disconnects between period of uptake and peak occurrence, as well as the potential for nocturnal uptake, should be considered by adding some weighting functions into the currently used exposure indices. Of particular consideration would be their inclusion in regional-to-national estimations of O$_3$ impacts on vegetation. Another useful approach to regional assessment for certain species
is to simulate growth effects with process-based models that account for seasonal climate and site factors that control conductance.

It is anticipated that, as the overlapping mathematical relationships of conductance, concentration, and defense mechanisms are better defined, O₃-flux-based models may be able to predict vegetation injury and/or damage at least for some categories of canopy-types with more accuracy than the exposure-response models.

**AX9.5 OZONE EXPOSURE-PLANT RESPONSE RELATIONSHIPS**

**AX9.5.1 Introduction**

Ambient O₃ concentrations have long been known to cause visible symptoms, decreases in photosynthetic rates, decreases in plant growth, and decreases in the yield of marketable organs (U.S. Environmental Protection Agency, 1978, 1986, 1996). Yet, despite considerable research in the U.S. and other countries during the past three decades, quantifying the effects of ambient O₃ exposure on vegetation remains a challenge. Numerous studies have related O₃ exposure to plant responses, with most effort focused on the yield of crops and the growth of tree seedlings. Most experiments exposed individual plants grown in pots or soil under controlled conditions to known concentrations of O₃ for a segment of daylight hours for some portion of the plant’s life span (Section AX9.1). The response of a plant species or variety to O₃ exposure depends upon many factors discussed in previous sections, including genetic characteristics (Section AX9.3.2), biochemical and physiological status (Section AX9.3), and previous and current exposure to other stressors (Sections AX9.3, AX9.4). Section AX9.3 describes how O₃ moves from the atmosphere into the leaf and the subsequent biochemical and physiological responses of plants. The current section focuses on the quantitative responses of plants to seasonal or multiyear exposures to known amounts of O₃. Quantitative responses include foliar symptoms and decreased growth of whole plants or decreased harvestable portions of them. Because of the available information, most of this section focuses on the response of individual plants, especially crop plants and tree seedlings, with limited discussion of mixtures of herbaceous species. The responses of natural ecosystems are discussed in Section AX9.6.

This section will pay particular attention to studies conducted since the publication of the 1996 AQCD (U.S. Environmental Protection Agency, 1996). However, because much O₃
research was conducted prior to the 1996 AQCD, the present discussion of vegetation response to \( \text{O}_3 \) exposure is largely based on the conclusions of the 1978, 1986, and 1996 criteria documents (U.S. Environmental Protection Agency, 1978, 1986, 1996). To provide a context for the discussion of recent research, the key findings and conclusions of those three documents are summarized below.

**AX9.5.2 Summary of Key Findings/Conclusions from Previous Criteria Documents**

Experimental data reviewed in the 1978 and 1986 \( \text{O}_3 \) AQCDs dealt primarily with the effects of \( \text{O}_3 \) on agricultural crop species (U.S. Environmental Protection Agency, 1978, 1986). The chapter on vegetation effects in the 1978 \( \text{O}_3 \) AQCD (U.S. Environmental Protection Agency, 1978) emphasized foliar symptoms and growth effects, but not those effects that affected yield, an emphasis dictated by the kind of data available at the time. The 1986 \( \text{O}_3 \) AQCD reviewed a substantial new body of evidence based on OTC experiments (see Section AX9.1) showing that ambient \( \text{O}_3 \) exposures reduced the growth and yield of herbaceous plants, again with a focus on major crop species. In the 1986 and 1996 \( \text{O}_3 \) AQCDs, data were presented from regression studies conducted to develop exposure-response functions for estimating yield loss of major crop species in different regions of the United States. The 1996 \( \text{O}_3 \) AQCD included results from additional herbaceous crop species as well as shrub and tree species. For a number of tree species, biomass growth of seedlings was related to growing season \( \text{O}_3 \) exposures to produce response functions for estimating \( \text{O}_3 \) exposures that reduce growth by 10 or 30%. Also, in the 1986 and 1996 \( \text{O}_3 \) AQCDs, data from studies using EDU as a protectant were reviewed. The 1978, 1986, and 1996 \( \text{O}_3 \) AQCDs also reviewed data on the response to \( \text{O}_3 \) exposures of forest ecosystems in the San Bernardino Mountains of southern California (U.S. Environmental Protection Agency, 1978, 1986, 1996). Because this region is exposed to high concentrations of \( \text{O}_3 \) and has shown evidence of ecosystem-level changes, it remains an important study area (see Section AX9.6).

Ozone can cause a range of effects, beginning with individual cells, leaves, and plants, and proceeding to plant populations and communities. These effects may be classified as either “injury” or “damage”. Injury encompasses all plant reactions, such as reversible changes in plant metabolism (e.g., altered photosynthetic rate), altered plant quality, or reduced growth that
does not impair yield or the intended use or value of the plant (Guderian, 1977). In contrast, damage includes all effects that reduce or impair the intended use or value of the plant. Damage includes reductions in aesthetic values as well as losses in terms of weight, number, or size of the plant part that is harvested (yield loss). Yield loss also may include changes in crop quality, i.e., physical appearance, chemical composition, or the ability to withstand storage. Losses in aesthetic values are difficult to quantify. Although foliar symptoms cannot always be classified as damage, their occurrence indicates that phytotoxic concentrations of O$_3$ are present, and, therefore, studies should be conducted to assess the risk to vegetation.

Visible symptoms due to O$_3$ exposures reduce the market value of certain crops and ornamentals for which leaves are the product, e.g., spinach, petunia, geranium, and poinsettia. The concept of limiting values used to summarize foliar symptoms in the 1978 O$_3$ AQCD (U.S. Environmental Protection Agency, 1978) was also considered valid in the 1986 O$_3$ AQCD (U.S. Environmental Protection Agency, 1986). Jacobson (1977) developed limiting values by assessing the available scientific literature and identifying the lowest exposure concentration/duration reported to cause foliar symptoms in a variety of plant species. Graphical analyses presented in those documents indicated that the limit for reduced plant performance was an exposure to 50 ppb for several hours per day for more than 16 days. Decreasing the exposure period to 10 days increased the concentration required to cause symptoms to 100 ppb; and a short, 6-day exposure further increased the concentration required to cause symptoms to 300 ppb. These limiting values established in 1978 were still deemed appropriate in the 1986 and 1996 O$_3$ AQCDs. Such foliar symptoms are caused by O$_3$ concentrations that occur in the United States as shown in Table AX9-13 (adapted from U.S. Environmental Protection Agency, 1996).

The 1986 O$_3$ AQCD emphasized that, although foliar symptoms on vegetation are often an early and obvious manifestation of O$_3$ exposure, O$_3$ effects are not limited to foliar symptoms. Other effects include reduced growth of many organs (including roots), changes in crop quality, and alterations in plant susceptibility to biotic stressors and sensitivity to abiotic stressors. The 1986 O$_3$ AQCD also emphasized that O$_3$ exerts phytotoxic effects only if a sufficient amount of O$_3$ reaches sensitive sites within the leaf (Section AX9.2). Ozone injury will not occur if the rate of O$_3$ uptake is low enough that the plant can detoxify or metabolize O$_3$ or its metabolites or if the plant is able to repair or compensate for the effects (Tingey and Taylor, 1982; U.S.
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<td>18.4%</td>
<td>124.5</td>
<td>19.4%</td>
<td>36.2</td>
<td>63.8%</td>
<td>37.0</td>
<td>50.3%</td>
</tr>
<tr>
<td>1986</td>
<td>118</td>
<td>0.048</td>
<td>20.3%</td>
<td>123.3</td>
<td>21.4%</td>
<td>34.9</td>
<td>70.7%</td>
<td>35.6</td>
<td>55.7%</td>
</tr>
<tr>
<td>1987</td>
<td>116</td>
<td>0.050</td>
<td>20.3%</td>
<td>128.7</td>
<td>20.4%</td>
<td>42.2</td>
<td>62.0%</td>
<td>41.8</td>
<td>50.3%</td>
</tr>
<tr>
<td>1988</td>
<td>134</td>
<td>0.054</td>
<td>18.7%</td>
<td>141.7</td>
<td>22.0%</td>
<td>58.0</td>
<td>50.5%</td>
<td>55.6</td>
<td>45.0%</td>
</tr>
<tr>
<td>1989</td>
<td>158</td>
<td>0.047</td>
<td>18.6%</td>
<td>127.8</td>
<td>22.5%</td>
<td>32.7</td>
<td>87.8%</td>
<td>35.2</td>
<td>64.1%</td>
</tr>
<tr>
<td>1990</td>
<td>172</td>
<td>0.049</td>
<td>19.8%</td>
<td>129.4</td>
<td>22.7%</td>
<td>34.6</td>
<td>82.7%</td>
<td>37.0</td>
<td>62.1%</td>
</tr>
<tr>
<td>1991</td>
<td>190</td>
<td>0.050</td>
<td>19.8%</td>
<td>130.6</td>
<td>23.6%</td>
<td>36.8</td>
<td>80.7%</td>
<td>38.8</td>
<td>62.9%</td>
</tr>
<tr>
<td><strong>Among Years</strong></td>
<td></td>
<td><strong>0.049</strong></td>
<td><strong>9.8%</strong></td>
<td><strong>129.0</strong></td>
<td><strong>9.9%</strong></td>
<td><strong>38.7</strong></td>
<td><strong>42.5%</strong></td>
<td><strong>39.6</strong></td>
<td><strong>29.8%</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup> Updated and additional years from data given in Table III of Tingey et al. (1991), where the spatial and temporal variation in ambient O₃ exposures is expressed in terms of several exposure indices.

<sup>b</sup> Indicates the number of separate monitoring sites included in the analysis; fewer sites had 5 months of available data than had 3 months of available data.

<sup>c</sup> The 2HDM index is calculated for sites with at least 3 months of available data. SUM00, SUM06, M7, SIGMOID, and 2HDM are the cumulative sum above 0.0 ppm, the cumulative sum above 0.06 ppm, the 7-h seasonal mean, the sigmoid weighted summed concentration, and the second highest daily maximum 1-h concentration, respectively.

<sup>d</sup> CV = coefficient of variation.

Environmental Protection Agency, 1986). Cellular disturbances that are not repaired or compensated for are ultimately expressed as foliar symptoms, reduced root growth, or reduced yield of fruits or seeds.

Beginning in the 1986 O₃ AQCD and continuing in the 1996 O₃ AQCD, OTC studies that better quantified the relationship between O₃ exposure and effects on crop species were reviewed, with a focus on yield loss. These studies can be grouped into two types, depending on the experimental design and statistical methods used to analyze the data: (1) studies that developed predictive equations relating O₃ exposure to plant response, and (2) studies that compared the effects of discrete treatment level(s) to a control. The advantage of the regression approach is that exposure-response models can be used to interpolate results between treatment levels.

Discrete treatment experiments were designed to test whether specific O₃ treatments were different from the control rather than to develop exposure-response equations, and the data were analyzed using analyses of variance. When summarizing these studies using discrete treatment levels, the lowest O₃ concentration that significantly reduced yield was determined from analyses done by the original authors. Often, the lowest concentration used in a study was the lowest concentration reported to reduce yield; hence, it was not always possible to estimate a no-effect exposure concentration. In general, the data indicated that 100 ppb O₃ (frequently the lowest concentration used in the studies) for a few hours per day for several days to several weeks usually caused significant yield reductions of 10 to 50%.

By the time the 1986 O₃ AQCD was prepared, much new information concerning the effects of O₃ on the yield of crop plants had become available through EPA’s NCLAN research program and through research funded by other agencies. The NCLAN project was initiated by the EPA in 1980 primarily to improve estimates of yield loss under field conditions and to estimate the magnitude of crop losses caused by O₃ throughout the United States (Heck et al., 1982; Heck et al., 1991). The cultural conditions used in the NCLAN studies approximated typical agronomic practices. The primary objectives were:

1. to define relationships between yields of major agricultural crops and O₃ exposure as required to provide data necessary for economic assessments and development of O₃ NAAQS;

2. to assess the national economic consequences resulting from O₃ exposure of major agricultural crops; and
(3) to advance understanding of cause-and-effect relationships that determine crop responses to pollutant exposures.

Using NCLAN data, the O$_3$ concentrations predicted to cause 10 or 30% yield loss were estimated using linear or Weibull response functions. The data in Table AX9-14 are from the 1996 document and were based on yield-response functions for 38 species or cultivars developed from studies using OTCs of the type developed by Heagle et al. (1973) (see Section AX9.1). Composite exposure-response functions for both crops and tree seedlings as a function of O$_3$ exposure expressed as SUM06 are shown in Figure AX9-19. Review of these data indicate that 10% yield reductions could be predicted for more than 50% of experimental cases when:

1. 12-h SUM06 values exceeded 24.4 ppm·h,
2. SIGMOID values exceeded 21.5 ppm·h, or
3. 7-h seasonal mean concentrations were 50 ppb. The SIGMOID index is very similar to the W126 index (see Section AX9.4 for further information about O$_3$ indices). Much lower values are required for each index to protect 75% of experimental cases (Table AX9-14). Grain crops were generally found to be less sensitive than other crops. The data summarized in the 1996 criteria document also indicated that the variation in sensitivity within species may be as great as differences between species.

The chemical protectant, EDU, was also used to provide estimates of yield loss. The impact of O$_3$ on yield was determined by comparing the yield data from plots treated with EDU versus untreated plots. Studies indicated that yields were reduced by 18 to 41% when daytime ambient O$_3$ concentrations exceeded 80 ppb for 5 to 18 days over the growing season. For this approach to be credible, the effects of EDU itself on a particular species must be preestablished under conditions without O$_3$ exposure (Kostka-Rick and Manning, 1992a).

The 1996 O$_3$ AQCD reviewed several experiments demonstrating that the seedlings of some tree species such as poplars (*Populus* spp.) and black cherry are as sensitive to O$_3$ as are annual plants, in spite of the fact that trees are longer-lived and generally have lower rates of gas exchange, and, therefore, a lower uptake of O$_3$. The 1996 document also reviewed data showing that O$_3$ exposures that occur at present in the United States are sufficient to affect the growth of a number of trees species. For example, exposure-response functions for 51 cases of tree seedling responses to O$_3$, including 11 species representing deciduous and evergreen growth habits, suggest that a SUM06 exposure for 5 months of 31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases studied (Table AX9-15). Similarly, a SUM06 exposure of

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Table AX9-14. Ozone Exposure Levels (Using Various Indices) Estimated to Cause at Least 10% Crop Loss in 50 and 75% of Experimental Cases

<table>
<thead>
<tr>
<th>50th PERCENTILE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SUM06</th>
<th>SE&lt;sup&gt;c&lt;/sup&gt;</th>
<th>SIGMOID</th>
<th>SE</th>
<th>M7</th>
<th>SE</th>
<th>2HDM</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCLAN Data (n = 49; wet and dry)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24.4</td>
<td>3.4</td>
<td>21.5</td>
<td>2.0</td>
<td>0.049</td>
<td>0.003</td>
<td>0.094</td>
<td>0.006</td>
</tr>
<tr>
<td>NCLAN Data (n = 39; wet only)</td>
<td>22.3</td>
<td>1.0</td>
<td>19.4</td>
<td>2.3</td>
<td>0.046</td>
<td>0.003</td>
<td>0.090</td>
<td>0.010</td>
</tr>
<tr>
<td>NCLAN Data (n = 54; wet and dry)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>26.4</td>
<td>3.2</td>
<td>23.5</td>
<td>2.4</td>
<td>NA</td>
<td>NA</td>
<td>0.099</td>
<td>0.011</td>
</tr>
<tr>
<td>NCLAN Data (n = 42; wet only)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>23.4</td>
<td>3.1</td>
<td>22.9</td>
<td>4.7</td>
<td>NA</td>
<td>NA</td>
<td>0.089</td>
<td>0.008</td>
</tr>
<tr>
<td>NCLAN Data (n = 10; wet)</td>
<td>25.9</td>
<td>4.5</td>
<td>23.4</td>
<td>3.2</td>
<td>0.041</td>
<td>0.001</td>
<td>0.110</td>
<td>0.042</td>
</tr>
<tr>
<td>NCLAN Data (n = 10; dry)</td>
<td>45.7</td>
<td>23.3</td>
<td>40.6</td>
<td>0.1</td>
<td>0.059</td>
<td>0.014</td>
<td>0.119</td>
<td>0.017</td>
</tr>
<tr>
<td>Cotton Data (n = 5)</td>
<td>23.6</td>
<td>2.3</td>
<td>19.3</td>
<td>2.3</td>
<td>0.041</td>
<td>0.001</td>
<td>0.066</td>
<td>0.032</td>
</tr>
<tr>
<td>Soybean Data (n = 13)</td>
<td>26.2</td>
<td>5.4</td>
<td>22.6</td>
<td>3.6</td>
<td>0.044</td>
<td>0.005</td>
<td>0.085</td>
<td>0.013</td>
</tr>
<tr>
<td>Wheat Data (n = 6)</td>
<td>21.3</td>
<td>15.2</td>
<td>19.3</td>
<td>12.7</td>
<td>0.061</td>
<td>0.018</td>
<td>0.098</td>
<td>0.059</td>
</tr>
<tr>
<td>Cotton Data (n = 5)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>30.0</td>
<td>12.7</td>
<td>27.2</td>
<td>12.8</td>
<td>NA</td>
<td>NA</td>
<td>0.075</td>
<td>0.012</td>
</tr>
<tr>
<td>Soybean Data (n = 15)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>23.9</td>
<td>6.5</td>
<td>22.0</td>
<td>8.0</td>
<td>NA</td>
<td>NA</td>
<td>0.088</td>
<td>0.008</td>
</tr>
<tr>
<td>Wheat Data (n = 7)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>25.9</td>
<td>10.5</td>
<td>21.4</td>
<td>9.4</td>
<td>NA</td>
<td>NA</td>
<td>0.097</td>
<td>0.028</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>75th PERCENTILE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SUM06</th>
<th>SE&lt;sup&gt;c&lt;/sup&gt;</th>
<th>SIGMOID</th>
<th>SE</th>
<th>M7</th>
<th>SE</th>
<th>2HDM</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCLAN Data (n = 49; wet and dry)</td>
<td>14.2</td>
<td>4.2</td>
<td>11.9</td>
<td>5.6</td>
<td>0.040</td>
<td>0.007</td>
<td>0.051</td>
<td>0.010</td>
</tr>
<tr>
<td>NCLAN Data (n = 39; wet only)</td>
<td>14.3</td>
<td>2.7</td>
<td>12.6</td>
<td>2.3</td>
<td>0.039</td>
<td>0.005</td>
<td>0.056</td>
<td>0.006</td>
</tr>
<tr>
<td>NCLAN Data (n = 54; wet and dry)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16.5</td>
<td>4.3</td>
<td>14.5</td>
<td>3.2</td>
<td>NA</td>
<td>NA</td>
<td>0.073</td>
<td>0.006</td>
</tr>
<tr>
<td>NCLAN Data (n = 42; wet only)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>17.2</td>
<td>3.0</td>
<td>14.7</td>
<td>2.4</td>
<td>NA</td>
<td>NA</td>
<td>0.070</td>
<td>0.006</td>
</tr>
<tr>
<td>NCLAN Data (n = 10; wet)</td>
<td>16.4</td>
<td>3.7</td>
<td>13.7</td>
<td>3.2</td>
<td>0.040</td>
<td>0.001</td>
<td>0.080</td>
<td>0.032</td>
</tr>
<tr>
<td>NCLAN Data (n = 10; dry)</td>
<td>24.0</td>
<td>0.8</td>
<td>22.3</td>
<td>0.1</td>
<td>0.053</td>
<td>0.022</td>
<td>0.093</td>
<td>0.003</td>
</tr>
<tr>
<td>Cotton Data (n = 5)</td>
<td>21.8</td>
<td>5.0</td>
<td>17.5</td>
<td>2.8</td>
<td>0.041</td>
<td>0.001</td>
<td>0.065</td>
<td>0.014</td>
</tr>
<tr>
<td>Soybean Data (n = 13)</td>
<td>14.2</td>
<td>0.1</td>
<td>12.4</td>
<td>0.1</td>
<td>0.041</td>
<td>0.006</td>
<td>0.069</td>
<td>0.004</td>
</tr>
<tr>
<td>Wheat Data (n = 6)</td>
<td>11.7</td>
<td>2.5</td>
<td>10.9</td>
<td>2.4</td>
<td>0.054</td>
<td>0.032</td>
<td>0.062</td>
<td>0.035</td>
</tr>
<tr>
<td>Cotton Data (n = 5)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>21.1</td>
<td>6.0</td>
<td>16.7</td>
<td>5.7</td>
<td>NA</td>
<td>NA</td>
<td>0.070</td>
<td>0.034</td>
</tr>
<tr>
<td>Soybean Data (n = 15)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>15.3</td>
<td>4.1</td>
<td>13.4</td>
<td>4.1</td>
<td>NA</td>
<td>NA</td>
<td>0.078</td>
<td>0.007</td>
</tr>
<tr>
<td>Wheat Data (n = 7)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.1</td>
<td>2.6</td>
<td>8.5</td>
<td>3.4</td>
<td>NA</td>
<td>NA</td>
<td>0.054</td>
<td>0.027</td>
</tr>
</tbody>
</table>

<sup>a</sup>See Appendix A for abbreviations and acronyms.

<sup>b</sup>The numbers in parentheses are the number of cases used in deriving the various exposure levels.

<sup>c</sup>Standard error (SE).

<sup>d</sup>NCLAN data refers to studies conducted as part of the NCLAN project. Wet and dry refer to watering regimes used in the studies, wet being well-watered, and dry meaning some level of drought stress was imposed.

<sup>e</sup>24-h exposure statistics reported in Lee et al. (1994b). Relative yield loss for 2HDM is relative to yield at 40 ppb rather than 0 ppb as was used in Tingey et al. (1991).

Figure AX9-19. Distribution of biomass loss predictions from Weibull and linear exposure-response models that relate biomass to \( \text{O}_3 \) exposure. Exposure is characterized with the 24-h SUM06 statistic using data from (a) 31 crop studies from National Crop Loss Assessment Network (NCLAN) and (b) 26 tree seedling studies conducted at U.S. Environmental Protection Agency’s Environmental Research Laboratory in Corvallis, OR; Smoky Mountains National Park, TN; Houghton, Michigan; and Delaware, Ohio. Separate regressions were calculated for studies with multiple harvests or cultivars, resulting in a total of 54 individual equations from the 31 NCLAN studies and 56 equations from the 26 seedling studies. Each equation was used to calculate the predicted relative yield or biomass loss at 10, 20, 30, 40, 50, and 60 ppm-h, and the distributions of the resulting loss were plotted. The solid line is the calculated Weibull fit at the 50th percentile.

Source: U.S. Environmental Protection Agency (1996); Hogsett et al. (1995).
Table AX9-15. SUM06 Levels Associated with 10 and 20% Total Biomass Loss for 50 and 75% of the Seedling Studies
(The SUM06 value is adjusted to an exposure length of 92 days per year.)

### Weibull Equations (all 51 seedling studies):

- 50th Percentile PRYL$^b = 1 - \exp(-[\text{SUM06}/176.342]^{1.34962})$
- 75th Percentile PRYL = 1 - \exp(-[\text{SUM06}/104.281]^{1.46719})

### Weibull Equations (27 fast-growing seedling studies):

- 50th Percentile PRYL = 1 - \exp(-[\text{SUM06}/150.636]^{1.43220})
- 75th Percentile PRYL = 1 - \exp(-[\text{SUM06}/89.983]^{1.49261})

### Weibull Equations (24 slow-to-moderate growing seedling studies):

- 50th Percentile PRYL = 1 - \exp(-[\text{SUM06}/190.900]^{1.49986})
- 75th Percentile PRYL = 1 - \exp(-[\text{SUM06}/172.443]^{1.14634})

### Weibull Equations (28 deciduous seedling studies):

- 50th Percentile PRYL = 1 - \exp(-[\text{SUM06}/142.709]^{1.48845})
- 75th Percentile PRYL = 1 - \exp(-[\text{SUM06}/87.724]^{1.53324})

### Weibull Equations (23 evergreen seedling studies):

- 50th Percentile PRYL = 1 - \exp(-[\text{SUM06}/262.911]^{1.23673})
- 75th Percentile PRYL = 1 - \exp(-[\text{SUM06}/201.372]^{1.01470})

<table>
<thead>
<tr>
<th>Levels Associated with Prevention of a 10 and 20% Total Biomass Loss for 50 and 75% of the Seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All 51 Seedling Cases</strong></td>
</tr>
<tr>
<td>Percent of Seedlings</td>
</tr>
<tr>
<td>50%</td>
</tr>
<tr>
<td>Relative 10%</td>
</tr>
<tr>
<td>Biomass Loss 20%</td>
</tr>
<tr>
<td><strong>27 Fast-Growing Seedling Cases</strong></td>
</tr>
<tr>
<td>Percent of Seedlings</td>
</tr>
<tr>
<td>50%</td>
</tr>
<tr>
<td>Relative 10%</td>
</tr>
<tr>
<td>Biomass Loss 20%</td>
</tr>
</tbody>
</table>
Table AX9-15 (cont’d). SUM06 Levels Associated with 10 and 20% Total Biomass Loss for 50 and 75% of the Seedling Studies (The SUM06 value is adjusted to an exposure length of 92 days per year.)

24 Slow-to-Moderate-Growth Seedling Cases

<table>
<thead>
<tr>
<th>Percent of Seedlings</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative 10%</td>
<td>42.6</td>
<td>24.2</td>
</tr>
<tr>
<td>Biomass Loss 20%</td>
<td>70.2</td>
<td>46.6</td>
</tr>
</tbody>
</table>

28 Deciduous Seedling Cases

<table>
<thead>
<tr>
<th>Percent of Seedlings</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative 10%</td>
<td>31.5</td>
<td>20.2</td>
</tr>
<tr>
<td>Biomass Loss 20%</td>
<td>52.1</td>
<td>33</td>
</tr>
</tbody>
</table>

23 Evergreen Seedling Cases

<table>
<thead>
<tr>
<th>Percent of Seedlings</th>
<th>50%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative 10%</td>
<td>42.6</td>
<td>21.9</td>
</tr>
<tr>
<td>Biomass Loss 20%</td>
<td>78.2</td>
<td>45.9</td>
</tr>
</tbody>
</table>

*See Appendix A for abbreviations and acronyms.

PRYL = predicted relative yield (biomass) loss


42.6 ppm·h should provide the same level of protection for evergreen seedlings. However, these results do not take into the account the possibility of effects on growth in subsequent years. Because multiple-year exposures may cause a cumulative effect on the growth of some trees (Simini et al., 1992; Temple et al., 1992), it is likely that a number of species are currently being affected even at ambient exposures (Table AX9-13).

In 1986, the EPA (U.S. Environmental Protection Agency, 1986) established that 7-h per day growing season mean exposures to O₃ concentrations above 50 ppb were likely to cause measurable yield loss in agricultural crops. At that time, few conclusions could be drawn about the response of deciduous or evergreen trees or shrubs, due to the lack of information about

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response of such plants to season-long exposures to \( \text{O}_3 \) concentrations of 40 to 60 ppb and above. However, the 1978 and 1986 \( \text{O}_3 \) AQCDs (U.S. Environmental Protection Agency, 1978, 1986) indicated that the limiting value for foliar symptoms on trees and shrubs was 60 to 100 ppb for 4 h. From 1986 to 1996, extensive research was conducted, establishing the sensitivity of many tree species. Based on research published since the 1986 \( \text{O}_3 \) AQCD (U.S. Environmental Protection Agency, 1986), a number of conclusions were drawn in 1996 \( \text{O}_3 \) AQCD (U.S. Environmental Protection Agency, 1996):

1. An analysis of 10 years of monitoring data from more than 80 to almost 200 nonurban sites in the United States established ambient 7-h growing season average concentrations of \( \text{O}_3 \) for 3 or 5 months of 51 to 60 ppb and 47 to 54 ppb, respectively. The SUM06 exposures ranged (a) from 24.8 to 45.2 ppm·h for 3 months and (b) from 32.7 to 58.0 ppm·h for 5 months (Tingey et al. (1991), Table AX9-13).

2. The results of OTC studies that compared yields at ambient \( \text{O}_3 \) exposures with those in filtered air and retrospective analyses of crop data (Table AX9-14) established that ambient \( \text{O}_3 \) concentrations were sufficient to reduce the yield of major crops in the United States. Research results since 1978 did not invalidate EPA conclusions (U.S. Environmental Protection Agency, 1978, 1986) that foliar symptoms due to \( \text{O}_3 \) exposures reduce the market value of certain crops and ornamentals where leaves are the product (such as spinach, petunia, geranium, and poinsettia) and that such damage occurs at ambient \( \text{O}_3 \) concentrations observed in the United States.

3. A 3-month SUM06 exposure of 24.4 ppm·h, corresponding to a 7-h mean of 49 ppb and a 2HDM of 94 ppb \( \text{O}_3 \) may prevent a 10% loss in 50% of the 49 experimental cases analyzed by Tingey et al. (1991). A 12-h growing season mean of 0.045 ppb should restrict yield losses to 10% in major crop species (Lesser et al., 1990).

4. Depending on duration, concentrations of \( \text{O}_3 \) and SUM06 exposures currently in the United States are sufficient to affect the growth of a number of tree species. Given the fact that multiple-year exposures may cause a cumulative effect on the growth of some trees (Simini et al., 1992; Temple et al., 1992), it is likely that a number of species currently are being impacted, even at ambient \( \text{O}_3 \) exposures (Tables AX9-13 and AX9-20).

5. Exposure-response functions for 51 cases of seedling response to \( \text{O}_3 \) (Hogsett et al., 1995), including 11 species representing deciduous and evergreen growth habits, suggest that a SUM06 exposure for 5 months of 31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases studied. A SUM06 exposure of 42.6 ppm·h should provide the same level of protection for evergreen seedlings. Note that these conclusions do not take into the account the possibility of effects on growth in subsequent years, an important consideration in the case of long-lived species.
Studies of the response of trees to O$_3$ have established that, in some cases (for instance, poplars and black cherry), trees are as sensitive to O$_3$ as are annual plants, in spite of the fact that trees are longer-lived and generally have lower gas exchange rates, and, therefore, lower O$_3$ uptake.

Use of the chemical protectant, EDU, is of value in estimating O$_3$-related losses in crop yield and tree growth, provided that care is exercised in establishing appropriate EDU dosages to protect the plants without affecting growth.

The major question to be addressed in the remainder of this section is whether new information supports or alters the 1996 criteria document conclusions summarized above. In particular, this section evaluates whether the response of plants to experimental treatments at or near O$_3$ concentrations characteristic of ambient levels in many areas of the United States (Tables AX9-13 and AX9-20) can be compared to a control or reduced O$_3$ treatment to establish a potential adverse effect. Before evaluating new information from the literature on O$_3$ effects on vegetation, O$_3$ exposure indices used in O$_3$ studies and trends in O$_3$ exposure patterns during the past two decades are briefly reviewed.

**AX9.5.3 Ozone Indices and Ambient Exposure**

As recognized in both the 1986 and the 1996 criteria documents, the characterization and representation of the exposure of vegetation to O$_3$ is problematic, because the specific aspects of pollutant exposure that cause injury or damage are difficult to quantify. This issue was addressed in Section AX9.4, and only a few points will be discussed here in order to provide a context for interpreting data on exposure-response relationships. The most important effects of O$_3$ on vegetation occur due to uptake of O$_3$ through stomata, with subsequent oxidative injury that appears to be rather nonspecific (Section AX9.2). As has been discussed by numerous authors during the last three decades, from a toxicological and physiological view, it is much more realistic to relate effects to internal (absorbed) O$_3$ dose rather than to exposure near the leaf or canopy (Fowler and Cape, 1982; Fuhrer et al., 1992; Grünhage et al., 1993, 1999; Legge et al., 1995; Massman et al., 2000; Musselman and Massman, 1999; Pleijel et al., 1995b; Runeckles, 1974; Taylor et al., 1982; Tingey and Taylor, 1982) (see also Section AX9.4). Theoretically, flux estimates should improve the assessment of O$_3$ effects, but despite recent attention to this topic, particularly in Europe, it remains difficult to estimate flux in the field outside of
experimental sites where continuous measurements of wind speed and other environmental conditions are made. This topic is discussed further below in Section AX9.5.4.5.

No simple exposure index can accurately represent all of the numerous factors operating at different timescales that affect O<sub>3</sub> flux into plants and subsequent plant response (Section AX9.4). Indices of peaks, such as the 2HDM, are not well suited for discerning exposure-response relationships, because they do not capture the effects of lower O<sub>3</sub> concentrations nor the cumulative effects of O<sub>3</sub> on vegetation (Heck and Cowling, 1997; U.S. Environmental Protection Agency, 1996). Therefore, peak indices have not been used in recent decades to develop exposure-response relationships for vegetation. Fortunately, other simple indices have shown substantial correlation with responses such as crop yield under experimental conditions. During the 1980s, the most commonly used indices for expressing O<sub>3</sub> exposure were 7-, 8-, or 12-h daytime average values over the duration of O<sub>3</sub> exposure, which was often 3 months or somewhat less for experimental studies with crops. These indices perform reasonably well for interpreting experimental data on the response of vegetation to O<sub>3</sub>, particularly for individual experiments, although they do not explain all of the variation among experiments in retrospective analysis of multiple experiments (Lesser et al., 1990).

Since the 1980s, cumulative indices such as the SUM06, AOT40, or W126 that preferentially weight higher concentrations have been used in conjunction with mean indices for developing exposure-response relationships (Tables AX9-14 and AX9-15, and Figure AX9-18). Such indices are often more suitable than mean values, because they are cumulative and because they preferentially weight higher concentrations. Thus, these indices generally provide somewhat better fits to experimental data than do mean indices, especially in retrospective analyses of multiple experiments on multiple species (Lee et al., 1994a; Lee et al., 1994b; Lee and Hogsett, 1999; Tingey et al., 1991). Unfortunately, no single index has been used consistently even in the recent literature, making it difficult to compare results among and between experiments and with ambient exposure data. However, Tables AX9-13 and AX9-20 provide summaries of ambient exposure data for several indices that can be compared to the experimental results reviewed in the remainder of this section. Of the cumulative indices that preferentially weight higher concentrations, the SUM06 index has been used most commonly in the U.S. literature, and it was selected in a meeting of scientific experts on O<sub>3</sub> effects on vegetation as suitable for a secondary standard to protect vegetation (Heck and Cowling, 1997).
However, it should be noted that the W126 index has been selected for use in protecting vegetation in Class 1 areas (Federal Land Manager's Air Quality Related Values Workgroup (FLAG), 2000). Even in recent studies, O$_3$ data are often presented using only a seasonal mean index value, and so mean values are frequently presented in this section. Such reporting of mean indices should not be interpreted as a preference for them, but rather as a limitation in the data reported in the literature. Additional information about O$_3$ exposure for individual experiments, including the number and type of O$_3$ treatments (addition of a constant concentration of O$_3$ or an amount proportional to ambient levels), and duration, are reported in Tables AX9-16 through AX9-19.

Since the 1996 O$_3$ AQCD, the use of the AOT40 index has become quite common in Europe for identifying and mapping areas of exceedance, but it has not been used much in the United States. Thus, studies reporting O$_3$ exposure only as AOT40 values are presented in tables summarizing effects on annual, herbaceous perennial, and woody vegetation. However, such studies are not as commonly cited in the text of this section, because AOT40 summary data on O$_3$ exposures in the United States are rarely available. This lack makes it difficult to compare experimentally derived exposure-response data expressed as AOT40 to ambient U.S. O$_3$ exposures. The development of critical levels in Europe has been based primarily on the AOT40 index, so this index is discussed in that context.

In addition to peak weighting, there is also evidence that the timing of exposure during plant growth is important. For example, the greatest effects on grain yield are due to exposure during grain filling, rather than earlier or later in the growing season (Lee et al., 1988; Pleijel et al., 1998; Soja et al., 2000; U.S. Environmental Protection Agency, 1996; Younglove et al., 1994). A recent study grew bush bean in OTCs with CF or above-ambient O$_3$ using exposure dynamics typical of the Midwestern United States for three time periods: (1) the entire season, (2) the period prior to anthesis, (3) during pod filling and maturation (Tingey et al., 2002). Ozone exposure prior to anthesis reduced growth by less than 1% per ppm·h (SUM06) while exposure during pod filling and maturation reduced growth by 4 to 7%. A meta-analysis of 53 studies of O$_3$ effects on soybean found that O$_3$ had greater effects with increases in developmental stage, with the greatest effect during seed filling (Morgan et al., 2003). The importance of respite times was discussed in the previous criteria documents (U.S. Environmental Protection Agency, 1978, 1986, 1996) but remains difficult to quantify.
<table>
<thead>
<tr>
<th>Species</th>
<th>Facility</th>
<th>Location</th>
<th>O₃ Concentration (units are ppb unless otherwise specified)¹</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean, cv. Pros</td>
<td>OTC</td>
<td>The Netherlands</td>
<td>CF to CF75: 9-h mean = 3-70, AOT40 = 0 to 17.7 ppm•h</td>
<td>62 days</td>
<td>Green pod yield</td>
<td>29 at 9-h mean = 44 (AOT40 = 3.6 ppm•h)</td>
<td>Tonneijck and Van Dijk (1998)</td>
</tr>
<tr>
<td>Bean, cv. Lit</td>
<td>OTC</td>
<td>Germany</td>
<td>CF, NF, CF-1×, CF-2×: mean = 1, 14, 15, 32</td>
<td>3 months</td>
<td>Pod yield</td>
<td>56 (CF, 2×)</td>
<td>Brunschon-Harti et al. (1995)</td>
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<tr>
<td>Bean, cv. Bush</td>
<td>OTC</td>
<td>Corvallis, OR</td>
<td>CF, +O₃: SUM06 = 0.0, 75.7 or 68.4 ppm•h; AOT40 = 0.0, 50.9 or 46.4 ppm•h; 7-h mean = 7, 89 or 85 (early and late season experiments)</td>
<td>63-65 days</td>
<td>Pod dry weight</td>
<td>51, 57 (early and late season experiments)</td>
<td>Tingey et al. (2002)</td>
</tr>
<tr>
<td>Bean, cvs.</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, 1.4×: 12-h mean = 23, 72</td>
<td>1 year</td>
<td>Pod dry weight</td>
<td>n.s. for Tenderette, 90 for S156</td>
<td>Heagle et al. (1999b)</td>
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<td>Tenderette, S156</td>
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<tr>
<td>Bean, cvs.</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, AA: 12-h mean = 31, 51, 49 in year 2000; 25, 46, 47 in year 2001</td>
<td>2 years</td>
<td>Pod dry weight</td>
<td>n.s. for R123 n.s. for Oregon-91 in 2001, 27 in 2001; 21, 45 for S156</td>
<td>Heagle et al. (1999b)</td>
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<td>R123, Oregon-91, S156</td>
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<tr>
<td>Corn</td>
<td>OTC</td>
<td>Beltsville, MD</td>
<td>CF, +40: 7-h mean = 20, 70</td>
<td>1 year</td>
<td>Grain yield</td>
<td>13</td>
<td>Mulchi et al. (1995) Rudorff et al. (1996c)</td>
</tr>
<tr>
<td>Cotton, cv.</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, 1.5×: 12-h mean = 21, 71</td>
<td>1 year</td>
<td>Seed-cotton weight</td>
<td>22</td>
<td>Heagle et al. (1999b)</td>
</tr>
<tr>
<td>Deltapine</td>
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<tr>
<td>Cotton, cv.</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 24, 51, 78</td>
<td>1 year</td>
<td>Seed-cotton weight</td>
<td>21, 49 (NF, 1.5×)</td>
<td>Heagle et al. (1999b)</td>
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<td>Deltapine</td>
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<tr>
<td>Oat, cv. Vital</td>
<td>OTC</td>
<td>Ostad, Sweden</td>
<td>CF, NF: 7-h mean = 12, 27</td>
<td>1 year</td>
<td>Grain yield</td>
<td>+2 (n.s.)</td>
<td>Pleijel et al. (1994a)</td>
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Table AX9-16 (cont’d). Summary of Selected Studies of Ozone Effects on Annual Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Facility</th>
<th>Location</th>
<th>O₃ Concentration (units are ppb unless otherwise specified)¹</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Potato, cvs. Superior, Dark Red Norland</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 15, 45, 80</td>
<td>1 year</td>
<td>Tuber yield</td>
<td>15, 31 for Norland in NF and 1.5x; 11 for Superior in 1.5x</td>
<td>Heagle et al. (1999b)</td>
</tr>
<tr>
<td>Potato²</td>
<td>OTC</td>
<td>6 sites N. Europe</td>
<td>AOT40 = 6-27 ppm•h</td>
<td>2 years (1 year at 2 sites)</td>
<td>Tuber yield</td>
<td>4% average for all experiments</td>
<td>Craigon et al. (2002)</td>
</tr>
<tr>
<td>Rape, oilseed</td>
<td>Open Air</td>
<td>Northumberland, UK</td>
<td>AA, +O₃: 7-h mean for 17 days Aug.-Sept = 30, 77, for 32 days in May-June = 31, 80</td>
<td>17 days in fall, overwinter, 32 days in spring</td>
<td>Seed yield</td>
<td>14</td>
<td>Ollerenshaw et al. (1999)</td>
</tr>
<tr>
<td>Rice, cvs. Koshi-hikari, Nippon-bare</td>
<td>OTC</td>
<td>Japan</td>
<td>CF, 1×, 1.5×, 2×, 2.75×: 7-h mean = 13.5-93.4</td>
<td>3 years</td>
<td>Grain yield</td>
<td>3 to 10 at 40 ppb</td>
<td>Kobayashi et al. (1995)</td>
</tr>
<tr>
<td>Soybean</td>
<td>OTC</td>
<td>Beltsville, MD</td>
<td>CF, +40: 7-h mean = 25, 72</td>
<td>2 years</td>
<td>Seed yield</td>
<td>25</td>
<td>Mulchi et al. (1995)</td>
</tr>
<tr>
<td>Soybean, cv. Essex</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, 1.5×: 12-h mean for 3 years = 23, 82</td>
<td>3 years</td>
<td>Seed yield</td>
<td>41</td>
<td>Fiscus et al. (1997)</td>
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<tr>
<td>Soybean, cvs. Forrest, Essex</td>
<td>OTC</td>
<td>Maryland</td>
<td>CF, +40: 7-h mean = 24 and 24, 63 and 62 for each year</td>
<td>2 years</td>
<td>Seed yield</td>
<td>10, 32 (2 cvs.)</td>
<td>Chernikova et al. (2000)</td>
</tr>
<tr>
<td>Soybean, cv. Essex</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 20, 50, 79</td>
<td>1 year</td>
<td>Seed yield</td>
<td>16, 37 (NF, 1.5×)</td>
<td>Heagle et al. (1998b)</td>
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<tr>
<td>Soybean, cv. Essex</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 18, 42, 69</td>
<td>1 year</td>
<td>Seed yield</td>
<td>15, 40 (NF, 1.5×)</td>
<td>Heagle et al. (1998b)</td>
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<td>Soybean, cv. Holladay</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 18, 42, 69</td>
<td>1 year</td>
<td>Seed yield</td>
<td>22, 36 (NF, 1.5×)</td>
<td>Heagle et al. (1998b)</td>
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<tr>
<td>Soybean, cv. NK-6955</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 18, 42, 69</td>
<td>1 year</td>
<td>Seed yield</td>
<td>+46, +4 (NF, 1.5×)</td>
<td>Heagle et al. (1998b)</td>
</tr>
<tr>
<td>Soybean, 3 cvs.</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 14, 36, 64</td>
<td>3 months</td>
<td>Seed yield</td>
<td>At ambient = +14, 11, 16 for 3 cvs.</td>
<td>Miller et al. (1994)</td>
</tr>
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</table>

¹ Concentrations are given in parts per billion (ppb).
<table>
<thead>
<tr>
<th>Species</th>
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<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Soybean, 3 cvs.</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 24, 49, 83</td>
<td>4 months</td>
<td>Seed yield</td>
<td>At ambient = 17, 13, 18 (3 cvs.)</td>
<td>Miller et al. (1994)</td>
</tr>
<tr>
<td>Soybean, cv. Essex</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>CF, NF, 1.5×: 12-h mean = 20, 50, 79</td>
<td>4 months</td>
<td>Seed yield</td>
<td>11, 22 (amb., 1.5×)</td>
<td>Miller et al. (1998)</td>
</tr>
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<td>Soybean, cvs, Essex, Forrest</td>
<td>OTC</td>
<td>Beltsville, MD</td>
<td>CF, NF+: 7-h mean = 24, 58</td>
<td>134 days</td>
<td>Seed yield</td>
<td>Essex = +11 (n.s.), Forrest = 21</td>
<td>Robinson and Britz (2000)</td>
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<tr>
<td>Soybean, cv. 93B15</td>
<td>FACE</td>
<td>Urbana-Champaign, IL</td>
<td>AA, +O₃ in 2002 8-h mean = 62, 75; SUM06 = 36, 70 ppm•h; AOT40 = 22, 42 ppm•h; in 2003 8-h mean = 50, 63; SUM06 = 22, 53 ppm•h; AOT40 = 14, 34 ppm•h</td>
<td>2 expts of 1 year</td>
<td>Seed yield</td>
<td>15, 25 (2002, 2003)</td>
<td>Morgan et al. (in press)</td>
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<td>Timothy</td>
<td>OTC</td>
<td>Sweden</td>
<td>AOT40 = 10, 20, 340; 12-h mean = 20, 152</td>
<td>1 year</td>
<td>Biomass</td>
<td>58</td>
<td>Danielsson et al. (1999)</td>
</tr>
<tr>
<td>Watermelon</td>
<td>OTC</td>
<td>Spain</td>
<td>CF (O₃ = 0), NF in 1988 AOT40 = 5.96 ppm•h, SUM06 = 0.29 ppm•h, AOT40 = 18.92 ppm•h, SUM06 = 4.95 ppm•h</td>
<td>2 expts of 1 year</td>
<td>Fruit yield</td>
<td>19, 39 (2 expts)</td>
<td>Gimeno et al. (1999)</td>
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<tr>
<td>Wheat¹, cv. Minaret</td>
<td>OTC</td>
<td>8 sites in N Europe</td>
<td>12-h mean (SD) low = 26.3 (12.2), 12-h mean (SD)-high = 51.4 (18.3)</td>
<td>13 studies of 1 year each</td>
<td>Grain yield</td>
<td>13 (n.s.)</td>
<td>Bender et al. (1999)</td>
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<td>AOT40 mean (SD) low = 6.18 (8.54) ppm•h, AOT40 mean (SD) high = 28.23 (23.05) ppm•h</td>
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<td>Hertstein et al. (1999)</td>
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<th>Species</th>
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<th>Location</th>
<th>O$_3$ Concentration (units are ppb unless otherwise specified)$^1$</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat$^1$</td>
<td>OTC</td>
<td>Sweden</td>
<td>AOT40 0 to 15 ppm$h$</td>
<td>7 years</td>
<td>Grain yield</td>
<td>23 at AOT40 = 15 ppm$h$</td>
<td>Danielsson et al. (2003)</td>
</tr>
<tr>
<td>Wheat, cv. Promessa</td>
<td>OTC</td>
<td>SE Ireland</td>
<td>CF, +50:  12-h total = 5.6, 32.6 ppm$h$</td>
<td>3 h/day, 5 days/week, 7 weeks</td>
<td>Grain yield</td>
<td>53</td>
<td>Finnan et al. (1996)</td>
</tr>
<tr>
<td>Wheat, cv. Promessa</td>
<td>OTC</td>
<td>SE Ireland</td>
<td>CF, +25:  12-h total = 6.2, 33.4 ppm$h$</td>
<td>6 h/day, 5 days/week, 7 weeks</td>
<td>Grain yield</td>
<td>+17</td>
<td>Finnan et al. (1996)</td>
</tr>
<tr>
<td>Wheat, cv. Promessa</td>
<td>OTC</td>
<td>SE Ireland</td>
<td>CF, +25, +50: 12-h total = 6.7, 34, 34 ppm$h$</td>
<td>+25 = 6 h/day, 5 days/week, +50 = 3 h/day, 5 days/week, both 7 weeks</td>
<td>Grain yield</td>
<td>Amb + 25 = 3 (n.s.); Amb + 50 = 17</td>
<td>Finnan et al. (1996)</td>
</tr>
<tr>
<td>Wheat, cvs. Massey, Saluda</td>
<td>OTC</td>
<td>Beltsville, MD</td>
<td>CF, +40: 7-h mean = 19, 20 and 61, 65 (2 years)</td>
<td>2 years</td>
<td>Grain yield</td>
<td>20</td>
<td>Mulchi et al. (1995) Rudorff et al. (1996b)</td>
</tr>
<tr>
<td>Wheat, cv. Turbo</td>
<td>OTC</td>
<td>Germany</td>
<td>8-h mean = 5.9, 61.2, 92.5</td>
<td>1 year</td>
<td>Grain yield</td>
<td>14, 40</td>
<td>Bender et al. (1994)</td>
</tr>
<tr>
<td>Wheat, cv. Turbo</td>
<td>OTC</td>
<td>Germany</td>
<td>8-h mean = 4.7, 86.4</td>
<td>1 year</td>
<td>Grain yield</td>
<td>20</td>
<td>Bender et al. (1994)</td>
</tr>
<tr>
<td>Wheat, cv. Turbo</td>
<td>OTC</td>
<td>Germany</td>
<td>7-h mean = 5, 41, 73</td>
<td>1 year</td>
<td>Grain yield</td>
<td>35</td>
<td>Fangmeier et al. (1994)</td>
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<tr>
<td>Wheat, winter, 8 cvs</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>12-h mean = 27, 47, 90</td>
<td>2 months</td>
<td>Grain yield</td>
<td>5 (n.s.)</td>
<td>Heagle et al. (2000)</td>
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<tr>
<td>Wheat, winter, 8 cvs</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>12-h mean = 22, 38, 74</td>
<td>2 months</td>
<td>Grain yield</td>
<td>16 (n.s.)</td>
<td>Heagle et al. (2000)</td>
</tr>
</tbody>
</table>

$^1$ Units are ppb unless otherwise specified.
### Table AX9-16 (cont’d). Summary of Selected Studies of Ozone Effects on Annual Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Facility</th>
<th>Location</th>
<th>O&lt;sub&gt;3&lt;/sub&gt; Concentration (units are ppb unless otherwise specified)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat, cv. Drabant</td>
<td>OTC</td>
<td>Finland</td>
<td>1992: 12-h mean = 14, 30, 61; AOT40 = 16.3, 34.8, 54.6 ppm-h. 1993: 12-h mean = 9, 21, 45; AOT40 = 10.2, 24.8, 40.6 ppm-h</td>
<td>2 years</td>
<td>Grain yield</td>
<td>At highest O&lt;sub&gt;3&lt;/sub&gt; = 13 each year</td>
<td>Ojanpera et al. (1998)</td>
</tr>
<tr>
<td>Wheat, cv. Riband</td>
<td>Open Air</td>
<td>Northumberland, UK</td>
<td>AOT40 for Mar to Aug 93 = 3.5, 6.2 ppm-h</td>
<td>1 year, overwinter</td>
<td>Grain yield</td>
<td>13</td>
<td>Ollerenshaw and Lyons (1999)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values for ambient or NF treatments are indicated in bold.

<sup>2</sup> Bold indicates that multiple experiments (more than just 2 years at a single site) were included in the analysis.
<table>
<thead>
<tr>
<th>Species</th>
<th>Facility</th>
<th>Location</th>
<th>O₃ Concentration (units are ppb unless otherwise specified)¹</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa, cvs. Apica, Team</td>
<td>OTC</td>
<td>Quebec, Canada</td>
<td>12-h mean: 1991 = 6, 39, 49, 110; 1992 = 0, 34, 42, 94</td>
<td>3 months</td>
<td>Biomass</td>
<td>For NF: Apica = 31, 21; Team = 14, 2 (n.s.)</td>
<td>Renaud et al. (1997)</td>
</tr>
<tr>
<td>Bahia grass</td>
<td>OTC</td>
<td>Auburn, AL</td>
<td>12-h mean = 22, 45, 91</td>
<td>24 weeks</td>
<td>Biomass at ambient O₃ for 1st, 2nd cutting of early and late season plantings.</td>
<td>34, 29 (n.s.), +6 (n.s.), 9 (n.s.)</td>
<td>Muntifinger et al. (2000)</td>
</tr>
<tr>
<td>Bent grass (Capillaris sp.)</td>
<td>OTC</td>
<td>United Kingdom</td>
<td>AOT40 = 0.8-15.0 ppm-h</td>
<td>8 h/day for 3 months</td>
<td>Biomass, in competition with 3 other spp. Total biomass in uncut pots, aboveground biomass in cut pots (cut every 14 days).</td>
<td>8 (uncut), +18 (cut)</td>
<td>Ashmore and Ainsworth (1995)</td>
</tr>
<tr>
<td>Clover, white</td>
<td>Ambient air</td>
<td>MA, OR, NC, CA (2 sites) and VA</td>
<td>SUM06 for 6-h/day = 10.2-39.4 ppm•h, AOT40 for 12-h/day = 0.6-50.1 ppm•h</td>
<td>2 growing seasons</td>
<td>Biomass ratio (sensitive/resistant)</td>
<td>4 at 6-h SUM06 = 39.4 ppm•h; 12 h AOT40 = 50.1 ppm•h</td>
<td>Heagele and Stefanski (2000)</td>
</tr>
<tr>
<td>Clover, white</td>
<td>Ambient air</td>
<td>14 European sites</td>
<td>AOT40 for 28 d = 0-12 ppm-h</td>
<td>3 growing seasons</td>
<td>Biomass ratio (sensitive/resistant)</td>
<td>5 at AOT40 for 28 days = 0.9-1.7 ppm•h</td>
<td>Mills et al. (2000)</td>
</tr>
<tr>
<td>Clover, white</td>
<td>OTC</td>
<td>Switzerland</td>
<td>CF, NF, NF++, NF+++; 12-h mean = 21, 39, 47, 65</td>
<td>3.5 months/ year for 2 years</td>
<td>Biomass, in managed pasture</td>
<td>24, 26, 52</td>
<td>Fuhrer et al. (1994)</td>
</tr>
<tr>
<td>Clover, white and red</td>
<td>OTC</td>
<td>Italy</td>
<td>CF, NF: AOT40 = 0.1; 8.9 ppm-h, 7-h mean = 24, 53</td>
<td>2 months</td>
<td>Biomass</td>
<td>20</td>
<td>Fumagalli et al. (1997)</td>
</tr>
</tbody>
</table>

¹ Units are ppb unless otherwise specified.
Table AX9-17 (cont’d). Summary of Selected Studies of the Effects of Ozone on Perennial Herbaceous Plants

<table>
<thead>
<tr>
<th>Species</th>
<th>Facility</th>
<th>Location</th>
<th>O₃ Concentration (units are ppb unless otherwise specified)¹</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fescue, red</td>
<td>OTC</td>
<td>United Kingdom</td>
<td>AOT₄₀ = 0.8-15.0 ppm-h</td>
<td>8 h/day for 3 months</td>
<td>Biomass, in competition with 3 other spp. Total biomass in uncut pots, aboveground biomass in cut pots (cut every 14 days).</td>
<td>+30 (uncut), +13 (cut)</td>
<td>Ashmore and Ainsworth (1995)</td>
</tr>
<tr>
<td>Lespedeza, Sericea</td>
<td>OTC</td>
<td>Auburn, AL</td>
<td>CF, NF, 2×: 12-h mean = 23, 40, 83, SUM₀₆ = 0.2, 9.1, 61.0, AOT₄₀ = 0.6, 7.0, 39.8</td>
<td>10 weeks</td>
<td>Biomass</td>
<td>n.s.</td>
<td>Powell et al. (2003)</td>
</tr>
<tr>
<td>Little bluestem</td>
<td>OTC</td>
<td>Auburn, AL</td>
<td>CF, NF, 2×: 12-h mean = 23, 40, 83 ppb, SUM₀₆ = 0.2, 9.1, 61.0, AOT₄₀ = 0.6, 7.0, 39.8</td>
<td>10 weeks</td>
<td>Biomass</td>
<td>n.s.</td>
<td>Powell et al. (2003)</td>
</tr>
<tr>
<td>Phleum alpinum</td>
<td>OTC</td>
<td>Sweden</td>
<td>AOT₄₀ = 0.01, 0.02, 0.34 ppm-h; 12-h mean = 20, 152</td>
<td>1 year</td>
<td>biomass</td>
<td>87</td>
<td>Danielsson et al. (1999)</td>
</tr>
<tr>
<td>Speedwell, Germander</td>
<td>OTC</td>
<td>United Kingdom</td>
<td>AOT₄₀ = 0.8-15.0 ppm-h</td>
<td>8 h/day for 3 months</td>
<td>Biomass, in competition with 3 other spp. Total biomass in uncut pots, aboveground biomass in cut pots (cut every 14 days).</td>
<td>14 (uncut), 26 (cut)</td>
<td>Ashmore and Ainsworth (1995)</td>
</tr>
<tr>
<td>Strawberry</td>
<td>OTC</td>
<td>United Kingdom</td>
<td>8-h mean = 27, 92; AOT₄₀ for +O₃ = 24.59 ppm-h</td>
<td>69 days</td>
<td>Fruit size, yield</td>
<td>Size = 14, yield = (n.s.)</td>
<td>Drogoudi and Ashmore (2000)</td>
</tr>
<tr>
<td>Sumac, winged</td>
<td>OTC</td>
<td>Alabama</td>
<td>SUM₀₆ = 0 to 132 ppm-h</td>
<td>6 months</td>
<td>Percent canopy cover (grown in old field community)</td>
<td>95</td>
<td>Barbo et al. (1998)</td>
</tr>
<tr>
<td>Timothy</td>
<td>OTC</td>
<td>Sweden</td>
<td>CF, NF, CF+: AOT₄₀ = 0.0, 1.3, 20.3 ppm-h; 12-h mean = 20, 68, 152</td>
<td>1 year</td>
<td>Biomass</td>
<td>n.s. in NF, 58 in CF+</td>
<td>Danielsson et al. (1999)</td>
</tr>
</tbody>
</table>

¹ Values for ambient or NF treatments are indicated in bold.

² Bold indicates that multiple experiments (more than just 2 years at a single site) were included in the analysis.
<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Facility</th>
<th>Location</th>
<th>O&lt;sub&gt;3&lt;/sub&gt; Concentration (units are ppb unless otherwise specified)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash, European</td>
<td>OTC</td>
<td>Hampshire, UK</td>
<td>NF, NF+: Mean = 17.7, 44.1; AOT40 for 24h = 1.9, 59.9 ppm-h</td>
<td>3 years for day 100 to day 162</td>
<td>Growth and biomass of organs</td>
<td>n.s.</td>
<td>Broadmeadow and Jackson (2000)</td>
<td></td>
</tr>
<tr>
<td>Ash, European</td>
<td>Seedling</td>
<td>OTC</td>
<td>Switzerland</td>
<td>0.5×, 0.85×, 1×, 0.5×+30: AOT40 = 0.1, 3.4, 7.1, 19.7 ppm-h</td>
<td>5 months</td>
<td>Biomass</td>
<td>26 at 1×, 50 at 0.5× + 30</td>
<td>Landolt et al. (2000)</td>
</tr>
<tr>
<td>Aspen</td>
<td>Cutting, Seedling</td>
<td>OTC</td>
<td>Michigan</td>
<td>CF, 1×, 2×: 3 months 7-h mean for 1990 =7, 43, 63 ; for 1991 (square wave exposure) = 11, 45, 66</td>
<td>98 days</td>
<td>Total biomass of 3 clones and seedlings</td>
<td>For 1990: 2-22 at 1x for clones (mean = 16), 14 for seedlings. For 1991: 23-39 at 1x for clones (mean = 30)</td>
<td>Karnosky et al. (1996)</td>
</tr>
<tr>
<td>Aspen</td>
<td>Cutting</td>
<td>OTC</td>
<td>Michigan</td>
<td>CF, 1×, 2×: SUM00 = 11, 58, 71 ppm-h</td>
<td>98 days</td>
<td>Total biomass</td>
<td>25-38 at 1×</td>
<td>Dickson et al. (2001)</td>
</tr>
<tr>
<td>Aspen</td>
<td>Cutting</td>
<td>FACE</td>
<td>Wisconsin</td>
<td>Ambient, 1.5×: 4-year ambient 12-h mean = 34.6, 36.9, 36.0, 36.6; 4-year 1.5× 12-h mean = 54.5, 51.1, 48.9, 52.8</td>
<td>7 years</td>
<td>Volume (d2*h)</td>
<td>21 after 3 years, 14 after 7 years at 1.5×</td>
<td>Isebrands et al. (2001)</td>
</tr>
<tr>
<td>Aspen</td>
<td>Cutting</td>
<td>Large OTC</td>
<td>New York</td>
<td>1×, 1.7×, 3×: SUM06 = 1, 20, 62 ppm-h; 9-h mean = 40, 74, 124</td>
<td>92 days</td>
<td>Shoot biomass</td>
<td>14, 25 for 2 clones at 1.7×</td>
<td>Yun and Laurence (1999a)</td>
</tr>
<tr>
<td>Aspen</td>
<td>First year</td>
<td>OTC</td>
<td>Pennsylvania</td>
<td>8-h mean = 39, 73</td>
<td>11 weeks</td>
<td>Biomass</td>
<td>14-30 for 3 of 6 N treatments</td>
<td>Pell et al. (1995)</td>
</tr>
<tr>
<td>Beech, European</td>
<td>OTC</td>
<td>Switzerland</td>
<td>0.5×, 0.85×, 1×, 0.5×+30: AOT40 = 0.1, 3.4, 7.1, 19.7 ppm-h</td>
<td>5 months</td>
<td>Biomass</td>
<td>6 at 1×, 30 at 0.5×+30</td>
<td>Landolt et al. (2000)</td>
<td></td>
</tr>
<tr>
<td>Beech, European</td>
<td>Seedling</td>
<td>OTC</td>
<td>Belgium</td>
<td>CF, NF, +30: 8-h mean = 5.29, 33; AOT40 = 0.406, 8.88 ppm-h</td>
<td>23 April - 30 Sept</td>
<td>Growth</td>
<td>No effect</td>
<td>Bortier et al. (2000c)</td>
</tr>
</tbody>
</table>
### Table AX9-18 (cont’d). Summary of Selected Studies of Ozone Effects on Deciduous Trees and Shrubs

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Facility</th>
<th>Location</th>
<th>**O₃ Concentration (units are ppb unless otherwise specified)**¹</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beech, European</td>
<td>Seedling</td>
<td>Growth chamber</td>
<td>Belgium</td>
<td>CF, CF+40, CF+100: Sum0 = 0.48, 8.93, 25.14 ppm·h; AOT40 of NF+100 = 13.91 ppm·h; uptake = 159, 2965, 7095 mol m²</td>
<td>7 episodes of 5 days</td>
<td>Biomass, diameter</td>
<td>No effect</td>
<td>Bortier et al. (2001b)</td>
</tr>
<tr>
<td>Beech, European</td>
<td>0-3 years</td>
<td>OTC</td>
<td>Switzerland</td>
<td>AOT40 for 24 h/days = 4-73 ppm·h</td>
<td>1-3 years</td>
<td>Total biomass</td>
<td>20 at AOT40 for 24 h = 32 ppm·h</td>
<td>Braun and Fluckiger (1995)</td>
</tr>
<tr>
<td>Beech, Japanese</td>
<td>4 years</td>
<td>Growth chamber</td>
<td>Japan</td>
<td>CF, +60 ppb for 7 h/day</td>
<td>156 days</td>
<td>Total biomass</td>
<td>19</td>
<td>Yonekura et al. (2001)</td>
</tr>
<tr>
<td>Birch, silver</td>
<td>Sapling</td>
<td>FACE</td>
<td>Finland</td>
<td>AOT40 = 1, 15 ppm·h; 7-h mean = 26, 40</td>
<td>5 years</td>
<td>Biomass</td>
<td>34 for root, n.s. for stem</td>
<td>Oksanen et al. (2001)</td>
</tr>
<tr>
<td>Birch, silver</td>
<td>Sapling</td>
<td>OTC</td>
<td>Sweden</td>
<td>NF, NF+, NF++; daylight mean 1997 = 29, 37, 54; 1998 = 25, 42, 71 ppb; AOT40 1997 = 2.4, 6.9, 35.1; 1998 = 0.6, 19.6, 74.7 ppm·h</td>
<td>2 years</td>
<td>Total biomass</td>
<td>Total biomass n.s. at NF+, 22 at NF++; root biomass 30 at NF++</td>
<td>Karlsson et al. (2003b)</td>
</tr>
<tr>
<td>Birch, [B. pubescens]</td>
<td>Seedling</td>
<td>Chamber in glasshouse</td>
<td>Norway</td>
<td>AOT40 = 0.1, 2.5, 7.1, 7.4, 17.8, 19.8 ppm·h</td>
<td>40 days</td>
<td>Biomass</td>
<td>Sig. decrease in root at AOT40 = 2.5 ppm·h, shoot at 7.1 ppm·h</td>
<td>Mortensen (1998)</td>
</tr>
<tr>
<td>Birch, paper</td>
<td>Sapling</td>
<td>FACE</td>
<td>Wisconsin</td>
<td>Ambient; 1.5x: 4 y Ambient 12-h mean = 34.6, 36.9, 36.0, 36.6; 4 y 1.5x 12-h mean = 54.5, 51.1, 48.9, 52.8</td>
<td>7 years (only 4 years of O₃ data reported)</td>
<td>Volume (d²·h)</td>
<td>No effect</td>
<td>Isebrands et al. (2001)</td>
</tr>
<tr>
<td>Cherry, black</td>
<td>2 years</td>
<td>OTC</td>
<td>Norris, TN</td>
<td>CF, 1×, 2×: 7-h mean = 21, 50, 97</td>
<td>April to August</td>
<td>Biomass</td>
<td>No effect</td>
<td>Samuelson (1994)</td>
</tr>
<tr>
<td>Species</td>
<td>Age</td>
<td>Facility</td>
<td>Location</td>
<td>O₃ Concentration (units are ppb unless otherwise specified)¹</td>
<td>Duration</td>
<td>Variable</td>
<td>Response (Decrease from lowest, %)</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------</td>
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<td>-------------------------------------------------------------</td>
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</tr>
<tr>
<td>Cherry, black</td>
<td>Seedling</td>
<td>OTC</td>
<td>GSMNP¹</td>
<td>CF, 1×, 1.5×, 2×: SUM₀₆ = 0-40.6 ppm-h, AOT₄₀ = 0.03-28.3 ppm-h</td>
<td>76 days</td>
<td>Biomass</td>
<td>n.s. at 1× and 1.5×, 38 at 2×</td>
<td>Neufeld et al. (1995)</td>
</tr>
<tr>
<td>Cherry, black</td>
<td>Seedling</td>
<td>OTC</td>
<td>GSMNP¹</td>
<td>CF, 0.5×, 1×, 1.5×, 2×: SUM₀₆ = 0-53.7 ppm-h; AOT₄₀ = 0.4 ppm-h</td>
<td>140 days</td>
<td>Biomass</td>
<td>n.s. at 1× and 1.5×, 59 at 2×</td>
<td>Neufeld et al. (1995)</td>
</tr>
<tr>
<td>Cherry, black</td>
<td>1 year</td>
<td>OTC</td>
<td>Delaware, OH</td>
<td>CF, 0.5, 1, 1.5, 2×: SUM₀₀ in 1990 = 17-107 ppm-h, in 1991 = 31-197 ppm-h</td>
<td>2 years (in 1990 for 3.5 months, 1991 for 4 months)</td>
<td>Total biomass</td>
<td>no effect at 1× and 1.5×, 32 at 2×</td>
<td>Rebbeck (1996)</td>
</tr>
<tr>
<td>Cherry, black</td>
<td>Seedling</td>
<td>OTC</td>
<td>Pennsylvania</td>
<td>CF, 0.75×, 0.97×: 7-h mean = 39 to 46, SUM₀₆ = 0-10.34 ppm-h</td>
<td>3 years for 17 weeks</td>
<td>Total biomass</td>
<td>6 at 0.75×, 14 at 0.97×</td>
<td>Kouterick et al. (2000)</td>
</tr>
<tr>
<td>Cottonwood,</td>
<td>Cutting</td>
<td>Ambient, in buried pots with irrigation</td>
<td>In and within 100 km of New York City, NY</td>
<td>12 h mean = 23-49 ppb</td>
<td>2 months each year, 3 10-years experiments</td>
<td>Total biomass</td>
<td>33% decrease at 38 ppb compared to 23 ppb</td>
<td>Gregg et al. (2003)</td>
</tr>
<tr>
<td>Eastern</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grape</td>
<td>3 years</td>
<td>OTC</td>
<td>Austria</td>
<td>CF, 1×, +30, +50: (AOT₄₀ = 0-50 ppm-h)</td>
<td>2 years (preflowering, past harvest)</td>
<td>Fruit yield</td>
<td>Calculated 10 at AOT₄₀ = 27 ppm-h</td>
<td>Soja et al. (1997)</td>
</tr>
<tr>
<td>Oak</td>
<td>Seedling</td>
<td>OTC</td>
<td>Hampshire, UK</td>
<td>NF, NF+: Mean = 17.7, 44, AOT₄₀ for 24h = 1.9, 59.9 ppm-h</td>
<td>3 years for day 100 to day 162</td>
<td>Biomass of organs</td>
<td>30 for total biomass</td>
<td>Broadmeadow and Jackson (2000)</td>
</tr>
<tr>
<td>Oak, red</td>
<td>Seedling</td>
<td>OTC</td>
<td>Norris, TN</td>
<td>SUM₀₆ for 3 years = 0, 29, 326 ppm-h; SUM₀₀ for 3 years = 147, 255, 507 ppm-h</td>
<td>3 years</td>
<td>Total biomass</td>
<td>n.s.</td>
<td>Samuelson et al. (1996)</td>
</tr>
</tbody>
</table>

¹ Units are ppm-h unless otherwise specified.
<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Facility</th>
<th>Location</th>
<th>O₃ Concentration (units are ppb unless otherwise specified)</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (Decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak, red</td>
<td>30 years</td>
<td>OTC</td>
<td>Norris, TN</td>
<td>SUM06 for 3 years = 0, 29, 326 ppm•h; SUM00 for 3 years = 147, 255 and 507 ppm•h</td>
<td>3 years</td>
<td>Stem increment</td>
<td>n.s. despite 50% reduction in net photosynthesis</td>
<td>Samuelson et al. (1996)</td>
</tr>
<tr>
<td>Maple, red</td>
<td>2 years</td>
<td>OTC</td>
<td>Norris, TN</td>
<td>CF, 1×, 2×: 7-h mean = 21, 50, 97 ppm•h</td>
<td>April to August</td>
<td>Biomass</td>
<td>No effect</td>
<td>Samuelson (1994)</td>
</tr>
<tr>
<td>Maple, sugar</td>
<td>1 year</td>
<td>OTC</td>
<td>Delaware, OH</td>
<td>CF, 0.5, 1.5, 2×: UM00 in 1990 = 17 to 107 ppm•h, in 1991 = 31 to 197 ppm•h</td>
<td>2 years (in 1990 for 3.5 months, 1991 for 4 months)</td>
<td>Total biomass</td>
<td>n.s., but linear trend</td>
<td>Rebbeck (1996)</td>
</tr>
<tr>
<td>Maple, sugar</td>
<td>Seedling</td>
<td>Large OTC</td>
<td>Ithaca, NY</td>
<td>CF, 1×, 1.5×, 2×: 3 years SUM00 = 148 to 591 ppm•h; daytime mean = 19.7 to 40.7</td>
<td>3 years</td>
<td>Biomass</td>
<td>No effect</td>
<td>Laurence et al. (1996)</td>
</tr>
<tr>
<td>Maple, sugar</td>
<td>Seedling</td>
<td>Large OTC</td>
<td>Ithaca, NY</td>
<td>1×, 1.7×, 3×: 3 years 12-h mean = 38, 69, 117</td>
<td>3 years</td>
<td>Total biomass</td>
<td>For 1.7× and 3×: 21, 64 in low light, 26 and 41 in high light</td>
<td>Topa et al. (2001)</td>
</tr>
<tr>
<td>Maple, sugar</td>
<td>Sapling</td>
<td>FACE</td>
<td>Wisconsin</td>
<td>Ambient, 1.5×: 4 y Ambient 12-h mean = 34.6, 36.9, 36.0, 36.6; 4y 1.5× 12-h mean = 54.5, 51.1, 48.9, 52.8</td>
<td>7 years</td>
<td>Volume (d2*h)</td>
<td>18</td>
<td>Isebrands et al. (2001)</td>
</tr>
<tr>
<td>Plum, Casselman</td>
<td>Sapling</td>
<td>Large OTC</td>
<td>Fresno, CA</td>
<td>CF, 1×, +O₃: 12-h mean = 31, 48, 91</td>
<td>4 years</td>
<td>Stem increment, fruit yield</td>
<td>Fruit yield 16 at 1×, stem +14 at +O₃</td>
<td>Retzlaff et al. (1997)</td>
</tr>
<tr>
<td>Poplar, black</td>
<td>Seedling</td>
<td>OTC</td>
<td>Belgium</td>
<td>CF, NF, +30: 8-h mean = 5, 29, 33; AOT40 = 0, 4, 8.9 ppm•h</td>
<td>23 April - 30 Sept</td>
<td>Diameter, height</td>
<td>29 for diameter in NF+, no effect on height</td>
<td>Bortier et al. (2000b)</td>
</tr>
<tr>
<td>Species</td>
<td>Age</td>
<td>Facility</td>
<td>Location</td>
<td>O₃ Concentration (units are ppb unless otherwise specified)¹</td>
<td>Duration</td>
<td>Variable</td>
<td>Response (Decrease from lowest, %)</td>
<td>Reference</td>
</tr>
<tr>
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</tr>
<tr>
<td>Poplar, hybrid (P. tremuloides × P. tremula)</td>
<td>0 year</td>
<td>FACE</td>
<td>Finland</td>
<td>AOT₄₀ = 0.07, 1.6 ppm•h; 7-h mean = 30, 38</td>
<td>2 months</td>
<td>Biomass, height</td>
<td>n.s. for biomass, 6 for height</td>
<td>Oksanen et al. (2001)</td>
</tr>
<tr>
<td>Poplar, hybrid</td>
<td>Cutting</td>
<td>OTC</td>
<td>Michigan</td>
<td>CF, CF+100: 12, 48 ppm•h</td>
<td>60 days</td>
<td>Total biomass</td>
<td>46 for average of 5 clones</td>
<td>Dickson et al. (1998)</td>
</tr>
<tr>
<td>Poplar, hybrid</td>
<td>Cutting</td>
<td>OTC</td>
<td>Michigan</td>
<td>CF, CF+100: 12, 48 ppm•h</td>
<td>60 days</td>
<td>Total biomass</td>
<td>46 for average of 5 clones</td>
<td>Dickson et al. (1998)</td>
</tr>
<tr>
<td>Yellow-poplar</td>
<td>1 year</td>
<td>OTC</td>
<td>Delaware, OH</td>
<td>CF, 0.5×, 1.5×, 2×: SUM₀₀ in 1990 = 17 to 107 ppm•h, in 1991 = 31 to 197 ppm•h</td>
<td>2 years (in 1990 for 3.5 months, 1991 for 4 months)</td>
<td>Total biomass</td>
<td>No effect</td>
<td>Rebbeck (1996)</td>
</tr>
<tr>
<td>Yellow-poplar</td>
<td>1-7 year</td>
<td>Large OTC</td>
<td>Delaware, OH</td>
<td>CF, 0.5×, 1.5×: SUM₀₀= 145, 583, 861 ppm•h ; SUM₀₆ = 0.3, 228.7, 661.8 ppm•h over 5 years</td>
<td>5 years</td>
<td>Total biomass</td>
<td>No effect</td>
<td>Rebbeck (1996)</td>
</tr>
</tbody>
</table>

¹ Values for ambient or NF treatments are indicated in bold.
² Bold indicates that multiple experiments (more than just 2 years at a single site) were included in the analysis.
Table AX9-19. Summary of Selected Studies of Ozone Effects on Evergreen Trees and Shrubs

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Facility</th>
<th>Location</th>
<th>O&lt;sub&gt;3&lt;/sub&gt; Concentration (units are ppb unless otherwise specified)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Duration</th>
<th>Variable</th>
<th>Response (decrease from lowest, %)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemlock, eastern</td>
<td>Seedling</td>
<td>OTC</td>
<td>GSMNP&lt;sup&gt;1&lt;/sup&gt;, TN</td>
<td>CF to 2×: SUM06 = 0.2-108.1 ppm•h, AOT40 = 0.2-63.9 ppm•h</td>
<td>3 years</td>
<td>Biomass</td>
<td>No effect</td>
<td>Neufeld et al. (2000)</td>
</tr>
<tr>
<td>Pine, loblolly</td>
<td>12 weeks</td>
<td>OTC</td>
<td>Oak Ridge, TN</td>
<td>CF to 2×: 24-h summer = 74, 137, 169, 206, 284 ppm•h</td>
<td>3 months</td>
<td>Biomass</td>
<td>14 in 1× (avg for all families)</td>
<td>McLaughlin et al. (1994)</td>
</tr>
<tr>
<td>Pine, loblolly</td>
<td>1 year</td>
<td>OTC</td>
<td>Alabama</td>
<td>1994: AOT40 = 2-112 ppm•h, SUM06 = 10-162 ppm•h, AOT40 = 3-83 ppm•h, SUM06 = 0-132 ppm•h</td>
<td>2 years, April to October</td>
<td>Dry weight, height, diameter</td>
<td>n.s.</td>
<td>Barbo et al. (2002)</td>
</tr>
<tr>
<td>Pine, loblolly</td>
<td>3 years</td>
<td>OTC</td>
<td>Raleigh, NC</td>
<td>Ambient, CF, NF, 1.5×, 2.5×: 12-h mean = 54, 29, 47, 76, 98</td>
<td>5 months</td>
<td>Height, diameter, needle length</td>
<td>No effect on stem height or diameter, decrease in needle length</td>
<td>Anttonen et al. (1996)</td>
</tr>
<tr>
<td>Pine, loblolly</td>
<td>4 weeks</td>
<td>Large OTC</td>
<td>Auburn, AL</td>
<td>CF, 1×, 2×: 12-h mean = 13, 47, 98 ppm•h in 1998; 12, 44, 97</td>
<td>2 12-week experiments</td>
<td>Shoot biomass, root biomass, foliar injury</td>
<td>Shoot = 15 in 1×, 22 in 2× in both years; Root = 26 in 2× in both years; Foliar sig. greater in 1× in 1999 and 2× in both years.</td>
<td>McLaughlin et al. (1994)</td>
</tr>
<tr>
<td>Pine, ponderosa</td>
<td>Seedling</td>
<td>OTC</td>
<td>Corvallis, OR</td>
<td>For CF 12-h SUM06 = 0 ppm•h; for +03 12-h SUMO6 = 22, 27, 31 ppm•h for 3 years</td>
<td>3 years: 16 weeks, 16 weeks, 14 weeks</td>
<td>Total biomass</td>
<td>No effect without grass, 25 with grass present</td>
<td>Andersen et al. (2001)</td>
</tr>
<tr>
<td>Species</td>
<td>Age</td>
<td>Facility</td>
<td>Location</td>
<td><strong>O3 Concentration</strong> (units are ppb unless otherwise specified)</td>
<td>Duration</td>
<td>Variable</td>
<td>Response (decrease from lowest, %)</td>
<td>Reference</td>
</tr>
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</tr>
<tr>
<td>Pine, ponderosa</td>
<td>39 to 45 years</td>
<td>Ambient gradient</td>
<td>CA</td>
<td>24-h mean for 3 weeks late July and early August for 1993 and 1994 = 70-90 ppb</td>
<td>Ambient gradient</td>
<td>Fine and medium root growth</td>
<td>85 at most polluted site.</td>
<td>Grulke et al. (1998a)</td>
</tr>
<tr>
<td>Pine, ponderosa</td>
<td>Seedling</td>
<td>OTC</td>
<td>CA</td>
<td>CF, 1×, 2×; 24-h mean approx. 20, 60, 120</td>
<td></td>
<td>Total biomass</td>
<td>n.s.</td>
<td>Takemoto et al. (1997)</td>
</tr>
<tr>
<td>Pine, Scots</td>
<td>Seedling</td>
<td>OTC</td>
<td>Hampshire, UK</td>
<td>NF, NF+: Mean = 17.7, 44.1; 24-h AOT40 = 1.9, 59.9 ppm•h</td>
<td>3 years for 62 days</td>
<td>Total biomass</td>
<td>15</td>
<td>Broadmeadow and Jackson (1999)</td>
</tr>
<tr>
<td>Pine, Scots</td>
<td>3-6 years</td>
<td>Free air</td>
<td>Finland</td>
<td>Amb, +O3: AOT40 = 0-1, 2-13 ppm•h</td>
<td>3 years</td>
<td>Biomass</td>
<td>No effect</td>
<td>Kainulainen et al. (2000b)</td>
</tr>
<tr>
<td>Pine, Scots</td>
<td>Seedling</td>
<td>OTC</td>
<td>Switzerland</td>
<td>0.5×, 0.85×, 1×, 0.5×+30: AOT40 = 0.1, 3.4, 7.1, 19.7 ppm•h</td>
<td>5 months</td>
<td>Biomass</td>
<td>14 at 1×, 22 at 0.5×+30</td>
<td>Landolt et al. (2000)</td>
</tr>
<tr>
<td>Pine, Scots</td>
<td>3 years</td>
<td>OTC</td>
<td>Finland</td>
<td>CF, 1×, +O3: 24 h AOT40 for 2 years = 0.5, 6, 73 ppm•h</td>
<td>2 years (4 months each)</td>
<td>Biomass</td>
<td>No effect</td>
<td>Utriainen et al. (2000)</td>
</tr>
<tr>
<td>Pine, Scots</td>
<td>3 years</td>
<td>Free air</td>
<td>Finland</td>
<td>1×, +O3: 24 h AOT40 for 2 years = 2, 37 ppm•h</td>
<td>3 years (3-4 months each)</td>
<td>Root and shoot biomass</td>
<td>32 only for root biomass in high N treatment</td>
<td>Utriainen and Holopainen (2001)</td>
</tr>
<tr>
<td>Pine, Mountain</td>
<td>Seedling</td>
<td>OTC</td>
<td>GSMNP², TN</td>
<td>CF to 2×: SUM06 = 0.2-116.4 ppm•h, AOT40 = 0.2-71.7 ppm•h</td>
<td>3 years</td>
<td>Biomass</td>
<td>Slight decrease in older needle mass only</td>
<td>Neufeld et al. (2000)</td>
</tr>
<tr>
<td>Pine, Virginia</td>
<td>Seedling</td>
<td>OTC</td>
<td>GSMNP², TN</td>
<td>CF to 2×: SUM06 = 0.1-32.8, 47.9, 56.2 ppm•h; AOT40 = 0.1-19.3, 27.1, 34.4 ppm•h</td>
<td>1-2 years (3 expts)</td>
<td>Biomass</td>
<td>No effect</td>
<td>Neufeld et al. (2000)</td>
</tr>
<tr>
<td>Species, Age</td>
<td>Facility</td>
<td>Location</td>
<td>O&lt;sub&gt;3&lt;/sub&gt; Concentration (units are ppb unless otherwise specified)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Duration</td>
<td>Variable</td>
<td>Response (decrease from lowest, %)</td>
<td>Reference</td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>Sequoia, giant 125 years</td>
<td>Branch chamber</td>
<td>California</td>
<td>0.25×, 1×, 2×, 3×: 24-h SUM00 approx. 10, 85, 180, 560 ppm•h</td>
<td>61 days</td>
<td>Branch growth</td>
<td>No effect</td>
<td>Grulke et al. (1996)</td>
<td></td>
</tr>
<tr>
<td>Spruce, Norway 4-7 years</td>
<td>Open air</td>
<td>Finland</td>
<td>Amb, +O&lt;sub&gt;3&lt;/sub&gt;: AOT40 = 0 1, 2-13 ppm•h</td>
<td>3 years</td>
<td>Biomass</td>
<td>No effect</td>
<td>Kainulainen et al. (2000b)</td>
<td></td>
</tr>
<tr>
<td>Spruce, Norway 3-7 years</td>
<td>OTC</td>
<td>Sweden</td>
<td>CF, 1.5×: 12-h mean for 4 years = 12, 44; AOT40 = 2, 23 ppm•h</td>
<td>4 years</td>
<td>Total biomass</td>
<td>8</td>
<td>Karlsson et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>Spruce, Norway Seedling</td>
<td>OTC</td>
<td>Switzerland</td>
<td>0.5×, 0.85×, 1×, 0.5×+30: AOT40 = 0.1, 3.4, 7.1, 19.7 ppm•h</td>
<td>5 months</td>
<td>Biomass</td>
<td>n.s.</td>
<td>Landolt et al. (2000)</td>
<td></td>
</tr>
<tr>
<td>Spruce, Norway 0-3 years</td>
<td>OTC</td>
<td>Switzerland</td>
<td>AOT40 for 24 h for 1 to 3 years = 22 to 63 ppm•h</td>
<td>1-3 years</td>
<td>Total biomass</td>
<td>n.s.</td>
<td>Braun and Fluckiger (1995)</td>
<td></td>
</tr>
<tr>
<td>Spruce, Norway 3 to 7 years</td>
<td>OTC</td>
<td>Sweden</td>
<td>CF, 1×, 1.5×: AOT40 daylight for 4 years = 1, 16, 79 ppm•h</td>
<td>4 years</td>
<td>Biomass</td>
<td>5.3 at 1.5×</td>
<td>Wallin et al. (2002)</td>
<td></td>
</tr>
<tr>
<td>Spruce, red Sapling</td>
<td>Large OTC</td>
<td>Ithaca, NY</td>
<td>CF, 1×, 1.5×, 2×: total for 4 years = 211 to 569 ppm•h; daytime mean = 21-71</td>
<td>4 years: 98-124 days/year</td>
<td>Biomass</td>
<td>No effect</td>
<td>Laurence et al. (1997)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Values for ambient or NF treatments are indicated in bold.
<sup>2</sup> Great Smoky Mountains National Park.
(Section AX9.4). Even when some of these aspects of O₃ exposure can be elucidated, it is difficult to apply this knowledge to developing exposure-response relationships based on data in the scientific literature, because O₃ exposure is often reported only in the form of a summary index such as a 12- or 24-h mean, SUM06, or AOT40.

Table AX9-13 presents summaries of ambient O₃ exposure patterns in the United States for 1982 to 1991 for several indices including the 7-h mean and SUM06. More recent summaries for the entire United States for these indices are not available, but Table AX9-20 summarizes more recent data for the central and eastern United States. As shown in Table AX9-20, from 1989 to 1995, mean 12-h 3-month SUM06 values (in ppm·h) at 41 rural sites in the Clean Air Status and Trends Network were 31.5 for the Midwest, 18.9 for the Upper Midwest, 33.2 for the Northeast, 13.2 for the Upper Northeast (New Hampshire, Maine), 34.5 for the South-Central, and 19.2 for the Southern Peripheral subregions (Baumgardner and Edgerton, 1998). These results are important because these sites were selected to represent rural areas, while many other monitoring sites represent urban or suburban areas. For these same subregions, W126 values ranged from 12.8 to 25.6 ppm·h. From 1989 to 1995, O₃ concentrations decreased about 5% for daily and 7% for weekly values for most of these sites, after adjusting for meteorological conditions (Holland et al., 1999). These trends were statistically significant at about 50% of the sites (p ≤ 0.05). However, because the trend analysis was intended to examine the efficacy of O₃ emissions controls, the trends were adjusted for meteorological conditions. Thus, they do not reflect the actual trends in O₃ exposure over time.

**AX9.5.4 Effects of Ozone on Annual and Biennial Species**

Much of the research on short-lived species during the last decade has been conducted in Europe. Several European studies have focused on wheat with an emphasis on developing critical levels as discussed in Section AX9.4.3 and reviewed briefly below in Section AX9.5.4.5.

An extensive search of the literature was performed using several electronic databases to identify scientific articles containing quantitative information on both the amount of O₃ exposure and its effects on vegetation. Greater emphasis is placed on studies with longer duration with O₃ exposure concentrations and environmental conditions that were as similar as possible to ambient conditions. Many of the studies reviewed herein were conducted in OTCs. In the United States, nearly all of such studies have used the type of OTC developed by Heagle et al.
Table AX9-20. Ozone Exposures at 35 Rural Sites in the Clean Air Status and Trends Network in the Central and Eastern United States From 1989 to 1995

<table>
<thead>
<tr>
<th>Subregion</th>
<th>SUM06 12-h, 3-Month Mean</th>
<th>SUM06 3-Month SD</th>
<th>W126 3-Month Mean</th>
<th>W126 3-Month SD</th>
<th>Max. 8 h &gt; 80 ppb Mean</th>
<th>Max. 8 h &gt; 80 ppb SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midwest</td>
<td>31.5</td>
<td>10.2</td>
<td>25.1</td>
<td>7.7</td>
<td>13.8</td>
<td>10.6</td>
</tr>
<tr>
<td>Upper Midwest</td>
<td>18.9</td>
<td>8.5</td>
<td>16.0</td>
<td>5.9</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Northeast</td>
<td>33.2</td>
<td>11.9</td>
<td>26.6</td>
<td>9.5</td>
<td>15.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Upper Northeast</td>
<td>13.2</td>
<td>8.6</td>
<td>12.8</td>
<td>6.5</td>
<td>3.3</td>
<td>5.5</td>
</tr>
<tr>
<td>South Central</td>
<td>34.5</td>
<td>16.6</td>
<td>25.6</td>
<td>11.5</td>
<td>7.1</td>
<td>10.0</td>
</tr>
<tr>
<td>Southern Periphery</td>
<td>19.2</td>
<td>7.6</td>
<td>15.2</td>
<td>5.4</td>
<td>1.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Units for SUM06 and W126 are ppm·h.

(1973). For the few studies in the U.S. that used other types of OTCs, they are described briefly in the text. In Europe, a wide variety of styles of OTCs have been used. See Section AX9.1 for further information about the use of OTCs. The emphasis in this subsection is on quantifying exposure-response relationships for annual plants, with a focus on the response of above-ground biomass and yield of species grown as crops or occurring as native or naturalized species in the United States. Emphasis is placed on studies not included in the 1996 AQCD (U.S. Environmental Protection Agency, 1996), including a few studies published prior to 1996. However, an attempt is made to compare the results of these more recent studies of individual species to those reviewed in the 1996 AQCD.

**AX9.5.4.1 Effects on Growth, Biomass, and Yield of Individual Species**

Most research on the effects of O$_3$ on herbaceous species has evaluated growth, biomass, or yield of commercial portions of crop or forage species. It is well established that reproductive organs such as seeds may be particularly sensitive to injury or biomass reductions due to O$_3$, as reviewed by (Black et al., 2000). As discussed in Section AX9.3, numerous analyses of
experiments conducted in OTCs and with naturally occurring gradients demonstrate that the effects of O$_3$ exposure vary depending on the growth stage of the plant. Plants grown for seed or grain are often most sensitive to exposure during the seed or grain-filling period (Lee et al., 1988; Pleijel et al., 1998; Soja et al., 2000; Younglove et al., 1994), whereas plants grown for biomass production, such as alfalfa, may be sensitive throughout the growth period (Younglove et al., 1994). However, because different species are sensitive during different periods of their growth and, because planting or germination dates vary throughout large regions even for a single species, no single phenological weighting scheme can appropriately and practically represent all vegetation in all locations in the United States. For natural populations, reductions in seed yield might be particularly important if subsequent seedling establishment is compromised by O$_3$.

Green beans (cv. Pros) were grown in pots in OTCs in the Netherlands for 62 days and exposed to 6 treatments consisting of constant O$_3$ additions to CF chambers (see Section AX9.1) for 9 h/day (Tonneijck and Van Dijk, 1998). Bean yield response to O$_3$ was nonlinear, with an apparent threshold near the CF30 (charcoal filtered with a constant addition of 30 ppb O$_3$) treatment with a 9-h mean O$_3$ concentration of 28 ppb and an AOT40 value of 0.1 ppm-h. Yield was reduced by 29% at a 9-h mean value of 44 ppb corresponding to an AOT40 value of 3.6 ppm-h (Table AX9-16). Beans were grown in pots in OTCs for 3 months with the following O$_3$ treatments: CF, nonfiltered (NF), CF with O$_3$ added up to ambient, and CF with 2×-ambient O$_3$ (Brunschen-Harti et al., 1995). Ozone reduced pod mass by 56% with a mean concentration of 32 ppb in the 2× ambient treatment as compared with 1 ppb in the CF treatment (daily averaging time not reported). A second treatment factor in this experiment was addition of EDU, as discussed below under the heading “Studies Using Ethylene Diurea as a Protectant.” Bush beans (cv Bush Blue Lake 290) were grown in pots in OTCs in Corvallis Oregon for 63 or 65 days in two experiments, one from May to July and one from August to October (Tingey et al., 2002). Plants were exposed to either CF air or CF air with above-ambient O$_3$ with temporal frequency and exposure dynamics typical of the Midwestern United States, with SUM06 values of 0.0 for the CF treatment and 75.7 or 68.4 ppm-h for the two experiments. Ozone exposure reduced pod dry weight by 51 and 57% in the two experiments. The sensitive cultivar S156 and the more resistant cultivar Tenderette were grown in pots in OTCs in Raleigh, NC at either CF (12-h mean of 23 ppb) or 1.4× (12-h mean of 72 ppb) (Heagle et al., 2002). At final harvest, the dry weight
of the sensitive cultivar was reduced 90% by the O₃ treatment, but the more resistant cultivar was not reduced. In two additional OTC experiments during 2000 and 2001 in Raleigh, NC, the sensitive cultivar S156, the moderately sensitive cultivar Oregon-91, and the more resistant cultivar R123 were grown in CF, NF, and ambient air treatments (Fiscus et al., 2005). For the NF treatment of the two experiments, the yield of S156 was reduced 21% and 45%, that of Oregon-91 by 27% only in 2001, while R123 was not significantly reduced. These yield reductions are greater than those previously reported in four similar studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental Protection Agency, 1996). Greater sensitivity in the more recent experiments may be due to cultivar differences or other differences in experimental protocols.

In a study with OTCs on silty loam soil in Beltsville, MD, corn yield was reduced by 13% with exposure to a 7-h mean concentration of 70 ppb O₃ compared to a CF treatment with a 7-h mean concentration of 20 ppb (Mulchi et al., 1995; Rudorff et al., 1996a,c). In this study, different amounts of O₃ were added above ambient levels for 5 days follows: 20, 30, 40, 50, 60 ppb, except that O₃ was not added to exceed a total concentration of 120 ppb (Rudorff et al., 1996a,c).

In two studies conducted in Raleigh, NC, cotton (cv. Deltapine 51) was grown in pots and exposed to CF and 1.5× (nonfiltered, see Section AX9.1) O₃ in one year, and CF, NF, and 1.5×-ambient O₃ in the second year, with ambient and elevated CO₂ concentrations (Heagle et al., 1999b) (Table AX9-16). In the first year, yield decreased by 22% with 1.5×-ambient O₃ (12-h mean value of 71 ppb). In the second year, yield decreased by 21% and 49% with exposure to ambient or 1.5× ambient O₃ (12-h mean values of 51 and 78 ppb, Table AX9-16). Increased CO₂ levels prevented or reduced this yield suppression (Heagle et al., 1999b). These yield reductions are similar to those reported previously in four similar studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental Protection Agency, 1996).

In a study of oats in OTCs in southern Sweden, exposure to ambient (NF) O₃ did not affect grain yield (Pleijel et al., 1994a). Ambient O₃ concentration expressed as a 7-h mean was 27 ppb, with only 1 h greater than 80 ppb and none above 90 ppb.

The interactive effects of elevated O₃ and CO₂ additions on potato yield (cv Bintje) were studied in OTCs at 6 sites in northern Europe (Craigon et al., 2002). Ozone was added to a target daily average value of 60 ppb, and AOT40 values across all years and experiments ranged AX9-219
from ~6 to 27 ppm·h. The O$_3$ treatment reduced total tuber yield an average of 4.8% with elevated O$_3$ treatment across all experiments (Craigon et al., 2002). This total effect was statistically significant even though the effects of individual experiments generally were not (Craigon et al., 2002), due to the increased statistical power of the pooled analysis. Several publications report other aspects of the “CHIP” experiments or present results of individual experiments (De Temmerman et al., 2002a,b; Donnelly et al., 2001a,b; Fangmeier et al., 2002; Finnan et al., 2002; Hacour et al., 2002; Lawson et al., 2002; Pleijel et al., 2002; Vandermeiren et al., 2002; Vorne et al., 2002). Resistant and susceptible cultivars of potato (Superior and Dark Red Norland, respectively) were grown for one season in Raleigh, NC and exposed to CF, NF, and 1.5× ambient O$_3$ treatments with 12-h mean values of 15, 45, 80 (Heagle et al., 2003). Tuber yield was decreased by 15 and 31% for Dark Red Norland in NF and 1.5x treatments, but by only 11% in only the 1.5x treatment for Superior.

The effect of an intermittent constant addition of O$_3$ using a free air exposure system in Northumberland, UK was investigated with the oilseed rape cultivar Eurol (Ollerenshaw et al., 1999). Ozone was added for 6 h/day for 17 days. The ambient treatment had a mean value of 30 ppb and the O$_3$ addition treatment had a mean of 77 ppb. After overwintering, O$_3$ was added for 32 days for 7 h/day between May and June (mean values of 31 and 80 ppb). Yield was reduced by 14% despite the lack of any foliar symptoms.

Field fumigation chambers ventilated with fans on both ends were used to assess effects of five O$_3$ treatments on rice over 3 years in Japan (Kobayashi et al., 1994, 1995). All O$_3$ treatments used CF air, and O$_3$ was added to the 0.5, 1.0, 1.5, 2.0, or 2.75× ambient concentration for 7 h/day. Based on a linear regression for the 3 years, yield decreased by 3 to 10% at a 7-h mean concentration of 40 ppb (Table AX9-16). This decrease is greater than that found for rice in earlier studies in California (Kats et al., 1985b), although whether this difference is due to differences in cultivars, experimental treatment, or environmental factors cannot be determined.

During 3 years in Beltsville, MD, the soybean cultivars Essex and Forrest were exposed to CF air and NF air in OTCs (Chernikova et al., 2000; Robinson and Britz, 2000) with O$_3$ added as described for experiments with corn and wheat at Beltsville (Mulchi et al., 1995; Rudorff et al., 1996c). During 1994 and 1995, as previously found for these cultivars, Essex was less sensitive than Forrest, with yield decreases of 10% (n.s.: p > 0.1) compared to 32% for Forrest (p < 0.01)
(Chernikova et al., 2000). There was no evidence of water stress in this experiment. In 1997, the two O\textsubscript{3} treatments were CF (7-h mean = 24 ppb) and NF with a constant addition of O\textsubscript{3} (7-h mean = 58 ppb) (Robinson and Britz, 2000). The yield of Essex was not significantly affected, while the yield of Forrest was decreased by 21% (Table AX9-16).

In a study in Raleigh, NC, the soybean cultivar Essex was grown in pots and exposed to CF and 1.5\times ambient O\textsubscript{3} concentrations during three growing seasons (Fiscus et al., 1997). Over the 3 years, exposure to an average 12-h mean O\textsubscript{3} concentration of 82 ppb reduced soybean yield by 41% (Table AX9-16). In similar studies also in Raleigh, NC, Essex was exposed to CF, NF, and 1.5\times ambient O\textsubscript{3} for two seasons (Heagle et al., 1998b). Yield decreased by 16% and 15% in the 2 years by ambient O\textsubscript{3} (12-h mean values of 50 and 42 ppb), and decreased by 37 and 40% with exposure to 1.5\times ambient O\textsubscript{3} (12-h mean values of 79 and 69 ppb, Table AX9-16). In this same experiment in the second year, similar yield reductions were observed for the cultivar Holladay, while the growth of cultivar NK-6955 was increased substantially by ambient O\textsubscript{3} exposure.

All three cultivars were grown in the same chambers in this experiment, and the authors suggested that NK-6955 plants may have shaded the other cultivars to some extent.

In a 2-year study using OTCs in Raleigh, NC, the soybean cultivars Coker 6955, Essex, and S53-34 were exposed to CF, NF, and 1.5\times ambient O\textsubscript{3} treatments (Miller et al., 1994). Seasonal mean 12-h O\textsubscript{3} concentrations ranged from 14 to 83 ppb. As compared to the CF treatment, ambient O\textsubscript{3} exposure (NF treatment) reduced seed yield by 11 to 18% except for Coker 6955 in the first year (1989), which showed a yield increase of 14%. The 1.5\times ambient O\textsubscript{3} treatment reduced yield by 32 to 56% in all cultivars in both years. In a similar subsequent experiment with the cultivar Essex, exposure to a 12-h mean ambient O\textsubscript{3} concentration of 50 ppb reduced yield by 11%, while exposure to 79 ppb reduced yield by 22% (Table AX9-16) (Miller et al., 1998). In similar experiments with Essex during 1999 and 2000, plants were exposed to 1.5\times ambient O\textsubscript{3} in OTCs either in pots or planted in the ground (Booker et al., 2005). Exposure to a 12-h mean ambient O\textsubscript{3} concentration of 75 ppb reduced yield by 27% in pots and 24% in the ground in 1999, and exposure to a 12-h mean ambient O\textsubscript{3} concentration of 67 ppb reduced yield by 41% in pots and 39% in the ground in 2000 (differences between results in pots and in the ground were not statistically significant).

In a study using a free air exposure system near Urbana-Champaign, IL, the soybean cultivar 93B15 was grown for two seasons (2002, 2003) and exposed to ambient ozone and
elevated ozone approximately 1.23 × ambient (Morgan et al., in press). The maximum 8-hour average ozone concentration was 62 and 50 ppb in the control plots for 2002 and 2003 respectively, and 75 and 63 ppb in the elevated ozone plots. Thus, the effective increase in ozone concentration in the elevated plots was 21 and 25% for 2002 and 2003, respectively. In 2003, a severe hailstorm in July removed many of the leaves in all plots early in the season, but the plants recovered and produced new foliage. Compared to the ambient treatment, the elevated treatment reduced yield by 15% in 2002 and 25% in 2003. The authors ascribe the greater proportional yield decrease seen in the second year despite lower O_3 exposure to the interactive effects of hail damage and O_3 exposure. Because free air exposure systems can only add O_3 above ambient concentrations, it should be noted that yield also might have been decreased in the ambient plots, and that the reduction in yield in the elevated O_3 treatment might have been greater in comparison to a lower background O_3 exposure regime. This FACE facility study is important, because it confirmed yield reductions reported previously with soybean plants grown in OTCs.

These yield reductions for soybean are generally similar to those reported previously in 13 similar studies summarized in the 1996 AQCD (Table 5-23 of U.S. Environmental Protection Agency, 1996). A meta-analysis of 53 studies of O_3 effects on soybean found that at an average O_3 exposure of 45 ppb, seed yield was decreased by 10% compared to the CF treatment, while at 70 ppb, seed yield was decreased by 24% (Morgan et al., 2003). The 95% confidence limits of these responses based on a bootstrap method did not include a value of zero yield loss. These results suggest that seasonal O_3 concentration patterns that occur in some years throughout many parts of the U.S. can reduce soybean seed yield.

A reanalysis of 7 years of data from OTC experiments with wheat in Ostad, Sweden showed that relative yield linearly decreased with increasing O_3, with a maximum yield loss of 23% at an AOT40 value of 15 ppm·h (Danielsson et al., 2003). A very similar response was found using the flux (stomatal conductance) model of Emberson et al. (2000b) and a similar amount of the variance was explained by the flux model (for AOT40 model, \( r^2 = 0.34 \) and for the Emberson flux model, \( r^2 = 0.39 \)). A modified flux model developed and calibrated for this site also had a similar linear response equation, but explained much more of the variance (\( r^2 = 0.90 \)).

During the 1990s, a major European research program investigated the combined effects of CO_2, O_3, and other physiological stresses on wheat (Bender et al., 1999; Hertstein et al., 1996,
The ESPACE-wheat program included 13 experiments in OTCs at eight sites in northern Europe over 3 years. Low- and high-O₃ exposures in these experiments had the following values: 12-h mean (SD) low = 26.3 ppb (12.2), 12-h mean (SD) high = 51.37 (18.3) ppb, AOT40 mean (SD) low = 6.2 (8.5) ppm-h, AOT40 mean (SD) high = 28.3 (23.0) ppm·h, as calculated from data presented in Table 3 of Hertstein et al. (1999). An analysis of all 13 experiments showed that high O₃ at ambient CO₂ reduced yield by 13% on average (Bender et al., 1999). However, this reduction was not statistically significant based on an ANOVA, and the authors concluded that the wheat cultivar Minaret may be relatively tolerant to O₃ (Bender et al., 1999). Results of some individual studies within this program have been reported previously (Donnelly et al., 1999; Fangmeier et al., 1996, 1999; Mulholland et al., 1997a, 1998a,b; Pleijel et al., 2000b).

In a study with OTCs on silty loam soil in Beltsville, MD, wheat yield was reduced by 20% on average over 2 years with 7-h mean concentrations of 61 and 65 ppb O₃, compared with CF treatment with a 7-h mean concentration of 20 ppb (Mulchi et al., 1995; Rudorff et al., 1996a,c). In the above study, different amounts of O₃ were added above ambient levels for 5 days as follows: 20, 30, 40, 50, 60 ppb, except that O₃ was not added to exceed a total concentration of 120 ppb (Rudorff et al., 1996c). Wheat grown in pots in OTCs was exposed to elevated O₃ and water stress in Germany, and yield was decreased by 35% in the 2×-ambient treatment with a 7-h mean O₃ concentration of 71 ppb, statistically significant effects were not seen in the 1×-ambient treatment (Fangmeier et al., 1994). In two studies conducted in Raleigh, NC, soft red winter wheat was grown in pots and exposed to CF, NF, and 1.5×-ambient O₃, with ambient and elevated CO₂ concentrations (Table AX9-16) (Heagle et al., 2000). In the first experiment, eight cultivars were exposed to 12-h mean O₃ concentrations of 27, 47, and 90 ppb, and in the second experiment two of these cultivars were exposed to 22, 38, and 74 ppb. There was a trend toward decreased yield in both experiments, but these trends were not statistically significant. The wheat cultivar Drabant was exposed to CF, NF, and a constant addition of 35 ppb during 1992 and 1993 in Finland (Ojanpera et al., 1998), using the Heagle-type OTCs (Heagle et al., 1973). The following 12-h mean O₃ exposures were observed in 1992: 14, 30, 61 ppb. In 1993, the values were 9, 21, and 45 ppb, (see Table AX9-16 for AOT40 values and other information). Yield was reduced 13% in each year by the added O₃ treatment.
The effect of an intermittent constant addition of O\textsubscript{3} using a free air exposure system was investigated with the winter wheat cultivar Riband in Northumberland, UK (Ollerenshaw and Lyons, 1999). Ozone exposures expressed as AOT40 values for September and October 1992 were 0.14 and 3.5 ppm\textperiodcentered h; while for April to August 1993, values were 3.5 and 6.2 ppm\textperiodcentered h. Yield was reduced by 13%.

These results provide an additional line of evidence supporting the OTC-studies that demonstrated yield reductions in wheat due to O\textsubscript{3} exposures that occur in the United States. These yield reductions for wheat are generally similar to those reported previously in 22 comparable studies summarized in the 1996 AQCD (Table 5-25 of U.S. Environmental Protection Agency, 1996).

\textbf{AX9.5.4.2 Effects on Plant Quality}

In addition to reductions in biomass or crop yield, O\textsubscript{3} may also reduce the quality or nutritive value of annual species. Many studies have shown effects of O\textsubscript{3} on various measures of plant organs that affect quality, with most studies focusing on characteristics important for food or fodder (U.S. Environmental Protection Agency, 1996).

The effect of a continuous intermittent addition of O\textsubscript{3} using a free air exposure system in Northumberland, UK was investigated with the oilseed rape cultivar Eurol as discussed above (Ollerenshaw et al., 1999). Ozone exposures expressed as AOT40 values for August to October 1991 were 0.2 and 3.8 ppm\textperiodcentered h; for June 1992 they were 0.7 and 8.1 ppm\textperiodcentered h. Yield quality measured as crude protein and oil content was decreased significantly. Because the price of the product is reduced in direct proportion to the oil content, such a decrease represents a substantial loss to growers (Ollerenshaw et al., 1999).

Two wheat cultivars, Massey and Saluda, were each grown for one year each in Beltsville, MD (Table AX9-16) and exposed to either CF or an addition of 40 ppb for 7 h/day for 5 days/week (Mulchi et al., 1995; Rudorff et al., 1996a,c). Milling and baking quality scores and flour protein were not significantly affected by elevated O\textsubscript{3} exposure, but the softness equivalent was increased slightly (2.4\%) in both experiments (Rudorff et al., 1996b). The authors concluded that these changes, along with other slight changes due to an increased CO\textsubscript{2} treatment, suggested that O\textsubscript{3} and CO\textsubscript{2} had only minor effects on wheat grain quality. In wheat grown in
Sweden, the harvest index was significantly decreased and the protein content increased due to exposure to a 12-h mean of 48 ppb (Gelang et al., 2000). In an analysis of 16 experiments conducted with spring wheat and either O₃ or CO₂ exposures in four Nordic countries, a negative linear relationship was found between grain yield and grain protein content (y = −0.38x + 138.6, expressed as percentages of the NF treatment (Pleijel et al., 1999a).

For three soybean cultivars grown in Raleigh, NC, O₃ significantly decreased oleic acid content, although the authors stated that the reduction was not large enough to be economically important (Heagle et al., 1998b).

In a UK study, potato exposed during 1998 to an AOT40 value of 12.5 ppm·h in OTCs (in Nottingham) resulted in the paste from tubers being more viscous (Donnelly et al., 2001b). In this study, an AOT40 exposure of 27.11 ppm·h in 1999 caused starch granules to be less resistant to swelling, and total glycoalkaloid content was increased due to an increase in a­solanine (Donnelly et al., 2001b). Such increases in glycoalkaloid content have been observed previously in potato (Pell and Pearson, 1984) and may be important, because glycoalkaloids cause bitter flavors and, at higher concentrations, toxicity. The authors indicated that levels found in this study approached those that may cause bitterness, but not those of concern for toxicity (Donnelly et al., 2001b).

In the CHIP program the effects of O₃ were studied using OTCs at six sites in northern Europe, and yield decreases were observed as described above. The reducing sugar and starch content of tubers decreased linearly due to O₃ exposure, while the ascorbic acid concentration increased linearly (Vorne et al., 2002). Compared to the CF treatment, exposure to an AOT40 value of 14 ppm·h decreased starch concentrations by 2%, decreased reducing sugar concentration by 30%, and increased ascorbic acid concentration by 20%. While the changes in reducing sugars and ascorbic acid increase tuber quality, the reduction in starch concentration decreases tuber quality.

In two 1-year studies using OTCs in commercial fields in Spain, the soluble solids content of watermelon was decreased 4 to 8% due to seasonal O₃ exposures as follows: AOT40 = 5.96 ppm·h and SUM06 = 0.295 ppm·h in one year; and AOT40 = 18.9, SUM06 = 4.95 in the second year (Gimeno et al., 1999).
AX9.5.4.3 Effects on Foliar Symptoms

For most annual crop species, the most important effects of O\textsubscript{3} are on yield of the commercially important part of the crop, expressed as the mass of the harvested portion. However, for some crops, foliar symptoms are important if they reduce the marketability of the crop. This is why efforts have been made to identify O\textsubscript{3} exposures associated with foliar symptoms. In Europe, Level I critical levels have been determined for such effects based on observations from experiments conducted in 15 countries under the auspices of the United Nations Economic Commission for Europe International Cooperative Programme on effects of air pollution and other stresses on crops and non-woody plants (UN ECE ICP-Vegetation; formerly ICP-Crops), as well as on observations of symptoms in commercial fields from 1993 to 1996 (Benton et al., 1995; Benton et al., 2000). Because the occurrence of symptoms increased with greater humidity, these levels took into account the VPD. Two short-term critical levels were derived from 1995 data: an AOT40 value of 0.2 ppm\textsubscript{h} over 5 days when mean VPD is below 1.5 KPa (0930 - 1630 h), and a value of 0.5 ppm\textsubscript{h} when the mean VPD is above 1.5 Kpa (Benton et al., 1996). The 1996 data supported the critical levels in 83% of observations, although symptoms occurred on three occasions when the AOT40 was less than 0.05 ppm\textsubscript{h} and the VPD was very low — less than 0.6 Kpa. The authors concluded that these critical levels are good indicators of the likelihood of foliar symptoms, but that further refinement may be required, such as including factors that modify O\textsubscript{3} uptake by stomata.

In a more recent study in Germany, 25 native herbaceous species were exposed to several square-wave O\textsubscript{3} exposures in CF OTCs (Bergmann et al., 1999). Six of the 25 species showed O\textsubscript{3}-specific symptoms, and five species responded to single-day peaks. The most sensitive species exhibiting O\textsubscript{3}-specific symptoms were Cirsium arvense and Sonchus asper, both of which responded to AOT40 values < 1.5 ppm\textsubscript{h} (Bergmann et al., 1999).

AX9.5.4.4 Other Effects

Several studies during recent decades have demonstrated O\textsubscript{3} effects on different stages of reproduction. Effects of O\textsubscript{3} have been observed on pollen germination, pollen tube growth, fertilization, and abortion of reproductive structures, as reviewed by Black et al. (2000). This issue is not addressed here, because reproductive effects will culminate for seed-bearing plants in seed production, and the substantial body of evidence relating O\textsubscript{3} exposure and reduced seed
production was discussed above. However, one example of a native species will be presented, because of its implications for extrapolating exposure-response data to noncommercial species. Spreading dogbane (*Apocynum androsaemifolium* L.) has been identified as a useful species for O$_3$ biomonitoring, because of O$_3$-induced diagnostic symptoms (Kohut et al., 2000). A study in Massachusetts found that exposure to O$_3$ in NF OTCs or ambient plots for 103 days produced significantly fewer flowers and that fewer of these flowers survived to produce mature fruits (Bergweiler and Manning, 1999). Because foliar symptoms were not common, the authors concluded they are not required for effects on reproduction to occur. Genotoxic effects and effects on population genetics were discussed in Section AX9.3.

**AX9.5.4.5 Scaling Experimental Data to Field Conditions**

Substantial effort has been invested in the design of OTCs for assessing the effects of air pollutants on vegetation under near-ambient conditions. The design, construction, and performance of many types of chambers has been reviewed extensively (Hogsett et al., 1987a,b). Despite such design efforts, the influence of experimental chambers on exposure-response functions has been debated for many years (e.g., Manning and Krupa, 1992), because several factors differ between OTC studies and actual fields. This issue was addressed in Sections AX9.1.2.2 and AX9.1.2.4, and only a few comments about the implications of chamber artifacts for interpreting exposure-response relationships are presented here.

While it is clear that chambers can alter some aspects of plant growth (for example, (Bytnerowicz et al., 2004; Elagöz and Manning, 2005b), the more important issue is whether they alter the response of plants to O$_3$. A review of such chamber studies done in California found that plants responded similarly to O$_3$ whether OTCs, closed-top chambers, or air exclusion systems were used; differences were found for fewer than 10% of growth parameters (Olszyk et al., 1986a). In another review of literature about Heagle-type OTCs (Heagle et al., 1988), the authors concluded that “Although chamber effects on yield are common, there are no results showing that this will result in a changed yield response to O$_3$.” A more recent study of chamber effects examined the responses of tolerant and sensitive white clover clones to ambient O$_3$ in greenhouse, OTCs, and ambient plots (Heagle et al., 1996). For individual harvests, O$_3$ reduced the forage weight of the sensitive clone 7 to 23% more in the greenhouse than in OTCs. However, the response in OTCs was the same as in ambient plots. A similar study with these
white clover clones near Naples, Italy also found no significant difference between \( \text{O}_3 \) effects measured in OTCs versus those measured by comparing the ratio of sensitive and resistant clones in ambient air (Fagnano et al., 2004). Several studies have shown very similar yield responses to \( \text{O}_3 \) for plants grown in pots or in the ground, suggesting that even such a significant change in environment does not alter the proportional response to \( \text{O}_3 \), at least as long as the plants are well-watered (Heagle, 1979; Heagle et al., 1983). As discussed in Section AX9.1, results from recent FACE studies are similar to those obtained from OTC studies, providing another line of evidence that chamber effects in OTCs do not substantially alter \( \text{O}_3 \) exposure-response relationships (Morgan et al., in press).

Most experiments investigating \( \text{O}_3 \) effects on annual vegetation provide adequate water to avoid substantive drought stress. Because drought stress has generally been shown to reduce the effect of \( \text{O}_3 \) on annual vegetation, such experiments may tend to overestimate \( \text{O}_3 \) effects on crops and especially on unmanaged or seminatural vegetation.

As mentioned above, the use of \( \text{O}_3 \) flux, rather than exposure, is theoretically more realistic, and such an approach would also address the vertical gradient issue (Section 3.3.2, Section AX9.1). A number of investigators have suggested that modeling \( \text{O}_3 \) flux can improve estimates of \( \text{O}_3 \) effects on vegetation. Models of \( \text{O}_3 \) flux can reduce the variation in the response to \( \text{O}_3 \) that is sometimes observed between years in an experiment (Emerson et al., 2000a, b; Fuhrer et al., 1992; Grünhage et al., 1993; Grünhage and Haenel, 1997; Pleijel et al., 2000a). In a study of \( \text{O}_3 \) deposition to an oat crop in OTCs, \( \text{O}_3 \) flux in the chamber was estimated to be up to twice that in an adjacent field based on a K-theory approach and measurements of stomatal conductance and environmental conditions (Pleijel et al., 1994b). These measurements were made for 2 hours on 5 days when the canopy was physiologically active and wind speeds were moderate. However, the \( \text{O}_3 \) flux in a plant-less chamber was nearly as high as that in the open field. The authors conclude that \( \text{O}_3 \) uptake in the chamber was between 100 and 200% of that in the field. These models of flux have a sound biological and meteorological basis and are useful for interpreting experimental data. Flux models have been successfully applied at intensive study sites with detailed site-specific data on stomatal conductance and micrometeorological conditions (e.g., Fredericksen et al., 1996b; Grünhage et al., 1993, 1994). Yet even at a single well-studied site, different methods can provide different estimates of \( \text{O}_3 \) flux. For example, at a site in a vineyard in California, an evapotranspiration-based method overestimated the \( \text{O}_3 \) flux as
compared to an eddy covariance approach by 20 to 26\% (Massman and Grantz, 1995). At a site in a nearby cotton field the evapotranspiration-based approach overestimated the eddy-covariance method by 8 to 38\%. Flux-response relationships have received substantial attention in Europe during the past decade, as part of an attempt to move beyond the exposure-based Level 1 critical levels to flux-based Level II critical levels (Section AX9.4). However, the database for flux-response relationships is very limited (Grünhage et al., 2004), and these approaches do not always explain greater amounts of response variation than do exposure-based approaches (Karlsson et al., 2004b). The critical level approach is discussed briefly below, because it has been used to extrapolate from field studies to landscapes, countries, and regions.

There has been criticism that the Level I critical level for crops overestimates O\(_3\) effects in the Mediterranean countries, because it was developed based on studies in Northern Europe (De Santis, 1999; De Santis, 2000). However, there is evidence of substantial crop loss due to O\(_3\) in some southern European counties, such as the Po valley in Northern Italy (Fumagalli et al., 2001). In these studies, Heagle-type OTCs were used. Losses in NF chambers as compared to CF chambers over several years at two sites ranged from 11.2 to 22.8\% for barley and wheat, from 0.3 to 31.5\% for other crop species, and from 4.1 to 19.8\% for forage species (Fumagalli et al., 2001). Surprisingly, the least effect was observed for soybean, despite AOT40 values of 9.32 ppm\(\cdot\)h, 3\times the Level I critical level. Similarly, a review of studies in Northern Italy found that ambient O\(_3\) episodes have been reported to cause foliar symptoms on 24 agricultural and horticultural crops in commercial fields (Fumagalli et al., 2001). Ambient O\(_3\) has also been reported to cause yield losses in several crop species, although no data on O\(_3\) exposure were presented by the reviewers (Fumagalli et al., 2001).

The Level I approach has also been criticized for focusing only on a single annual species (wheat) and a single woody perennial species (beech). However, this species focus is appropriate, because the goal was to determine an exposure-response relationship for a sensitive species based on available data. In support of standards in Germany, an effort was made to combine data from different species, and consisted of a meta-analysis of studies conducted in both closed and OTCs (Grünhage et al., 2001). In this study, experiments published between 1989 and 1999 were included and analyzed if they met the following conditions: (1) a significant O\(_3\) effect was determined; (2) exposure conditions were well defined; (3) foliar symptoms, growth, or yield was measured; and (4) plant species were relevant to Europe.

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(Grünhage et al., 2001). Despite the focus on European species, many of the species studies also occur in the United States. Separate regressions for herbaceous plants and for tree species were created as a function of duration of exposure at a given level of O$_3$ exposure at the top of the plant canopy. These regression equations, with confidence limits and with correction for the vertical gradient in O$_3$ from the top of the quasi-laminar boundary layer, can be used to define whether effects are unlikely (below the lower confidence limit), probable (between the confidence limits), or highly likely (above the upper confidence limit) to occur near a given O$_3$-monitoring station.

A further concern about the Level 1 approach is that foliar symptoms, rather than biomass, may be an important endpoint, because foliar symptoms may be more sensitive (VanderHeyden et al., 2001). In an OTC study in southern Switzerland, it was shown that a number of tree species show foliar symptoms at AOT40 values lower than the Level 1 value of 10 ppm·h (VanderHeyden et al., 2001).

Exposure-response relationships developed primarily from OTC experiments, with confirming evidence from other approaches such as resistant and sensitive clover clones exposed in ambient air and FACE experiments, are useful for estimating the effects of ambient O$_3$ on vegetation in the U.S. However, despite the substantial number of experimental studies listed in Table AX9-16 and in previous Air Quality Criteria Documents for O$_3$ (for example, U.S. Environmental Protection Agency, 1996), most studies have been conducted in only a few locations with only a few species of economically important crops. While these studies provide strong evidence that ambient O$_3$ in the U.S. is likely reducing crop yields and plant growth significantly in many regions in many years, it remains difficult to extrapolate to all landscapes and all plant species and to determine whether exposure-response relationships based on existing studies will protect all species in all locations from significant deleterious effects of O$_3$.

**AX9.5.4.6 Summary of Effects on Short-Lived Species**

For annual vegetation, the data summarized in Table AX9-16 show a range of growth and yield responses both within species and among species. Nearly all of these data were derived from studies in OTCs, with only two studies using open-air systems in the UK (Ollerenshaw et al., 1999; Ollerenshaw and Lyons, 1999). It is difficult to compare studies that report O$_3$ exposure in different indices such as AOT40, SUM06, or 7-h or 12-h mean values. However,
when such comparisons can be made, the results of this more recent body of research confirm
the earlier results summarized in the 1996 O_3 AQCD (U.S. Environmental Protection Agency,
1996). A summary of earlier literature concluded that a 7-h, 3-month mean of 49 ppb
corresponding to a SUM06 exposure of 24.4 ppm-h would cause 10% loss in 50% of 49
experimental cases (Tingey et al., 1991). A similar study using a 24-h, rather than 7-h,
averaging period found that a SUM06 exposure of 26.4 ppb would cause 10% loss in 50% of
54 experimental cases (Lee et al., 1994a,b). Recent data summarized in Table AX9-16 support
this conclusion, including data from a FACE study which confirmed results found previously
using OTCs (Morgan et al., in press). These values represent ambient exposure patterns that
occur in some years over large portions of the United States. Some annual species such as
soybean are more sensitive, and greater losses may be expected (Table AX9-16). Thus, the
recent scientific literature supports the conclusions of the 1996 AQCD that ambient O_3
concentrations are reducing the yield of major crops in the United States.

Much research in Europe has used the AOT40 exposure statistic, and substantial effort has
gone into developing Level-1 values for vegetation. Based on regression analysis of 15 OTC
studies of spring wheat, including one study from the United States and 14 from locations
ranging from southern Sweden to Switzerland, an AOT40 value of 5.7 ppm-h was found to
correspond to a 10% yield loss, and a value of 2.8 ppm-h corresponded to a 5% yield loss
(Fuhrer et al., 1997). Because a 4 to 5% decrease could be detected with a 99% confidence
level, a critical level of an AOT40 value of 3 ppm-h was selected in 1996 (Kärenlampi and
Skärby, 1996).

In addition to reductions in crop yield, O_3 may also reduce the quality or nutritive value of
annual species. Many studies have shown effects of O_3 on various measures of plant organs that
affect quality, with most studies focusing on characteristics important for food or fodder. These
studies indicate that there may be economically important effects of ambient O_3 on the quality of
crop and forage species. Previous criteria documents have concluded that foliar symptoms on
marketable portions of crops and ornamental plants can occur with seasonal 7-h mean O_3
exposures of 40 to 100 ppb (U.S. Environmental Protection Agency, 1978, 1986, 1996). The
recent scientific literature does not refute this conclusion.

The use of OTCs may reverse the usual vertical gradient in O_3 that occurs within a few
meters above the ground surface (Section 3.3.2, Section AX9.1). Such a reversal suggests that
OTC studies may overestimate, to some degree, the effects of an O$_3$ concentration measured several meters above the ground, because the O$_3$ concentration is slightly lower at the canopy height than at the height at which O$_3$ is monitored. For example, as shown in Figure 3.6 (Section 3.3.2), under unstable atmospheric conditions that are typical during daylight in agricultural fields, there was an average decrease of 7% in ozone concentration from 4 m to 0.5 m above the surface. However, such considerations do not invalidate the conclusion of the 1996 AQCD (U.S. Environmental Protection Agency, 1996) that ambient O$_3$ exposures (Tables AX9-13 and AX9-21) are sufficient to reduce the yield of major crops in the United States. Recent studies using OTCs confirmed previous results that ambient ozone exposures can significantly reduce the growth of annual species and the yield of commercially important crop species. Additionally, a two-year study using a free air exposure system with soybean confirmed yield reductions found previously using OTCs (Morgan et al, in press).

**AX9.5.5 Effects of Ozone on Long-Lived (Perennial) Species**

Although there has been considerable research in Europe on annual species during the past 10 years, much research in the United States has focused on perennial species. In Europe, and in a few studies in the United States, effects of O$_3$ on mixtures of annual and perennial herbaceous species have been investigated using growth chambers, greenhouses, and OTCs. Section AX9.5.5.1 reviews such studies, with an emphasis on studies using OTCs.

**AX9.5.5.1 Herbaceous Perennial Species**

Two alfalfa cultivars were grown in pots and exposed to CF, NF, 1.5×-ambient and 2×-ambient O$_3$ concentrations in two 1-year studies in Quebec, Canada (Renaud et al., 1997). One cultivar, Apica, is commonly grown in the region, and another, Team, is normally grown farther south and is more tolerant to O$_3$. For Apica in both years and for Team in 1991, O$_3$ exposure caused a linear reduction in above-ground biomass. In the NF treatment, growth of Apica was decreased by 31 and 21% in the 2 years, while the growth of Team was reduced by 14% in 1991, but not reduced in 1992. The authors suggested that the differing effects on Team could be due to different progenies and propagation methods in the 2 years or to more rapid growth in 1991 along with higher O$_3$ peak values in 1991. In 1991, O$_3$ maxima exceeded 60 ppb in 15 days, whereas in 1992 there were only three such days. At the end of the growing season,
total starch reserves in roots were decreased by O$_3$, due primarily to a decrease in root mass, that the authors suggested could accelerate decline in alfalfa yields. These yield reductions are generally similar to those reported previously in five similar studies summarized in the 1996 AQCD (Table 9-25 of U.S. Environmental Protection Agency, 1996).

A study in Alabama exposed early- and late-season-planted Bahia grass (*Paspalum notatum* cultivar Pensacola) in OTCs to CF, NF, or 2×-ambient O$_3$ treatments (Muntifering et al., 2000). Ozone exposures expressed as 12-h mean values over the 24-week experiment were 22, 45, and 91 ppb, and the highest ambient O$_3$ concentrations were recorded in late June, late July, late August and mid-September at approximately 90 ppb. Above-ground biomass growth was reduced by the NF treatment for the first and second harvest by 34% and 29% for the early-season planting, but statistically significant effects were not observed in the late-season planting (Table AX9-17). The 2×-ambient treatment did not cause further significant reductions in biomass. The authors suggested that the lack of a significant O$_3$ effect in the late planting may have been due to the shorter total O$_3$ exposure time as well as to the lower O$_3$-exposure concentrations during the weeks immediately preceding harvest. These results are important, because this is an economically important species and because previous studies have focused on grass species that use the C$_3$, rather than C$_4$, metabolic pathway.

An investigation of the use of different O$_3$ indices and averaging times on the correlation with growth effects was undertaken with the North Carolina clover system (Heagle et al., 1995). For 2 years of data at six sites in Massachusetts, Oregon, North Carolina, California (2 sites), and Virginia, averaging time was found to be more important than the choice of the type of index including mean, SUM06, and AOT40 (Heagle and Stefanski, 2000). The best correlation between O$_3$ exposure and the ratio of sensitive-to-tolerant clover types was found for the 6-h period from 1000 to 1600 h. For this period, very similar $r^2$ values (0.91 to 0.94) were found for SUM06, W126, and AOT40 (Heagle and Stefanski, 2000). For the above indices, a linear relationship was found, with no effect in Corvallis, OR with exposure to a SUM06 value of 10.2 ppm·h and a ratio of 0.53 (sensitive/tolerant) at San Bernardino with a SUM06 exposure of 39.4 ppm·h (Heagle and Stefanski, 2000).

In a study of the biomass ratio of O$_3$-sensitive versus O$_3$-insensitive clover at 14 sites in Europe during 1996 to 1998, a model was developed using ANN techniques (see Section AX9.1) that had $r^2$ values for the training data of 0.84 and for unseen validation data of 0.71 (Mills et al., AX9-233)
The predictive factors in the model were AOT40, 24-h mean O$_3$, daylight mean temperature, and 24-h mean temperature. This model was selected after a thorough investigation of a number of models using many more or fewer parameters using both ANN and multiple linear regression techniques. This model predicted that a 5% reduction in biomass ratio was associated with AOT40 values in the range 0.9 to 1.7 ppm h accumulated over 28 days, with plants being most sensitive under conditions of low NO$_x$, moderate temperature, and high 24-h mean O$_3$ concentration.

Exposure to a square-wave 8-h mean O$_3$ concentration of 92 ppb for 62 days in an experiment in OTCs in the UK did not significantly reduce the total yield of strawberry fruits, but did decrease the average size of the fruits by 14% (Drogoudi and Ashmore, 2000). This contrasts with an increase in total yield (fruit weight) found in a previous study in California (Takemoto et al., 1988).

When timothy was exposed in OTCs in Sweden to NF, CF, and CF+O$_3$ treatments, there was no effect of a 12-h mean O$_3$ exposure of 68 ppb (NF treatment), but a 12-h mean exposure of 152 ppb decreased yield by 58% (Danielsson et al., 1999). A similar lack of effect of exposure to a 12-h mean O$_3$ exposure of 62 ppb was found in a previous study in the United States (Kohut et al., 1988).

Although most investigations of O$_3$-response relationships focus on growth or yield of marketable portions of plants, some studies also investigate effects on plant quality. In the study of bahia grass in Alabama discussed above, in addition to the effects on yield, there were significant effects on quality for ruminant nutrition (Muntifering et al., 2000). Concentrations of neutral detergent fiber (NDF) were higher in primary-growth and regrowth forages from the early-season planting when exposed to 2×-ambient O$_3$ than when exposed to the NF treatment. The concentration of acid detergent fiber was higher in the 2×-ambient treatment than in NF treatment regrowth, whereas acid detergent lignin concentration was higher in 2×-ambient than in NF primary-growth forage. Crude protein concentrations were lower in CF-exposed than in NF-exposed regrowth forage from the early planting and in CF- than in NF-exposed primary-growth forage from the initial harvest of the late-season planting. No differences were observed among treatments in concentrations of total phenolics in primary-growth or regrowth forages from either planting, although concentrations of total phenolics tended to be higher in CF-exposed than in NF-exposed primary-growth forage from the late-season planting. The
authors concluded that the alterations in quality of primary-growth and vegetative regrowth forages were of sufficient magnitude to have nutritional and possibly economic implications to their use for ruminant animal feed.

*Sericea lespedeza* and little bluestem were exposed to CF, NF, and 2×-ambient O$_3$ in OTCs in Alabama for 10 weeks (Powell et al., 2003). Ozone treatments expressed as 12-h mean concentrations were 23, 40, and 83 ppb and expressed as seasonal SUM06 values were 0.2, 9.1, and 61.0 ppm-h. Although there were few statistically significant effects of O$_3$ on yield (the yield of only the 2×-ambient compared to NF for *Sericea lespedeza* in the last of six harvests), plant quality as feed for ruminants was reduced. The nutritive quality of *Sericea lespedeza* was decreased by 7% and that of little bluestem by 2% as a result of increased cell wall constituents and decreased in vitro digestibility.

For some annual species, particularly crops, the endpoint for an assessment of the risk of O$_3$ exposure can be defined as yield or growth; e.g., production of grain. For plants grown in mixtures such as hayfields, and natural or seminatural grasslands (including native nonagricultural species), endpoints other than production of biomass may be important. Such endpoints include biodiversity or species composition and measures of plant quality such as total protein and effects may result from competitive interactions among plants in mixed-species communities. Most of the available data on non-crop herbaceous species are for grasslands.

In a study of two perennial grasses (bent grass and red fescue) and two forbs (white clover, Germander speedwell [*Veronica chamaedrys* L.]) grown in pots in OTCs, O$_3$ effects differed among species and cutting treatments (Ashmore and Ainsworth, 1995) (see also Table AX9-17). Fescue biomass increased with higher O$_3$ treatments both in pots that were not cut during the growing season (mid-June to mid-September) and those that were cut every two weeks. However, bent grass biomass decreased with higher O$_3$ exposure in the uncut treatment and increased in the cut treatment. White clover and Germander speedwell biomass decreased substantially with higher O$_3$ exposure with and without cutting, with greater decreases in the cut treatment. The authors cautioned that the experiment did not replicate field circumstances. The plants were all cut to 1 cm above the ground, which does not simulate grazing, and there may have been effects due to growing the plants in pots. However, two key results of this study likely apply to mixtures of species growing in hay or forage fields or seminatural and natural communities. First, O$_3$ exposure increased the growth of O$_3$-tolerant species, while exacerbating
the growth decrease of O₃ sensitive species. Second, the total biomass of the mixed-species community was unaffected by O₃ exposure due to the differential effects on O₃-sensitive and O₃-tolerant species.

In a 2-year study using OTCs placed over managed pasture in Switzerland, the above-ground biomass of clover (red and white) was reduced linearly in response to increased O₃ exposure (Table AX9-17) (Fuhrer et al., 1994). Exposure to a 12-h mean concentration of 39 ppb O₃ reduced biomass by 24% as compared to the CF treatment with a 12-h mean concentration of 21 ppb O₃. There was a trend toward increased above-ground biomass of grasses (primarily orchard grass), but this trend was not statistically significant. As often found in other studies of mixtures of species, by O₃ exposure did not significantly affect total above-ground biomass O₃ exposure.

A field-grown grass/clover mixture was exposed to CF, NF, and two O₃ addition treatments for two growing seasons in OTCs in southern Sweden (Pleijel et al., 1996). The mixture consisted of 15% (by seed weight) red clover cv. Fanny, 60% timothy cv. Alexander, and 25% fescue cv. Svalofs Sena. Ozone concentrations expressed as AOT40 ranged from 0 to approximately 47 ppm-h and expressed as 7-h mean from 11 to 62 ppb. Over this range, a slight, but statistically significant, linear decrease of 4% in total above-ground biomass was seen growth over six harvests. No significant decrease was seen in the proportion of clover, and the authors ascribed this lack of effect to the relatively higher O₃ sensitivity of timothy and lower sensitivity of this clover cultivar as compared to previously published results for other grass/clover mixtures (e.g., Fuhrer et al., 1994).

A mixture of species in an old farm field in Alabama was exposed to O₃ for two growing seasons in large OTCs (4.8 m high and 4.5 m diameter); and a similar lack of effect of O₃ was found on total plant community growth measured as both canopy cover and vertical canopy density (Barbo et al., 1998). Of the 40 species in the plots, O₃ effects were examined only on the most common species: blackberry, broomsedge bluestem, bahia grass, Panicum spp., and winged sumac (second year only). Of these species, a 2×-ambient O₃ treatment increased the percent canopy cover of blackberry over 2 years by 124%, while that of winged sumac was decreased by 95% (Table AX9-17). Blackberry showed no significant effect on biomass, but ripe fruit mass was decreased by 28% (Chappelka, 2002). However, there was a significant chamber effect for this latter response. Biomass was not reported for other species in this study.
due to a hurricane. Effects on loblolly pine grown in this experiment are discussed subsequently in Section AX9.5.5.5.

In summary, results of studies on perennial herbaceous species conducted since the 1996 criteria document was prepared are presented in Table AX9-17. As for single-season agricultural crops, yields of multiple-year forage crops are reduced at O₃ exposures that occur in some years over large areas of the United States (Tables AX9-13, AX9-21). This result confirms that reported in the 1996 AQCD (U.S. Environmental Protection Agency, 1996). When species are grown in mixtures, O₃ exposure can increase the growth of O₃-tolerant species while exacerbating the growth decrease of O₃-sensitive species (e.g., Ashmore and Ainsworth, 1995; Fuhrer et al., 1994). Because of this competitive interaction, the total growth of the mixed-species community may not be affected by O₃ exposure (Ashmore and Ainsworth, 1995; Barbo et al., 1998; Fuhrer et al., 1994). However, in some cases mixtures of grasses and clover species have shown significant decreases in total biomass growth in response to O₃ exposure in studies in the United States (Heagle et al., 1989; Kohut et al., 1988) and in Sweden (Pleijel et al., 1996). In Europe, a provisional critical level for perennials of an AOT40 value of 7 ppm·h over 6 months has been proposed to protect sensitive plant species from the adverse effects of O₃.

**AX9.5.5.2 Deciduous Woody Species**

It is extremely difficult and costly to study entire mature trees under controlled conditions such as those in OTCs, with the possible exception of some species managed for fruit or nut production. For this reason, the great majority of investigations have been of seedlings in growth chambers, greenhouses, or OTCs. A few investigations have been carried out on saplings or more mature trees using free air exposure systems (Haeberle et al., 1999; Isebrands et al., 2000, 2001; Werner and Fabian, 2002). Exposure-response functions based on 28 experimental cases of seedling response to O₃ suggest that a SUM06 exposure for 3 months of 31.5 ppm·h would protect hardwoods from a 10% growth loss in 50% of the cases (Table AX9-18). However, there is a substantial range in sensitivity among species. A risk analysis was undertaken to predict tree biomass growth reductions due to O₃ based on exposure-response equations for seedlings of individual species combined with the species’ spatial distribution across the eastern United States and interpolated O₃ exposure expressed as SUM06 (Hogsett et al., 1997). The growth of sensitive species such as aspen and black cherry was predicted to be
reduced by at least 20% across 50% of their ranges in a high \( \text{O}_3 \) year and approximately 10% in a lower-than-average \( \text{O}_3 \) year (Hogsett et al., 1997).

A few investigations reported since the last criteria document was prepared have examined saplings or mature trees, notably of oak species in the southern Appalachian Mountains and pine species in California. Most of these studies have been of natural (uncontrolled) \( \text{O}_3 \) exposures. Additional studies have examined foliar symptoms on mature trees, and in recent years such surveys have become more common and with greater attention to the standardization of methods and the use of reliable indicator species (Campbell et al., 2000; Smith et al., 2003). Previous criteria documents have noted the difficulty in relating foliar symptoms to effects on individual tree growth, stand growth, or ecosystem characteristics (U.S. Environmental Protection Agency, 1996). This difficulty still remains to the present day.

Some investigators have suggested that a comprehensive risk assessment of the effects of \( \text{O}_3 \) on mature tree species might best be accomplished by extrapolating measured effects of \( \text{O}_3 \) on seedlings to effects on forests using models based on tree physiology and forest stand dynamics (Chappelka and Samuelson, 1998; Laurence et al., 2000, 2001). Several such efforts are discussed in Sections AX9.3 and AX9.6.

In this subsection, emphasis will be placed on experimental evidence of \( \text{O}_3 \) effects on the growth of woody species under controlled conditions with some information from observational studies under ambient conditions in forests. Experimental results are summarized for deciduous species in Table AX9-18; the species are discussed below in the order in which they appear in this table.

A series of studies in Michigan and Wisconsin during the 1990s on clones of trembling aspen previously demonstrated that they differ in their \( \text{O}_3 \) sensitivity (Coleman et al., 1995a,b, 1996; Dickson et al., 2001; Isebrands et al., 2000, 2001; Karnosky et al., 1996, 1998, 1999; King et al., 2001). Several of those studies were undertaken with plants in pots or in the ground in OTCs and additional studies were undertaken at three sites selected to differ primarily in \( \text{O}_3 \) exposure (Karnosky et al., 1999). An ongoing study was undertaken using a FACE carbon dioxide and \( \text{O}_3 \) enrichment facility in Rhinelander, WI (Isebrands et al., 2000, 2001). These studies showed that \( \text{O}_3 \)-symptom expression was generally similar in OTCs, FACE, and gradient sites, supporting the previously observed variation among aspen clones (Karnosky et al., 1999). In the Michigan OTC study, plants were grown in pots and exposed to CF, 0.5× ambient, 1×
ambient, 1.5× ambient, 2×-ambient O\textsubscript{3} treatments for 98 days (Karnosky et al., 1996). Ozone concentrations expressed as 3-month, 7-h mean values were 7 ppb (CF), 43 ppb (1×) and 63 ppb (2×). Ozone decreased total plant biomass between 2 and 22% for three clones previously selected to represent high, intermediate and low O\textsubscript{3} tolerance based on previous studies of larger populations (clones 216, 271 and 259; Karnosky et al., 1996). Seedlings produced from 15 parent trees responded similarly as did the 3 clones, with an average biomass reduction of 14% in the 1×-ambient treatment for the seedlings compared to 16% for the clones. In a second experiment using square wave exposures, biomass reduction for the clones was ranged from 23 to 39% (mean = 31%) at a 7-h mean O\textsubscript{3} concentration of only 45 ppb, which is similar to the response to the 2×-ambient treatment in the previous experiment at a 7-h mean O\textsubscript{3} concentration of 66 ppb.

The FACE study evaluated the effects of multiple years of exposure to combinations of elevated CO\textsubscript{2} and 1.5× ambient O\textsubscript{3} on growth responses in mixture of five trembling aspen clones (Isebrands et al., 2000, 2001). Height, diameter, and stem volume (diameter\textsuperscript{2} × height) were decreased by elevated O\textsubscript{3}. On average for all clones, stem volume was decreased by 20% over the first 3 years in the elevated O\textsubscript{3} treatment as compared with the 1×-ambient treatment. However, one clone showed increased growth in response to O\textsubscript{3}. Ozone concentrations were not reported. Over the first 7 years of the study, average stem volume was decreased by 14% with 12-h mean O\textsubscript{3} concentrations between 49 and 55 ppb as compared with effects at ambient O\textsubscript{3} concentrations with 12-h mean values of 35 to 37 ppb (O\textsubscript{3} exposure data are for the first 4 years, as they have not been reported for subsequent years) (Karnosky et al., 2003b, 2005). This FACE facility study is important, because it confirmed responses reported previously with these clones grown in pots or soil in OTCs, without the alterations of microclimate induced by chambers. Currently, this is the only U.S. study using this technology to have examined the effects of O\textsubscript{3} under these conditions. This study is also significant, because the elevated O\textsubscript{3}-exposure pattern used was intended to reproduce the 6-year average pattern from Washtenaw County, Michigan (Karnosky et al., 1999).

Rooted cuttings of two aspen clones from Acadia National Park in Maine were exposed to 1×-ambient, 1.7×-ambient, and 3×-ambient O\textsubscript{3} concentrations in large OTCs in Ithaca, NY for much of one growing season (15 June to 15 September) (Yun and Laurence, 1999a). Both circular (4.7 m diameter, 3.7 m height) and rectangular (7.4 m × 2.75 m × 3.7 m height)
chambers were used (Mandl et al., 1989). Exposure to 1.7×-ambient O₃ (SUM06 = 20 ppm·h, 9-h mean = 74 ppb) reduced shoot growth by 14 and 25% compared to ambient O₃ for the two clones (Yun and Laurence, 1999a). Total dry weight was reduced by 55 and 35% in the two clones by the 3×-ambient treatment (SUM06 = 62 ppm·h, 9-h mean = 124 ppb) compared to the ambient O₃ treatment.

When black poplar cuttings in OTCs in Belgium were exposed to 8-h mean O₃ concentrations of 5, 29, and 33 ppb, diameter growth decreased by 29% in the highest O₃ treatment, but height growth was unaffected (Bortier et al., 2000b). A 2-month study of hybrid poplar (Populus tremuloides × P. tremul) in a free air exposure system in Finland with 7-h mean O₃ concentrations of 30 and 38 ppb found a 6% decrease in height with no effect on biomass (Oksanen et al., 2001). Eastern cottonwood cuttings in pots buried in the ground with drip irrigation were exposed to ambient O₃ at several sites in and near New York City in three 2-month experiments during three summers (Gregg et al., 2003). Ozone concentrations were lower at urban sites than at rural sites within 100 km of the urban sites. Total biomass growth was greater in urban than rural sites, with a strong linear decrease in biomass with increasing O₃ across all sites and years (r² = 93). Total biomass decreased 33% with 12-h mean O₃ levels of 38 ppb compared to 23 ppb. Multiple regression analysis showed no significant temperature effect on biomass. Therefore, the authors suggested that O₃ exposures were the most likely explanation for the reduced biomass in rural areas. The overall growth reductions and the variation among genotypes seen on all of the above aspen and poplar studies is similar to those previously reported in three OTCs studies summarized in the 1996 O₃ AQCD (Table 9-26 of U.S. Environmental Protection Agency, 1996).

For paper birch, over the first 7 years of the Wisconsin FACE study, average stem volume was unaffected by 12-h mean O₃ concentrations between 49 and 55 ppb as compared to effects at ambient O₃ concentrations with 12-h mean values of 35 to 37 ppb (based on O₃ exposure data for the first 4 years, as they have not been reported for subsequent years) (Karnosky et al., 2003b, 2005). In contrast, significant effects were found in this study for aspen and sugar maple, so these results indicate that paper birch is relatively insensitive to O₃ compared to these other species.

Black cherry seedlings grown in pots were exposed in OTCs in the Great Smoky Mountain National Park in Tennessee to O₃ treatments ranging from CF to 2×-ambient in two experiments.
during 1989 and 1992 (Neufeld et al., 1995). Ozone exposure, expressed as SUM06, ranged from 0 to 40.6 ppm h in 1989 and from 0 to 53.7 ppm h in 1992. Corresponding AOT40 values were 0.0 to 28.3 ppm h in 1989 and 0 to 40.4 ppm h in 1992. In 1989, total biomass was decreased in the 1.5×-ambient treatment by 18% and in the 2×-ambient treatment by 38%. In 1992, total biomass was decreased in the 1.5×-ambient treatment by 27%, and in the 2×-ambient treatment by 59%. In this study, SUM06 and AOT40 provided better fits than did SUM00 with Weibull regression analyses to log-transformed biomass data. Although a Weibull model was used, responses to O<sub>3</sub> expressed as SUM06 and AOT40 appeared to be linear. The O<sub>3</sub> exposures in the 1.5×-ambient and 2×-ambient treatments were reported to be similar to those for a site near Charlotte, NC in a high-O<sub>3</sub> year (1988). In a 2-year experiment in OTCs in Ohio, seedlings of black cherry, sugar maple, and yellow poplar were exposed to O<sub>3</sub> treatments with SUM00 values ranging from 16 to 107 ppm h in 1990 and 31 to 197 ppm h in 1991 (Rebbeck, 1996). After two seasons of exposure, only black cherry showed a growth decrease: total biomass was reduced by 32% in the 2×-ambient O<sub>3</sub> treatment compared to the CF treatment; root biomass was decreased by 39%. These results contrast with those of a previous study with black cherry seedlings in which significant biomass reductions with exposures up to 2×-ambient were not observed (7-h mean = 21 to 97 ppb), perhaps because of the small sample size (3 seedlings per chamber (Samuelson, 1994) in the earlier study.

A multiyear study of effects of O<sub>3</sub> on both seedling and mature (30-year-old) red oak trees was conducted in Norris, TN in large OTCs with three replicates per O<sub>3</sub> treatment. Trees were exposed for 3 years to CF, 1×-ambient, and 2×-ambient treatments, with the following O<sub>3</sub> exposures: SUM06 for 3 years = 0, 29, 326 ppm h; SUM00 for 3 years = 147, 255, and 507 ppm h. The net photosynthetic rate in mature trees was reduced by 25% in the ambient treatment and by 50% in the 2×-ambient treatment (Samuelson and Edwards, 1993; Hanson et al., 1994; Wullschleger et al., 1996). Despite these large decreases, no significant effects on stem increment at the base, stem increment in the canopy, or leaf mass were observed for the mature trees (Samuelson et al., 1996). Similarly, seedling biomass was not significantly reduced by O<sub>3</sub> exposure. The difficulty in replicating experiments with mature trees makes it difficult to detect changes in growth or biomass. However, the mean values of the stem increment at the base and within the canopy in the ambient treatment were larger than those in the CF treatment, although those in the 2×-ambient treatment were lower. Therefore, this study of mature trees
does not provide evidence that these ambient concentrations reduced above-ground tree growth, even after 4-years exposure.

Sugar maple seedlings were exposed for 3 years to ambient, 1.7×-ambient, and 3×-ambient O$_3$ treatments at both high-light (35% of ambient) and low-light levels (15% of ambient) (Topa et al., 2001). This experiment was conducted in large OTCs near Ithaca, NY. Over the 3 years, O$_3$ exposures expressed as SUM00 for the three treatments were 88, 126, and 185 ppm-h. After 3 years, total seedling biomass in the 3×-ambient treatment was reduced by 64% and 41% in the low- and high-light treatments, respectively (compared to the 1×-ambient treatment). The larger reduction of biomass under low-light conditions suggests that seedlings growing under closed canopies may be substantially more sensitive to O$_3$ than are seedlings exposed to higher-light levels in gaps or clearings. These results differ from other studies in which seedling biomass was unaffected by exposure to SUM00 values of 304 ppm-h over 2 years (Rebbeck, 1996) or 591 ppm-h over 3 years (daytime mean of 40.7) (Laurence et al., 1996). However the latter two studies used much higher light levels, which may have reduced the O$_3$ effect, based on the results of Topa et al. (2001). Over the first 7 years of the Wisconsin FACE study, average stem volume of sugar maple was decreased by 14% with 12-h mean O$_3$ concentrations between 49 and 55 ppb as compared with effects at ambient O$_3$ concentrations with 12-h mean values of 35 to 37 ppb (based on O$_3$ exposure data for the first 4 years, as they have not been reported for subsequent years) (Karnosky et al., 2003b, 2005). These growth effects were not statistically significant for the first 3 years, but became significant subsequently. These results are important because they demonstrate that 3 years of exposure may not be long enough to evaluate effects of O$_3$ on the growth of tree species.

Although most studies demonstrate that O$_3$ decreases biomass growth, occasional results indicate that O$_3$ can increase growth of some portions of woody perennials. When Casselman plum trees near Fresno, CA were exposed to O$_3$ in large, rectangular OTCs to three O$_3$ treatments (CF, 1×-ambient, and an above-ambient O$_3$ treatment) for 4 years (12-h mean = 31, 48, 91 ppm-h), stem increment increased 14% in the highest O$_3$ treatment compared to the CF treatment; and this effect was statistically significant (Retzlaff et al., 1997). However, fruit yield decreased in this treatment by 42% and also decreased by 16% in the 1×-ambient-O$_3$ treatment. Root growth was not measured in this study. Hence, the increase in stem diameter may have been at the expense of other organs. However, in a fifth year, all plants were exposed to
1×-ambient O₃, and there were no differences in fruit yield, suggesting that trees were able to recover to some extent from the effects of O₃ exposure in prior years.

When potted yellow poplar seedlings were exposed to O₃ concentrations up to SUM00 values of 107 ppm·h in one year and 197 ppm·h in a second year, no effects on biomass were observed (Rebbeck, 1996). In a study at the same location with seedlings planted in the ground and exposed to O₃ concentrations with SUM06 values of 0.3, 228.7, and 661.8 ppm·h over 5 years, no effects on biomass were found (Rebbeck and Scherzer, 2002).

Many studies have demonstrated that root growth is more sensitive to O₃ exposure than is stem growth. For example, in a study with black cherry seedlings exposed in OTCs in Tennessee in 1989, root biomass in a 2×-ambient treatment was decreased by 42%, while stem biomass was decreased by only 24%. However, in a second experiment in 1992, root and stem growth reductions in the 2×-ambient treatment were similar (65% versus 60%) (Neufeld et al., 1995). In Finland, reduced root growth was found for a number of clones of silver birch (Oksanen and Saleem, 1999). After 5 years, root growth was decreased by 33%, but shoot growth was not affected by O₃ exposures of a 7-h mean of 15 ppm·h over 5 years in a FACE system (Oksanen, 2001). When first-year poplar seedlings (P. tremuloides) were exposed in OTCs to two O₃ concentrations and six N concentrations, the root/shoot ratio was decreased soon after exposure to O₃ in all N treatments, even though O₃ effects on total biomass were not detected in the low-N and very high-N treatments (Pell et al., 1995). These results suggest that effects on the root/shoot ratio occur before significant growth effects arise. In a series of OTC experiments lasting 1 to 3 years at 3 different elevations in Switzerland, fine root growth in European beech was found to be more sensitive to O₃ than was shoot or total biomass (Braun and Fluckiger, 1995). Although the estimated effect of O₃ on fine root biomass was similar to that for total biomass, fine root biomass was significantly decreased at AOT40 (24-h) values of only 3 ppm·h, while total biomass was not significantly decreased until AOT40 values reached 30 to 40 ppm·h.

**AX9.5.5.3 European Critical Levels**

In Europe, a Level I critical level has been set for forest trees based on OTC studies of saplings. This level is discussed here because it was based on a deciduous tree species. For consistency with the approach used for crops, an AOT40 index value was selected. A few
studies have shown that O$_3$ can be taken up by tree species at nighttime, e.g., young birch trees (Matyssek et al., 1995). However, because most evidence suggests that O$_3$ deposition at nighttime is low (Coe et al., 1995; Rondon et al., 1993), a value for only daylight hours was selected in Europe (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996). European beech was selected for development of a Level I critical level, because data from several studies were available for this species and because deciduous tree species were judged to be more sensitive to O$_3$ compared to evergreen tree species (Fuhrer et al., 1997; Kärenlampi and Skärby, 1996). A critical level was defined as an AOT40 value of 10 ppm·h for daylight hours for a 6-month growing season (Kärenlampi and Skärby, 1996). However, other studies have shown that other species such as silver birch may be more sensitive to O$_3$ than beech (Pääkkönen et al., 1996).

Level I critical values are not designed for making quantitative estimates of the O$_3$ effects on vegetation at the regional scale, instead a so-called Level II critical value is required for this purpose. For long-lived perennials, additional problems complicate extrapolation. As discussed below (Section AX9.5.5.7), considerable scaling is required to extrapolate from experiments conducted with tree seedlings to estimate effects on mature trees in forests. Because of these scaling issues, there is greater uncertainty in estimating effects on forest trees than on annual plants such as crops. While some information is available for addressing issues such as scaling from seedlings to mature trees and estimating O$_3$ uptake, this information may still be insufficient for developing a Level II approach that can provide quantitative estimates of forest growth losses due to O$_3$ (Broadmeadow, 1998).

**AX9.5.5.4 Summary of Effects on Deciduous Woody Species**

Recent evidence from free air exposure systems and OTCs supports results observed previously in OTC studies (Table AX9-15, Figure AX9-18). Specifically, a series of FACE studies undertaken in Rhinelander, WI (Isebrands et al., 2000, 2001) showed that O$_3$-symptom expression was generally similar in OTCs, FACE, and ambient-O$_3$ gradient sites, supporting the previously observed variation among aspen clones using OTCs (Karnosky et al., 1999). This study also found no effects on sugar maple growth after 3 years, but in years 4 to 7 found significant growth reduction due to O$_3$ (Karnosky et al., 2005). These results are important, because they indicate results obtained from OTCs are supported by results from free air exposure systems and also that more than 3 years may be required to adequately investigate the effects of
O₃ on the growth of tree species. Finally, this study found that competition may alter the effect of O₃, depending on environmental conditions and genotype (McDonald et al., 2002). New evidence is also available comparing various aspects of O₃ sensitivity between seedlings and mature trees of some species, notably red oak. As has been observed in previous O₃ criteria documents, root growth is often found to be the most sensitive indicator in terms of biomass response to O₃.

Results since 1996 support the conclusions of the 1996 AQCD (U.S. Environmental Protection Agency, 1996) that individual deciduous trees are generally less sensitive to O₃ than are most annual plants, with the exception of a few genera such as *Populus*, which are highly sensitive. However, the data presented in Table AX9-18 suggest that ambient exposures that occur in different regions of the United States can sometimes reduce the growth of seedlings of deciduous species. Results from multiple-year studies sometimes show a pattern of increasing effects in subsequent years. Although, in some cases, growth decreases due to O₃ become less significant or even disappear over time. While some mature trees show greater O₃ sensitivity in physiological parameters such as net photosynthetic rate compared to seedlings, these effects may not translate into measurable reductions in biomass growth. Because even multiple-year experiments do not expose trees to O₃ for more than a small fraction of their life span and because competition may, in some cases, exacerbate the effects of O₃ on individual species, determining the effects on mature trees remains a significant challenge. Effects on mature trees under natural conditions are discussed after the review of evergreen species below and more fully in Section AX9.6, in the context of extrapolating from controlled studies to forest ecosystems.

**AX9.5.5.5 Evergreen Woody Species**

Most investigations have shown evergreen tree species to be less sensitive to O₃ compared to deciduous species (U.S. Environmental Protection Agency, 1996). For example, exposure-response functions based on 23 experimental cases of seedling response to O₃, suggest that a SUM06 exposure for 3 months of 42.6 ppm·h would protect evergreen species from a 10% growth loss in 50% of the cases (Table AX9-15). For deciduous species, the corresponding SUM06 value was 31.5 ppm·h (Table AX9-15). As another example, experiments in the Great Smoky Mountains National Park found black cherry seedlings to demonstrate substantial
decreases in biomass, as discussed above and shown in Table AX9-18 (Neufeld et al., 1995). However, exposure for up to three growing seasons did not decrease the biomass of eastern hemlock, Table Mountain pine, and Virginia pine seedlings exposed to O$_3$ under similar conditions in this location, as shown in Table AX9-19 (Neufeld et al., 2000).

As for deciduous species, there is a substantial range in sensitivity among evergreen species. As discussed above for deciduous species, a risk analysis was undertaken to predict tree biomass growth reductions due to O$_3$ based on exposure-response equations for tree seedlings combined with species distribution across the eastern United States and interpolated O$_3$ exposure (Hogsett et al., 1997). While some species such as Virginia pine were predicted to be affected only slightly even in a high O$_3$ year, the growth of sensitive evergreen species such white pine was predicted to be reduced by 5% in a lower-than-average O$_3$ year and 10% in a high O$_3$ year across 50% of its range (Andersen et al., 1997). The remainder of this section discusses experimental results for evergreen species in the order shown in Table AX9-19.

Douglas fir seedlings were exposed to elevated O$_3$ concentrations in a free air zonal air pollution system in British Columbia, Canada for two growing seasons with 12-h mean values in 1988 of 18 to 41 ppb and in 1989 of 27 to 66 ppb (Runecles and Wright, 1996). Although substantial variation was seen in effects among the different treatments, there was a significant decrease in the growth of the second flush weight in the second year, with reductions of 55% at the highest O$_3$ exposure, based on a linear regression. This result contrasts with the lack of effect seen in a previous study with seedlings of this species grown in pots for 134 days and exposed to 7-h mean O$_3$ concentrations up to 71 ppb (Table 9-30 in U.S. Environmental Protection Agency, 1996).

First-year loblolly pine seedlings of 53 open-pollinated families were exposed to 1×-ambient O$_3$ in OTCs for a single growing season, and average growth volume was decreased by 14% compared to a CF treatment (McLaughlin et al., 1994). The 1×-ambient O$_3$ exposure in this study expressed as 24-h SUM00 was 137 ppm·h, and the CF treatment reduced O$_3$ by 47%. In this study, the root-to-shoot ratio was decreased significantly in six of the nine families examined. Exposure to O$_3$ with SUM06 values up to 162 ppm·h and 132 ppm·h in 2 successive years in OTCs had no effect on seedlings grown in competition with various species of grasses and forbs (Barbo et al., 2002). Exposure of 3-year-old seedlings to O$_3$ exposures of up to

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2.5×-ambient (12-h mean of 98 ppb) also had no significant effect (Anttonen et al., 1996). Four-week-old loblolly pine seedlings were grown in large OTCs in Alabama and exposed to CF, 1× ambient, 2× ambient O₃ treatments in 2 one year experiments, with seasonal 12-h mean O₃ concentrations of 13, 47, 98 ppm·h in 1998 and 12, 44, 97 ppm·h in 1999 (Estes et al., 2004). Shoot biomass was decreased 15% in the 1× treatment and 22% in 2× in both years, while root biomass was decreased by 26% in the 2× treatment in both years. Foliar symptoms were significantly greater in the 1× treatment in 1999 and in the 2× treatment in both years. Information summarized in the 1996 AQCD (U.S. Environmental Protection Agency, 1996), indicated that significant effects on seedling growth were observed in several studies of seedlings exposed to elevated O₃ concentrations for one or more years. Several studies, including that of McLaughlin et al. (1994), demonstrate considerable variation in O₃ sensitivity among different genotypes of loblolly pine.

For Ponderosa pine seedlings, the 1996 AQCD reviewed a number of studies with exposures to elevated O₃ concentrations for one to three growing seasons (U.S. Environmental Protection Agency, 1996). More recent similar studies support the earlier results (Table AX9-19) (Andersen et al., 2001; Takemoto et al., 1997). The 1996 criteria document also discussed at some length the ongoing work examining effects of O₃ across a naturally-occurring O₃ gradient in the San Bernardino Mountains in California. Since that time, much research on ponderosa pine has focused on interactive effects of additional stresses such as nitrogen and on effects of O₃ on physiological parameters (Sections AX9.3, AX9.6). Effort has also been focused on the effects of O₃ on root growth because such effects could alter sensitivity to drought or nutrient stress. Ecosystem level effects of O₃ are discussed further in Section AX9.6, but some information relevant to exposure-response relationships is discussed below.

For several tree species, O₃ has been shown in experimental studies with seedlings to reduce root growth more than shoot growth (U.S. Environmental Protection Agency, 1996). Ponderosa pine has been shown to be sensitive to O₃, and studies with seedlings have shown reduced root growth, decreases root-to-shoot ratios, and decreased allocation to roots (Andersen et al., 1991, 1997; Andersen and Rygiewicz, 1995; Andersen and Scagel, 1997; Tingey et al., 1976b). Data from a long-studied pollution gradient in the San Bernardino Mountains of southern California suggests that O₃ substantially reduces root growth in natural stands of
ponderosa pine. Root growth in mature trees was decreased at least 87% in a high pollution site as compared to a low pollution site (Grulke et al., 1998a), and a similar pattern was found in a separate study with whole tree harvest along this gradient (Grulke and Balduman, 1999). Because other potential influences on root growth, including shading by competing trees, soil temperature, soil moisture, phenology, were not correlated with the observed pattern of reduced root growth, the authors conclude that $O_3$ was the cause of the observed decline in root growth. Further results of field investigations with ponderosa pine and other pine species native to California are discussed below under the heading “Scaling experimental data on long-lived species to field conditions” as well as in Section AX9.6.

Table Mountain pine, Virginia pine, and eastern hemlock seedlings were exposed to various levels of $O_3$ (CF to 2× ambient) in OTCs for in a series of experiments two or three years in Great Smoky Mountains National Park in Tennessee (Neufeld et al., 2000). There were no statistically significant effects of $O_3$ exposure on stem or root biomass, and only slight effects on the biomass of the oldest needles in Table Mountain pine in the 2× ambient treatment.

As reviewed in the 1996 criteria document, studies of the response of red spruce to $O_3$ exposures generally have not found effects on growth of seedlings or saplings, even after exposure to high concentrations (12-h mean of 90 ppb) for up to 4 years. A report since that time confirms that this slow-growing species is $O_3$ insensitive for at least several years (Laurence et al., 1997).

For perennial vegetation, cumulative effects over more than one growing season may be important. For 3-year-old Norway spruce in Sweden, exposure to elevated $O_3$ for three growing seasons decreased total biomass by 18% and stem biomass by 28% (Karlsson et al., 1995). However, after a fourth season of exposure, total biomass decreased significantly by only 8% (Karlsson et al., 2002). In this experiment, the $O_3$ exposures expressed as 12-h mean values averaged over four growing seasons were 12 and 44 ppb for the CF and 1.5×-ambient treatments, respectively; and AOT40 values were 2 and 23 ppm·h, respectively. Despite 4 years of exposure, this experiment did not demonstrate a consistent trend in the $O_3$ effect on biomass that would suggest a significant carry-over effect. However, a study of 3- to 7-year-old Norway spruce in OTCs in Finland found a 5.3% decrease in total plant biomass after 7 years, with an elevated $O_3$ AOT40 exposure value of 79 ppm·h (Ottosson et al., 2003; Wallin et al., 2002).
AX9.5.5.6 Summary of Effects on Evergreen Woody Species

In summary, the O$_3$ sensitivity of different genotypes within species and between species of evergreen vegetation varies widely. Based on studies with evergreen seedlings in OTCs, major species in the United States are generally less sensitive than are most deciduous trees, and slower-growing species are less sensitive than faster-growing ones. However, exposure to ambient O$_3$ may reduce the growth of seedlings of commonly occurring species. Because tree species are long-lived, most experiments have only covered a very small portion of the life span of a tree, making estimation of any effect on mature trees difficult. Considerations for scaling the results of seedling studies to mature forest trees as well as additional information from field surveys and studies of mature trees under natural conditions are discussed below and in Section AX9.6.

AX9.5.5.7 Scaling Experimental Data to Mature Trees

As compared with annual crop species, it is much more difficult to define appropriate exposure-response relationships for tree species. For annual species, an experiment may cover the whole life span of the plant, but it is difficult and expensive to provide controlled-exposure conditions for long-lived plants for any significant portion of their life spans, although a few FACE studies have demonstrated that it is feasible. However, FACE studies cannot investigate the effects of ambient O$_3$ exposures, because lower-than-ambient O$_3$ treatments cannot be applied. Most studies have used small seedlings, because they are manageable under experimental conditions; but seedlings and mature trees may have different sensitivities to O$_3$. For perennial species, effects of O$_3$ may accumulate over more than 1 year, and may interact with other stresses such as drought stress over multiple growing seasons. As for annual species (Section AX9.3.2), substantial variability occurs among evergreen genotypes and this variation may interact with other stress responses differently in different landscapes and regions. Despite these difficulties, investigators have addressed some of these issues since the 1996 AQCD (U.S. Environmental Protection Agency, 1996). New information is available on the response of mature evergreen trees to O$_3$ under field conditions, and models based on tree physiology and stand dynamics have been used to predict O$_3$ effects on forest stands and regions. The following issues are reviewed briefly below: (1) interaction of drought and O$_3$ stress, (2) scaling data from
seedlings to mature-tree studies. Two additional scaling issues are addressed in Section AX9.6: (1) scaling data to forest stands, and (2) scaling data to ecosystems and regions.

**AX9.5.5.7.1 Interactive Effects of Drought and Ozone**

Many interacting factors may influence the effect of O$_3$ on vegetation. For crop plants, environmental conditions are often managed such that nutrients and water are not strongly limiting; but for native vegetation, including most perennial species, such factors are likely to limit growth. The effects of interacting stresses on vegetation were reviewed in Section AX9.3. However, because drought is common in many forests, and because there is substantial evidence that it alters the response of trees to O$_3$, it is discussed in this section in the context of determining exposure-response relationships for trees.

Controlled experiments with seedlings provide direct evidence that drought can reduce the impact of O$_3$. For example, for 3-year-old Norway spruce in Sweden, exposure to elevated O$_3$ for three growing seasons decreased total biomass by 18% and stem biomass by 28% (Karlsson et al., 1995). However, for droughted trees, both total and stem biomass decreased only 5%, with a statistically significant interaction with O$_3$ for stem biomass. Yet after a fourth season of exposure, there was no longer any interaction between drought and O$_3$, while there was a significant decrease of 8% in the biomass when both drought and well-watered data were combined (Karlsson et al., 2002). In this study, seedlings were grown in sand in 120-L pots and for the drought treatment, water was withheld for 4 weeks during the first year and for 7 to 8 weeks during each of the last 3 years. In this experiment, the O$_3$ exposures expressed as 12-h seasonal daylight mean averaged over four growing seasons were 12 and 44 ppb for the CF and 1.5×-ambient treatments, respectively. Over this period, the AOT40 values for the treatments averaged 2 and 23 ppm·h respectively. Despite 4 years of exposures, this experiment did not demonstrate a consistent trend in drought O$_3$ interactions. The difference in effects seen between the third and fourth season suggest that scaling drought-O$_3$ interactions from seedlings to mature trees may be difficult. However, evidence from biomonitoring surveys supports an interaction between drought and O$_3$ effects, at least for foliar symptoms. In systematic surveys of foliar symptoms on species selected as biomonitors throughout much of the eastern United States, symptoms were more common and more severe in areas with high O$_3$ concentrations (Smith
et al., 2003). However, in very dry years, such as 1999, the occurrence and severity of symptoms was greatly reduced, even in areas with high ambient O$_3$ concentrations.

**AX9.5.5.7.2 Scaling from Seedlings to Mature Trees**

Because most experiments are conducted with seedlings, various methods are required to scale experimental data on seedlings to mature trees. An overview of physiological differences between young and old plants, and the consequences of these differences for O$_3$ sensitivity, was provided in Section AX9.3.5.3. The discussion below focuses on information relevant to developing exposure-response relationships for mature trees. Information from a few experimental studies, as well as scaling efforts based on physiological characteristics incorporated into models, are discussed in Section AX9.6.

Although most studies continue to examine the effects of O$_3$ on seedlings, during the 1990s some studies examined the effects of O$_3$ on the response of mature trees. Studies of mature trees demonstrate differences in some aspects of O$_3$ sensitivity between seedlings and mature trees. For some species, such as red oak, seedlings are less sensitive to O$_3$ than are mature trees (Hanson et al., 1994; Samuelson and Edwards, 1993; Wullschleger et al., 1996). Both red oak seedlings and genetically related mature trees were exposed to CF, 1×-ambient, or 2×-ambient O$_3$ exposures in OTCs in Tennessee for two growing seasons (Hanson et al., 1994). Nine large chambers (4.6 × 8.2 m) were used to enclose individual mature trees and standard EPA-style OTCs were used for potted seedlings. Ozone exposures expressed as a 24-h SUM00 were 34, 79, and 147 ppm·h in 1992 and 37, 95, 188 ppm·h in 1993 for the sub-ambient, and 2×-ambient treatments. Mature trees had a greater light-saturated net photosynthetic rate and stomatal conductance compared to seedling foliage at physiological maturity. By the end of the growing season, exposure to 1×-ambient and 1×-ambient O$_3$ reduced the light-saturated net photosynthetic rate and stomatal conductance of mature trees by 25 and 50%, respectively, compared with the CF treatment (35 ppm·h). In seedlings, however, light-saturated net photosynthetic rate and stomatal conductance were less affected by O$_3$ exposure. The authors concluded that extrapolations of the results of seedling-exposure studies to foliar responses of mature forests without considering differences in foliar anatomy and stomatal response between juvenile and mature foliage may introduce large errors into projections of the O$_3$ responses of mature trees.
In a study of ponderosa pine in California, seedlings and branches of mature trees (in branch chambers) were exposed to O$_3$ concentrations of 0.5-, 1-, and 2×-ambient O$_3$ concentrations (Momen et al., 1997). Net photosynthetic rate of 1-year-old, but not current-year, foliage was reduced in mature trees but not significantly reduced in seedlings. This effect was not due to alteration of stomatal conductance by O$_3$. This result contrasts with those with earlier studies of red spruce (Rebbeck et al., 1993).

In contrast to the findings for red oak and Ponderosa pine, giant sequoia seedlings had higher rates of stomatal conductance, CO$_2$-exchange rate, and dark respiration than did mature trees (Grulke and Miller, 1994). As compared to older trees, stomatal conductance was more than 7-fold greater in current-year, and 4-fold greater in 2-year-old, seedlings (Grulke and Miller, 1994). The authors concluded that giant sequoia seedlings are sensitive to atmospheric O$_3$ until ~5 years of age. Low conductance, high water use efficiency, and compact mesophyll all contribute to a natural O$_3$ tolerance, or to O$_3$ defense, or to both, in the foliage of older trees. Similarly, lower stomatal conductance was found in mature Norway spruce in Austria compared to seedlings grown with optimal water and nutrients in a growth chamber (Wieser, 1997). In this study, net photosynthetic rate was less sensitive to added O$_3$ in mature trees compared to seedlings. In a related study, the average rate of O$_3$ uptake of 17-year-old trees ~0.6 nmol m$^{-2}$ s$^{-1}$, decreasing linearly in older trees, such that rates were only ~0.1 m$^{-2}$ s$^{-1}$ in 216-year-old trees (Wieser et al., 1999).

Based on a review of studies of stomatal conductance in both seedlings and mature trees, Samuelson and Kelly (2001) concluded that O$_3$ uptake in oak species, black cherry, sugar maple, and American beech averaged 47% lower in potted seedlings than in mature trees. For evergreen species, they concluded that O$_3$ uptake in seedlings averaged 26% higher than in mature trees. They also suggested that artifacts introduced by growth in pots confound these differences that exposure-response functions derived from seedlings grown in situ are more applicable to mature trees than are studies of seedlings grown in pots (Samuelson and Kelly, 2001).

As discussed above for annual vegetation, it has long been noted that internal O$_3$ dose is more appropriate than external O$_3$ exposure for assessing the effects of O$_3$ on vegetation, because effects occur primarily via the uptake of O$_3$ through the stomata (Section AX9.2.2). However, external O$_3$ exposure sometimes has been shown to explain O$_3$ effects as well or better than calculated internal O$_3$ dose. For ponderosa pine, Grulke and others (2002b) found little
difference in the response of net photosynthetic rate and stomatal conductance to O\textsubscript{3} exposure as compared to calculated O\textsubscript{3} uptake; and estimated O\textsubscript{3} uptake by ponderosa pine and O\textsubscript{3} exposure at several sites were highly correlated \((r^2 = 0.92)\). For red oak, Hanson and others (Hanson et al., 1994) found that SUM00 explained 83% of the variance in the response of light-saturated photosynthetic rate to O\textsubscript{3} levels, while estimated internal dose explained only 76% of the variance. In this same study, SUM06 explained only 49% of the variance. Due to genetic variation or other factors, individual mature trees will vary in their response to similar O\textsubscript{3} exposures. For example, in 125-year-old giant sequoia trees exposed to \(~230\) ppm•h of O\textsubscript{3} in branch chambers, O\textsubscript{3} uptake in one individual was \(~5\) mmol m\(^{-2}\), while in another it was \(~9.5\) mmol m\(^{-2}\) (Grulke et al., 1996).

Based on these results, stomatal conductance, O\textsubscript{3} uptake, and O\textsubscript{3} effects cannot be assumed to be equivalent in seedlings and mature trees. In general, mature deciduous trees are likely to be more sensitive to O\textsubscript{3} compared to seedlings, while mature evergreen trees are likely to be less sensitive than seedlings. However, even when differences in physiological traits occur, concomitant effects on stem growth may not be detected in the field. Additionally, complex interactions may occur between environmental conditions and O\textsubscript{3} responses; and artifacts may occur for seedling studies, especially for seedlings grown in pots. Finally, competition between species or genotypes within a species can either exacerbate or ameliorate the effects of O\textsubscript{3}. Such effects are predicted by models of the growth of mixed species forests, as discussed in Section AX9.6, and various interactions between competitive ability and O\textsubscript{3} effects have been found for aspen clones in the Wisconsin FACE study (McDonald et al., 2002). Section AX9.6 further discusses issues that must be addressed when scaling data from individual mature trees to forests and regions.

**AX9.5.6 Studies With the Chemical EDU**

The chemical EDU has been used with the goal of protecting plants from O\textsubscript{3} effects without controlling O\textsubscript{3} exposure (Table AX9-21) (U.S. Environmental Protection Agency, 1986, 1996). As discussed in Section AX9.1.3.3, the use of EDU has the potential to be a low-cost, practical method of evaluating ambient O\textsubscript{3} exposures on plants grown under natural conditions without the limitations imposed by methodologies such as OTCs (Section AX9.1.3.3). However, because EDU is phytotoxic, and may have effects on plants other than antioxidant protection,
<table>
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<td>Soil drench 200 mL of 150 ppm solution per plant every 14 days</td>
<td>CF, NF, CF-1×, CF-2×: mean = 1, 14, 15, 32 ppb</td>
<td>O3 reduced pod, shoot, and root mass. EDU increased root, leaf, and shoot mass, but a significant interaction with O3 occurred only for root weight.</td>
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<td>Bean, cv. BBL-290</td>
<td>2 expts in 5.5 L pots in OTCs with 4 O3 treatments</td>
<td>Soil drench every 14 days, in expt 1 = 0.14, 28, 56, 120 mg/L potting medium; expt 2 = 0, 8, 16, 32 mg/L</td>
<td>2 expts with CF, NF and 2 constant additions of O3. 7-h mean O3 (ppb) for Expt 1 = 34, 70, 95, 121; Expt 2 = 19, 42, 74, 106</td>
<td>Visible injury and reduced total biomass or yield, even in CF treatment. Within an O3 treatment, sometimes increased yield (Expt 2 only).</td>
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<td>Bean, cv. Bush Blue Lake 290, Bush Blue Lake 274, lines S156, R123</td>
<td>Field-grown in fine sandy loam in Massachusetts, in 2 one-year experiments.</td>
<td>Foliar spray at 300 ppm every 7 days between full expansion of primary leaves and pod senescence.</td>
<td>Ambient, with 181 and 141 h &gt; 40 ppb, 74 and 95 h &gt; 60 ppb, and 23 h &gt; 80 ppb in 2001 and 2002 respectively</td>
<td>EDU increased final above-ground biomass in S156 in 2001 and 2002, but significantly decreased above-ground biomass in R123 in both years and BBL 274 in 2002.</td>
<td>Elagöz and Manning et al. (2005a)</td>
</tr>
<tr>
<td>Bean</td>
<td>Pots with potting mix at 3 locations in Spain (2 years at 1 site)</td>
<td>Soil drench of 200 mL of increasing concentrations of 100, 150, 200, 250 ppm every 14 days (4-10 mg l:1 soil)</td>
<td>AOT40 = 0.4-1.8 ppm-h</td>
<td>0 to 50% increase in pod mass, but did not restore yield at sites with higher O3.</td>
<td>Ribas and Penuelas (2000)</td>
</tr>
<tr>
<td>Bean, cv. Lit</td>
<td>Pots with potting mix at 4 sites in the Netherlands</td>
<td>Soil drench of 200 mL of increasing concentrations of 100, 150, 200, 250 ppm every 14 days (4-10 mg l:1 soil)</td>
<td>AOT40 = 0.64-0.98, 7-h mean = 49-55 ppb</td>
<td>Average 20% yield increase at all sites.</td>
<td>Tonneijck and Van Dijk (1997)</td>
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<tr>
<td>Bean, cv. Lit</td>
<td>Pots with potting mix at 1 site in Belgium</td>
<td>Soil drench of 200 mL of increasing concentrations of 100, 150, 200, 250 ppm every 14 days (4-10 mg l:1 soil)</td>
<td>AOT40 = 0.81 ppm-h</td>
<td>16% yield increase.</td>
<td>Vandermeiren et al. (1995)</td>
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<td>Species</td>
<td>Description</td>
<td>EDU Application</td>
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<td>Effects of EDU</td>
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<tr>
<td>Clover, subterranean</td>
<td>Plants in 10-cm pots at 4 rural sites in the Netherlands for 3 years</td>
<td>100 ml of 150 ppm solution as soil drench every 14 days for 2 months</td>
<td>AOT40 = 0-0.56 ppm-h for 4-week periods</td>
<td>Injury, but not leaf biomass was affected by EDU and O₃ exposure.</td>
<td>Tonneijck and Van Dijk (2002a)</td>
</tr>
<tr>
<td>Clover, white</td>
<td>15-cm pots in field, well watered, 12 locations throughout Europe, 3 years</td>
<td>100 mL of 150 ppm solution as soil drench every 14 days for 3 months</td>
<td>AOT40 (28 days) = 0-20 ppm-h</td>
<td>Change in biomass ratio, weak linear relationship ($r^2 = 0.16$) stronger relationship using ANN and climatic factors</td>
<td>Ball et al. (1998)</td>
</tr>
<tr>
<td>Clover, white, cv. Menna</td>
<td>2 expts, 10-cm pots in field in Italy, see also companion OTC expt</td>
<td>100 mL of 150 ppm solution as soil drench every 14 days for 2 months</td>
<td>AOT40 = 15.5, 12.1 ppm-h; 7-h mean = 69, 60</td>
<td>n.s.</td>
<td>Fumagalli et al. (1997)</td>
</tr>
<tr>
<td>Clover, white, cv. Menna</td>
<td>2 expts, 10-cm pots in OTCs in Denmark</td>
<td>Soil drench 100 mL of 150 ppm solution every 14 days</td>
<td>CF, NF, NF+25, NF+50 ppb, O₃ exposure not reported</td>
<td>No effect of EDU despite highly significant effect of O₃ on above-ground biomass</td>
<td>Mortensen and Bastrup-Birk (1996)</td>
</tr>
<tr>
<td>Poplar, hybrid</td>
<td>Stem injections, field, cuttings, 1 or 2 years</td>
<td>Approx. 125 or 250 mg/leaf (low, high EDU treatments) 5 times every 14 days</td>
<td>1991: 7-h mean = 56, AOT40 = 23; 1992: 7-h mean = 59, AOT40 = 27</td>
<td>No effect on biomass; 6%, 12% more severely O₃ damaged leaves in high EDU for 2 years</td>
<td>Ainsworth et al. (1996)</td>
</tr>
<tr>
<td>Pine, loblolly</td>
<td>1 year old half-sib seedlings in field in TX for 3 years</td>
<td>150, 300, 450 ppm every 14 days</td>
<td>1995, 1996, 1997, no. h &gt; 40 ppb = 1723, 2297, 2052; no. h &gt; 60 ppb = 378, 584, 528; peak = 113, 102, 118</td>
<td>For EDU 450 trt, above-ground biomass increased approx 46% (n.s. in other treatments</td>
<td>Manning et al. (2003)</td>
</tr>
<tr>
<td>Radish, cv. Cherry Belle</td>
<td>Plants in pots in potting mix exposed for 5 weeks in southern Sweden.</td>
<td>Soil drench containing 20 mg EDU applied 2 times, 14 days apart</td>
<td>24-h mean = 31 ppb, 7-h mean = 36 ppb, AOT40 = 1.3 ppm-h.</td>
<td>24% increase in hypocotyl mass, 18% increase in shoot mass</td>
<td>Pleijel et al. (1999b)</td>
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</table>
it is crucial that the correct dosage for protection from O₃ be determined without the direct
effects of EDU. For example, a study in Massachusetts applied EDU to foliage of field-grown
bush beans of two lines (S156, R123) and two cultivars (Bush Blue Lake 290, Bush Blue Lake
274) (Elagöz and Manning, 2005a). EDU increased the above-ground biomass of one line but
decreased the above-ground biomass in the other line and in one of the cultivars. Other studies
have shown that EDU does not always have greater effects at higher O₃ exposures (Ribas and
Penuelas, 2000; Tonneijck and Van Dijk, 1997). Such results suggest that it may be difficult to
quantify ambient O₃ effects using EDU, because the amount of plant growth or yield expected at
a low (background) O₃ concentration cannot be inferred from EDU-treated plants grown at
locations with higher O₃ exposures. Unfortunately, although many studies with EDU have been
conducted in recent decades, very few have used multiple EDU application levels along with
multiple O₃ exposures to characterize the EDU system for a given plant species. Therefore, the
text of this section focuses on how data from existing studies can be used for developing or
validating exposure-response relationships, rather than reviewing results of all individual studies.
Data from individual studies on O₃ exposure, EDU application rates, and the effects of EDU are
presented in Table AX9-21. In addition to EDU, sodium erythorbate has been used in a few
studies as a protectant chemical. Since very few published studies have used sodium erythorbate
and attempts to establish appropriate doses for individual species are even more limited, the use
of this chemical is not reviewed here.

In summary, it is difficult to use data from existing EDU studies to develop exposure-
response relationships or to quantify the effects of ambient O₃ exposure. Despite these
limitations, the EDU studies reviewed in previous criteria documents (U.S. Environmental
Protection Agency, 1986, 1996) and the more recent studies summarized in Table AX9-21
provide another line of evidence that ambient O₃ exposures occurring in many regions of the
United States are reducing the growth of crops and trees.

**AX9.5.7 Summary**

Data published during the last decade support the conclusions of previous criteria
documents that there is strong evidence that ambient concentrations of O₃ cause injury and
damage to numerous common and economically valuable plant species. For annual vegetation,
the data summarized in Table AX9-16 show a range of growth and yield responses both within
and among species. Nearly all of these data were derived from studies in OTCs, with only two studies using open-air systems in the UK (Ollerenshaw et al., 1999; Ollerenshaw and Lyons, 1999). It is difficult to compare studies that report O<sub>3</sub> exposure using different indices, such as AOT40, SUM06, or 7-h or 12-h mean values. However, when such comparisons can be made, the results of more recent research confirm earlier results summarized in the 1996 O<sub>3</sub> AQCD (U.S. Environmental Protection Agency, 1996). A summary of earlier literature concluded that a 7-h, 3-month mean of 49 ppb corresponding to a SUM06 exposure of 26 ppm·h would cause 10% loss in 50% of 49 experimental cases (Tingey et al., 1991). More recent data summarized in Table AX9-16 support this conclusion, and more generally indicate that ambient O<sub>3</sub> exposures can reduce the growth and yield of annual species. Some annual species such as soybean are more sensitive, and greater losses may be expected (Table AX9-16). A two-year study using a free-air exposure system with soybean confirmed yield reductions found previously using OTCs (Morgan et al., in press). Thus, the more recent scientific literature supports the conclusions of the 1996 O<sub>3</sub> AQCD (U.S. Environmental Protection Agency, 1996) that ambient O<sub>3</sub> concentrations are probably reducing the yield of major crops in the United States.

Much research in Europe has used the AOT40 exposure statistic, and substantial effort has gone into developing Level-1 critical levels for vegetation using this index. Based on regression analysis of 15 OTC studies of spring wheat, including one study from the United States and 14 from locations ranging from southern Sweden to Switzerland, an AOT40 value of 5.7 ppm·h was found to correspond to a 10% yield loss, and a value of 2.8 ppm·h corresponded to a 5% yield loss (Fuhrer et al., 1997). Because a 4 to 5% decrease could be detected with a 99% confidence level, a critical level of an AOT40 value of 3 ppm·h was selected in 1996 (Kärenlampi and Skärby, 1996).

In addition to likely reductions in crop yield, O<sub>3</sub> may also reduce the quality or nutritive value of annual species. Many studies have shown effects of O<sub>3</sub> on various measures of plant organs that affect quality, with most studies focusing on characteristics important for food or fodder. These studies indicate that there may be economically important effects of ambient O<sub>3</sub> on the quality of crop and forage species. Previous O<sub>3</sub> criteria documents have concluded that visible symptoms on marketable portions of crops and ornamental plants can occur with seasonal 7-h mean O<sub>3</sub> exposures of 40 to 100 ppb (U.S. Environmental Protection Agency, 1978, 1986, 1996). The more recent scientific literature does not refute this conclusion.
The use of OTCs may reverse the usual vertical gradient in O$_3$ that occurs within a few meters above the ground surface (Section 3.3.2, Section AX9.1). This reversal suggests that OTC studies may somewhat overestimate the effects of an O$_3$ concentration measured several meters above the ground. However, such considerations do not invalidate the conclusion of the 1996 AQCD (U.S. Environmental Protection Agency, 1996) that ambient O$_3$ exposures (Tables AX9-13 and AX9-21) are sufficient to reduce the yield of major crops in the United States. Recent studies using OTCs confirmed previous results that ambient ozone exposures can significantly reduce the growth of annual species and the yield of commercially important crop species. Additionally, a two-year study using a free air exposure system with soybean confirmed yield reductions found previously using OTCs (Morgan et al, in press).

As for single-season agricultural crops, yields of multiple-year forage crops are reduced at O$_3$ exposures that occur over large areas of the United States. This result is similar to that reported in the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996). When species are grown in mixtures, O$_3$ exposure can increase the growth of O$_3$-tolerant species and exacerbate the growth decrease of O$_3$-sensitive species (e.g., Ashmore and Ainsworth, 1995; Fuhrer et al., 1994). Because of this competitive interaction, the total growth of the mixed-species community may not be affected by O$_3$ exposure (Ashmore and Ainsworth, 1995; Barbo et al., 1998; Fuhrer et al., 1994). However, in some cases, mixtures of grasses and clover species have shown significant decreases in total biomass growth in response to O$_3$ exposure in studies in the United States (Heagle et al., 1989; Kohut et al., 1988) and in Sweden (Pleijel et al., 1996). In Europe, a provisional critical level for herbaceous perennials of an AOT40 value of 7 ppm·h over 6 months has been proposed to protect sensitive plant species from adverse effects of O$_3$.

For deciduous tree species, recent evidence from free air exposure systems and OTCs supports results observed previously in OTC studies. For example, a series of FACE studies undertaken in Rhinelander, WI (Isebrands et al., 2000, 2001) showed that O$_3$-symptom expression was generally similar in OTCs, FACE, and also at sites along an ambient O$_3$ gradient, supporting the previously observed variation among aspen clones using OTCs (Karnosky et al., 1999). This study also found no effects on sugar maple growth after 3 years, but in years 4 to 7 found significant growth reduction due to O$_3$ (Karnosky et al., 2005). These results are important because, they indicate results obtained from OTCs are supported by results from free air exposure systems and also that more than 3 years may be required to adequately investigate
effects of $O_3$ on the growth of tree species. As has been observed in previous criteria documents, root growth often is found to be the most sensitive biomass response indicator to $O_3$.

Results reported since 1996 support the conclusion of the 1996 $O_3$ AQCD (U.S. Environmental Protection Agency, 1996) that deciduous trees are generally less sensitive to $O_3$ than are most annual plants, with the exception of a few very sensitive genera such as *Populus* and sensitive species such as black cherry. However, the data presented in Table AX9-18 suggest that ambient $O_3$ exposures that occur in the United States can potentially reduce the growth of seedlings of deciduous species. Results from multiple-year studies sometimes show a pattern of increased effects in subsequent years. In some cases, however, growth decreases due to $O_3$ may become less significant or even disappear over time. While some mature trees show greater $O_3$ sensitivity in physiological parameters such as net photosynthetic rate than do seedlings, these effects may not translate into measurable reductions in biomass growth. However, because even multiple-year experiments do not expose trees to $O_3$ for more than a small fraction of their life span, and because competition may in some cases exacerbate the effects of $O_3$ on individual species, determining effects on mature trees remains a significant challenge.

In Europe, a Level I critical level has been set for forest trees based on OTC studies of European beech seedlings. A critical level was defined as an AOT40 value of 10 ppm·h for daylight hours for a 6-month growing season (Kärenlampi and Skärby, 1996). However, other studies show that some species such as silver birch may be more sensitive to $O_3$ compared to beech (Pääkkönen et al., 1996).

For evergreen tree species, as for other tree species, the $O_3$ sensitivity of different genotypes and different species varies widely. Based on studies with seedlings in OTCs, major species in the United States are generally less sensitive than are most deciduous trees, and slower-growing species are less sensitive than are faster-growing species. Interacting stresses such as competition stress may increase the sensitivity of trees to $O_3$. As for deciduous species, most experiments with evergreen species have only covered a small portion of the life span of a tree and have been conducted with seedlings, making estimating effects on mature trees difficult.

For all types of perennial vegetation, cumulative effects over more than one growing season may be important; furthermore, studies for only a single season may underestimate effects. Mature trees may be more or less sensitive to $O_3$ than are seedlings, depending on the
species, but information on physiological traits may be used to predict some such differences. In some cases, mature trees may be more sensitive to O$_3$ than seedlings due to differences in their gas exchange rates, growth rates, greater cumulative exposures, or due to the interaction of O$_3$ stress with other stresses.

**AX9.6 EFFECTS OF OZONE EXPOSURE ON NATURAL ECOSYSTEMS**

**AX9.6.1 Introduction**

The preceding section on species-level responses (AX9.5) provides a lead-in to address the response of ecosystems to O$_3$. The conclusion of the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) was that aside from the results from the San Bernardino NF, there was no direct evidence that O$_3$ is altering natural ecosystems in the United States. This conclusion is generally valid today, except that our understanding of the effects of O$_3$ in the San Bernardino NF has been tempered by additional understanding of the complicating role that N deposition plays in this system. Despite the lack of any new, direct information linking O$_3$ with ecosystem changes, numerous publications since 1996 have highlighted ways in which O$_3$ may affect ecosystem structure and/or function. This section addresses new and (where appropriate) older literature in order to illustrate possible shifts in energy or material flow through ecosystems as a result of O$_3$ exposure.

An ecosystem is defined as comprising all of the organisms in a given area interacting with the physical environment, so that a flow of energy leads to a clearly defined trophic structure, biotic diversity, and cycling of materials between living and nonliving parts (Odum, 1963). Individuals within a species and populations of species are the building blocks from which communities and ecosystems are constructed. Classes of natural ecosystems, e.g., tundra, wetland, deciduous forest, and conifer forest, are distinguished by their dominant vegetation forms. Ecosystems boundaries are delineated when an integral unit is formed by their physical and biological parts. Defined pathways for material transport and cycling and for the flow of energy are contained within a given integrated unit.

Each level of organization within an ecosystem has functional and structural characteristics. At the ecosystem level, functional characteristics include, but are not limited, to energy flow; nutrient, hydrologic, and biogeochemical cycling; and maintenance of food chains.
The sum of the functions carried out by ecosystem components provides many benefits to mankind, as in the case of forest ecosystems (Smith, 1992). These include food, fiber production, aesthetics, genetic diversity, and energy exchange.

Ecosystems are functionally highly integrated. Changes in one part of an ecosystem, such as the primary producer component, may have consequences for connected parts, such as the consumer and decomposer components. For example, when needles are shed prematurely as a result of \(O_3\) exposure, successional development of phyllosphere fungi inhabiting the surface of Ponderosa pine needles may be truncated (Bruhn, 1980). In addition, decomposer populations in the litter layer may be capable of higher rates of decomposition, due to the higher N content of the younger age classes of needles falling from \(O_3\)-damaged pines (Fenn and Dunn, 1989).

Because ecological systems integrate the effects of many influences, the results of \(O_3\) exposure may depend on co-occurring influences that predispose an ecosystem to stress (Colls and Unsworth, 1992). One important change in our thinking since the 1996 \(O_3\) AQCD is that, at the high levels of \(O_3\) exposure that are known to result in detectable plant responses (>250 ppm h accumulated over a growing season), N deposition must also be considered as a concurrent stressor. Both \(O_3\) exposure and increased N deposition can cause changes in N cycling and compartmentalization within ecosystems.

The vast majority of \(O_3\)-effects literature addresses individual species responses (see Section AX9.6.4.3), as was also true in 1996 (U.S. Environmental Protection Agency, 1996). This section differs from the preceding one in that the physiological stress of individual species is considered only within the context of its natural ecosystem. Changes in function at the individual level propagate through the higher levels of organization, resulting in changes in ecosystem structure and function. However, since ecosystem-level responses result from the interaction of organisms with one another and with their physical environment, it takes longer for a change to develop to a level of prominence at which it can be identified and measured. The paucity of scientific literature on \(O_3\) effects at the ecosystem level is a result of both the complexity of ecological systems, and long response times. In addition, “indirect” effects of \(O_3\) on plants (e.g., effects that alter the plants’ ability to integrate environmental stresses) may be more important than the direct effects on photosynthesis and respiration at the leaf level (Johnson and Taylor, 1989).
A conceptual framework (see Table AX9-22) for discussing the effects of O₃ on ecosystems was developed by the EPA Science Advisory Board (Young and Sanzone, 2002). Their six essential ecological attributes (EEAs) include landscape condition, biotic condition, organism condition, ecological processes, hydrological and geomorphological processes, and natural disturbance regimes (see Table AX9-22). The major ecological effects of O₃, and gaps in our knowledge of O₃ exposure effects at the ecosystem level, are summarized at the end of this chapter. While the main focus is O₃ effects newly described since the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996), many key historical papers are cited to demonstrate ecosystem response, particularly where they remain the only examples in the literature. Although the vast majority of published studies focus on individuals, six case studies (five field examples and one FACE experiment) have measured several ecosystem components simultaneously to better understand the overall ecosystem response to O₃. We provide an overview of these six studies up-front to provide a context for the subsequent discussion on possible ecosystem effects on an EEA basis.

AX9.6.2 Case Studies
AX9.6.2.1 Valley of Mexico

The first evidence of air pollution impacts on vegetation in the Valley of Mexico (Mexico City Air Basin) were observations of foliar injury symptoms in bioindicator plants attributed to O₃, PAN, SO₂, and possibly other pollutants (De Bauer, 1972). Subsequently, O₃ injury to foliage and crowns of pine trees were reported in forests to the south and southwest of Mexico City (De Bauer and Hernández-Tejeda, 1986; De Bauer and Krupa, 1990). Ozone is considered to be the pollutant with the most severe impacts on vegetation within the Mexico City urban zone and in forests downwind of the city. *Pinus hartwegii* is the most O₃-sensitive pine species and is severely impacted by high O₃ exposures encountered to the south/southwest of the Mexico City metropolitan area (Miller et al., 1994). The potential for O₃ injury is particularly high in this area, because O₃ levels are high during the summer rainy season when soil moisture availability and stomatal conductance are greatest; these factors enhance O₃ uptake and injury. Decline of *Abies religiosa* (oyamel) in the Desierto de los Leones NP is a well-known example of dramatic dieback and mortality of entire forest stands due primarily to air pollution stress (Alvarado et al., 1993). Other factors, such as a lack of stand thinning, also contribute to forest...
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<td>Mixed conifer forest</td>
<td>Community composition, Stand structure</td>
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<td>Pinus ponderosa</td>
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<td>Soil microbial community</td>
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<td>Blotch leaf miner performance</td>
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<td>Disease susceptibility</td>
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### Table AX9-22 (cont’d). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

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<td>% population sensitive</td>
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<td>Grulke and Lee (1997); Arbaugh et al. (1998); Salardino and Carroll (1998); Temple et al. (1992) Grulke et al. (2003)</td>
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<td>Chappelka et al. (1999a,b)</td>
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<td>and <em>Rubus cuneifolius</em></td>
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<td>Nutrient availability and O₃</td>
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| Water Budget               | *Picea rubens*               | Water-use efficiency no effect                                                      | Laurence et al. (1997)      |
|                          | *Pinus armandi*              | Water-use efficiency                                                                | Shan et al. (1996)           |
|                          | *Pinus jeffreyi*             | Canopy transpiration                                                                | Grulke et al. (2003a)        |
|                          | *Picea abies*                | Transpiration (xylem sap flow)                                                      | Maier-Maercker (1997)        |
|                          | *Fraxinus excelsior*         | Water stem flow                                                                     | Wiltshire et al. (1994)      |
|                          | *Betula pendula*             | Water-use efficiency                                                                | Maurer and Matyssek (1997)   |
|                          | *Populus hybrids*            | Water-use efficiency                                                                | Reich and Lassoie (1984)     |
Table AX9-22 (cont’d). Essential Ecological Attributes for Natural Ecosystems Affected by O₃

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<td>Frequency of fire</td>
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<td>• Duration</td>
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<td>Duration of bark beetle attack</td>
<td>Minnich et al. (1995)</td>
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decay. Lead in automobile gasoline was phased out in 1990, and foliar concentrations of heavy metals in forest species are not now at phytotoxic levels (Fenn and De Bauer, 1999). Sulfur dioxide concentrations decreased in the early 1990s as a result of regulatory mandates limiting their emissions. Sensitive plants in the northeast and northwest sectors of the Mexico City urban zone where concentrations are highest may still be impacted by exposure to ambient SO₂ levels. Deposition of ionic forms of N and S are high in forested areas southwest of Mexico City. However, the effects of these chronic nutrient inputs to the forest are only beginning to be investigated and understood.

The ecological perturbations caused by severe air pollution exposures in forests located downwind of Mexico City are expected to continue for the near future (the next 5 to 10 years), largely as a result of high O₃ concentrations as well as emissions of N oxides. The longer-term response is more uncertain and depends largely on the effectiveness of regulatory emissions control strategies. Currently, pollutant levels are declining. Forest responses to this trend will
depend on how long it takes to reduce levels sufficiently to allow sensitive species to recover. Some of the change to the ecosystem is probably irreversible, such as the loss of lichen diversity and the loss of other O$_3$-sensitive species (Zambrano and Nash, 2000).

**AX9.6.2.2 San Bernardino Mountains**

The San Bernardino Mountains lie east of the Los Angeles Air Basin in California, and significant levels of pollution have been transported into the mountain range, including into a Class I wilderness area. The effects of O$_3$ exposure on the mixed conifer forest of the San Bernardino Mountains is perhaps the longest and most thoroughly documented O$_3$ ecological effects evaluation (Miller and McBride, 1999a). In this classic case study linking tropospheric O$_3$ exposure to damage to an entire forest ecosystem (U.S. Environmental Protection Agency, 1996) (Table AX9-23), Miller et al. (1963) first identified the unique foliar chlorotic mottle that was occurring on two of the dominant tree species, *Pinus ponderosa* and *P. jeffreyi*. Levels of O$_3$ averaging 100 to 120 ppb over 24 h with 1-h peaks well into the 200 ppb range were common in the region in the 1960s and 1970s (Miller and McBride, 1999a). Single-hour peak values have declined in recent years due to heavily regulated pollution control (Arbaugh et al., 1998; Lee et al., 2003; Takemoto et al., 2001).

Since the 1996 O$_3$ AQCD (U.S. Environmental Protection Agency, 1996) was written, the concurrent role of N deposition in modifying ecosystem response to O$_3$ exposure in the San Bernardino Mountains has been further elucidated (Arbaugh et al., 2003; Bytnerowicz et al., 1999; Bytnerowicz, 2002; Fenn et al., 1996; Takemoto et al., 2001). Both O$_3$ exposure and N deposition reduce foliar retention (Grulke and Balduman, 1999) and alter tissue chemistry of both needles and litter (Poth and Fenn, 1998). In addition, confounding factors such as drought and fire suppression add to the complexity of ecosystem response (Arbaugh et al., 2003; Minnich et al., 1995; Takemoto et al., 2001). Extensive crown injury measurements have also been made, linking ambient O$_3$ exposure data to chlorotic mottle and fascicle retention (Arbaugh et al., 1998). Ozone exposure and N deposition reduce carbon allocation to stems and roots (Grulke et al., 1998a, 2001), further predisposing trees to drought stress, windthrow, root diseases, and insect infestation (Takemoto et al., 2001). Increased mortality of susceptible tree species (Ponderosa and Jeffrey pine) has shifted community composition toward white fir and incense-cedar (*Abies concolor, Calocedrus decurrens*) and has altered forest stand structure.
<table>
<thead>
<tr>
<th>Study</th>
<th>Keystone Species</th>
<th>Study Type</th>
<th>Period Studied</th>
<th>Key Ecological Findings</th>
</tr>
</thead>
</table>
| Valley of Mexico | *Pinus hartwegii*, *Abies religiosa* | Field transects | 35 yrs | • Significant foliar injury (De Bauer, 1972; De Bauer and Hernández-Tejeda, 1986; De Bauer and Krupa, 1990)  
• Community composition changes (Alvarado et al., 1993)  
• Species richness changes (Zambrano and Nash, 2000) |
| San Bernardino Mountains | *Pinus ponderosa*, *P. jeffreyi* | Field transects | 40 yrs | • Community composition changes (Arbaugh et al., 2003; Miller, 1973; Minnich et al., 1995)  
• Population changes (McBride and Laven, 1999)  
• $O_3$-pine-bark beetle interaction (Pronos et al., 1999)  
• Altered C flows (Arbaugh et al., 1999; Grulke et al., 1998a, 2001, 2002b; Grulke and Balduman, 1999)  
• Interaction of $O_3$, drought, N deposition (Fenn et al., 1996; Grulke, 1999; Takemoto et al., 2001)  
• Altered carbon cycling (Arbaugh et al., 1999) |
| Sierra Nevada Mountains | *Pinus ponderosa*, *P. jeffreyi* | Field | 35 yrs | • Wide-scale nature of effects (Edinger et al., 1972; Miller and Millecan, 1971)  
• Link to decreased growth (Peterson et al., 1987, 1991, 1995)  
• Quantification of $O_3$ flux (Bauer et al., 2000; Goldstein et al., 2003; Panek et al., 2002)  
• Cumulative $O_3$ effects (Takemoto et al., 1997)  
• Canopy level responses (Grulke et al., 2003a,b)  
• Population changes (Carroll et al., 2003) |
| Appalachian Mountains | *Fraxinus americana*, *Liriodendron tulipfera*, *Pinus strobus*, *Prunus serotina* | Field | 25 yrs | • Link of visible symptoms to growth decreases (McLaughlin et al., 1982; Somers et al., 1998)  
• Wide-scale nature of effects (Chappelka et al., 1999a; Hildebrand et al., 1996) |
Table AX9-23 (cont’d). Case Studies Demonstrating the Ecological Effects of O₃

<table>
<thead>
<tr>
<th>Study</th>
<th>Keystone Species</th>
<th>Study Type</th>
<th>Period Studied</th>
<th>Key Ecological Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspen FACE</td>
<td><em>Acer saccharum</em>, <em>Betula papyrifera</em>, <em>Populus tremuloides</em></td>
<td>Open-air O₃ exposure</td>
<td>6 yrs</td>
<td>• Competitive interactions (McDonald et al., 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• O₃-aspen-rust interaction (Karnosky et al., 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Plant-insect interactions (Holton et al., 2003; Percy et al., 2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• C and N cycling (King et al., 2001; Lindroth et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Moderation of CO₂ responses by O₃ (Isebrands et al., 2001; Karnosky et al., 2003b; McDonald et al., 2002; Wustman et al., 2001)</td>
</tr>
<tr>
<td>Plantago</td>
<td><em>Plantago major</em></td>
<td>Field</td>
<td>20 yrs</td>
<td>• Population structure (Davison and Reiling, 1995; Lyons et al., 1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• O₃ resistance (Reiling and Davison, 1992c)</td>
</tr>
<tr>
<td>Carpathian Mountains</td>
<td><em>Pinus sylvestris</em>, <em>Picea abies</em></td>
<td>Field</td>
<td>15 yrs</td>
<td>• Significant foliar injury (Davison and Reiling, 1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Community composition changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Species diversity changes</td>
</tr>
</tbody>
</table>

(Miller et al., 1989) (Table AX9-25). Ozone exposure is implicated in projected changes in stand composition (McBride and Laven, 1999) toward a predominance of oaks, rather than mixed conifer forests. Forest understory species have also been affected (Temple, 1999). These individual species responses collectively have affected trophic structure and food web dynamics (Dahlsten et al., 1997; Pronos et al., 1999), as well as C and N cycling (Arbaugh et al., 2003) (Table AX9-24).

**AX9.6.2.3 Sierra Nevada Mountains**

Like the San Bernardino Mountains, the western slope of the Sierra Nevada Mountains in central and southern California has also been exposed to elevated O₃ for a long time, although the effects have been much less. Symptoms of O₃ injury have been found on Ponderosa and Jeffrey pines in all of the Sierra Nevada national forests and parks (Carroll et al., 2003). First identified as a problem in the 1970s (Miller et al., 1972), elevated O₃ with daytime means of 60-80 ppb are common (Bauer et al., 2000; Böhml et al., 1995; Bytnerowicz et al., 2002c; Panek...

<table>
<thead>
<tr>
<th>Pollutant Occurrence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_3$ exposure and N deposition</td>
<td>Fenn et al. (1996; 2000); Grulke et al. (1998a, 2003a); Bytnerowicz et al. (1999)</td>
</tr>
</tbody>
</table>

**Cellular, Biochemical**

- Foliar pigments  
  - Grulke and Balduman (1999); Grulke and Lee (1997); Tausz et al. (1999a,b,c, 2001)

**Foliar Responses**

- Foliar Symptoms  
  - Arbaugh et al. (1998); Grulke and Lee (1997); Miller and Rechel (1999)

**Gas Exchange**

- Photosynthesis and Conductance  
  - Grulke (1999); Grulke et al. (2002a,b); Grulke and Retzlaff (2001)

**Whole Organism**

- Growth/Biomass
  - Above-ground  
    - Grulke and Balduman (1999)
  - Below-ground  
    - Grulke et al. (1998a); Grulke and Balduman (1999)
  - Root/shoot ratio  
    - Grulke et al. (2001); Arbaugh et al. (1998); Miller and Rechel (1999)
  - Carbon allocation  
    - Miller and McBride (1999b); Minnich et al. (1995); Minnich (1999)
  - Crown vigor  
    - Miller and Rechel (1999)

**Ecosystem**

- Community dynamics/succession simulations  
  - Arbaugh et al. (2003); Arbaugh et al. (1999); McBride and Laven (1999)

- Understory vegetation  
  - Temple (1999)

**Pest interactions**

- Bark beetle/predators  
  - Dahlsten et al. (1997); Pronos et al. (1999)
- Disease occurrence  
  - Miller and Rechel (1999); Pronos et al. (1999)
- Litter decomposition

**Disturbance**

- Bark beetle occurrence  
  - Miller and McBride (1999b); Minnich et al. (1995); Minnich (1999)
- Fire frequency

AX9-273
Table AX9-25. Effects of Ozone, Ozone and N Deposition, and Ozone and Drought Stress on *Pinus ponderosa* and *Pinus jeffreyi* in the Sierra Nevada and the San Bernardino Mountains, California. Citations are focused on research published since U.S. EPA (1996).

<table>
<thead>
<tr>
<th>Foliar Biochemistry and Tissue Chemistry</th>
<th>O₃</th>
<th>O₃ + N deposition</th>
<th>O₃ + Drought</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ascorbate</td>
<td>d¹</td>
<td>d</td>
<td>i</td>
<td>Grulke et al. (2003b); Tausz et al. (2001)</td>
</tr>
<tr>
<td>Dehydroascorbate</td>
<td>i</td>
<td>n.d.</td>
<td>d</td>
<td>Grulke et al. (2003b)</td>
</tr>
<tr>
<td>Total glutathione</td>
<td>d</td>
<td>i</td>
<td>d</td>
<td>Tausz et al. (2001)</td>
</tr>
<tr>
<td>Oxidized glutathione</td>
<td>i</td>
<td>i</td>
<td>d</td>
<td>Tausz et al. (2001)</td>
</tr>
<tr>
<td>α Carotenoids</td>
<td>i</td>
<td>n.d.</td>
<td>d</td>
<td>Grulke et al. (2003b)</td>
</tr>
<tr>
<td>Foliar nitrogen</td>
<td>d</td>
<td>i</td>
<td>d</td>
<td>Grulke et al. (1998a); Grulke and Lee (1997); Poth and Fenn (1998)</td>
</tr>
<tr>
<td>Starch</td>
<td>n.d.</td>
<td>d</td>
<td>i</td>
<td>Grulke et al. (2001)</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>d</td>
<td>id</td>
<td>d</td>
<td>Grulke et al. (1998b, 2003b); Grulke and Lee (1997); Takemoto et al. (1997)(Grulke (1999); Tausz et al. (2001)</td>
</tr>
</tbody>
</table>

Gas Exchange

| Max lower canopy                        | n.d. | i | d | Grulke (1999); Grulke et al. (2002b); Grulke and Retzlaff (2001); Panek (2004) |
| Max whole canopy                        | d    | n.d. | d | Grulke et al. (2003b); Panek and Goldstein (2001) |
| Stomatal limitation                     | n.d. | n.d. | i | Panek and Goldstein (2001) |
| Stomatal conductance                    | d    | di  | d | Grulke (1999); Grulke et al. (2003a); Panek (2004) |
| Foliar respiration                      | n.s. | i | d | Grulke (1999); Grulke et al. (2002a) |
Table AX9-25 (cont’d). Effects of Ozone, Ozone and N Deposition, and Ozone and Drought Stress on *Pinus ponderosa* and *Pinus jeffreyi* in the Sierra Nevada and the San Bernardino Mountains, California. Citations are Focused on Research Published Since U.S. EPA (1996).

<table>
<thead>
<tr>
<th></th>
<th>O₃</th>
<th>O₃ + N deposition</th>
<th>O₃ + Drought</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth and Productivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foliar biomass</td>
<td>n.d.</td>
<td>i</td>
<td>d</td>
<td>Grulke and Balduman (1999)</td>
</tr>
<tr>
<td>Height growth</td>
<td>n.d.</td>
<td>i</td>
<td>d</td>
<td>Grulke and Balduman (1999)</td>
</tr>
<tr>
<td>Bole diameter growth</td>
<td>d</td>
<td>i</td>
<td>d</td>
<td>Grulke and Balduman (1999)</td>
</tr>
<tr>
<td>Fine root biomass</td>
<td>d</td>
<td>d</td>
<td>i</td>
<td>Grulke et al. (1998a)</td>
</tr>
<tr>
<td>Leaf Surfaces</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal occlusion</td>
<td>i</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Bytnerowicz et al. (1999); Bytnerowicz and Turunen (1994)</td>
</tr>
<tr>
<td><strong>Trophic Interactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bark beetle</td>
<td>n.s.</td>
<td>i</td>
<td>i</td>
<td>Pronos et al. (1999)</td>
</tr>
<tr>
<td><strong>Ecosystem Level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competitive indices</td>
<td>n.d.</td>
<td>d</td>
<td>i</td>
<td>Miller and Rechel (1999)</td>
</tr>
</tbody>
</table>

1 Responses are shown as significant increases (i), significant decreases (d), both significant decreases and increases reported (di), nonsignificant effects (n.s.), and no data (n.d.) compared to trees or seedlings at field sites with lower O₃, drought stress, or lack of significant N deposition (<10 kg ha⁻¹ yr⁻¹). Frequently n.d. was used for lack of a control site without compounding high N deposition. Foliar analyses and leaf surface properties were largely determined from previous year needles. Gas exchange data were generally from previous year needles at peak growing season, prior to late summer drought (mid- to late July).

2 Abbreviations: C = carbon; N = nitrogen; $A_{\text{max}}$ = maximum photosynthesis rate.

et al., 2002). The west-slope Sierra Nevada forests are also exposed to a wide range of additional gaseous and particulate pollutants, including various S and N compounds (Bytnerowicz et al., 1999; Fenn et al., 2003b; Takemoto et al., 2001), but at levels much lower than in the San Bernardino Mountains. Typical O₃-induced visible foliar symptoms, including chlorotic mottle, chlorophyll degradation, and premature senescence, are commonly found on O₃-sensitive genotypes of Ponderosa pine (Arbaugh et al., 1998; Peterson et al., 1991; Staszak et al., 2004) and Jeffery pine (Arbaugh et al., 1998; Grulke et al., 2003b; Patterson and Rundel, 1995; Peterson et al., 1987). Other important conifers in the region, such as giant sequoia, appear to be relatively O₃-tolerant (Grulke et al., 1996). The symptoms of foliar injury and...
growth reductions have been verified on seedlings in O₃ exposure chambers (Momen et al., 2002; Momen and Helms, 1996; Temple, 1988).

Ozone foliar injury of dominant pine species in the Sierra Nevada Mountains is correlated to decreased radial growth in both Ponderosa pine (Peterson et al., 1991) and Jeffrey pine (Patterson and Rundel, 1995; Peterson et al., 1987). Because of the large amount of intraspecific variation in O₃ sensitivity in these two species, O₃ exposure may be a selective agent (Patterson and Rundel, 1995), with differential mortality rates for sensitive individuals (Carroll et al., 2003). The region’s forests may also be experiencing subtle changes in species composition and community structure (Patterson and Rundel, 1995; Takemoto et al., 2001).

Based on fire scar dating, reconstructions of stand age classes, historical records, and present stand structure, fire has been largely excluded in western forests for the last 75 to 100 years (Minnich, 1999; Minnich and Padgett, 2003). Fire exclusion has resulted in fewer large stand-replacing fires rather than a mosaic of smaller low-intensity fires. The change in fire intensity may have selectively altered stand structure, fitness and competitiveness of component species, along with their susceptibility to atmospheric pollutants and other stressors (Minnich, 1999). Short-lived (50 to 80 years) species such as knobcone (Pinus attenuata) and Coulter pine (Pinus coulteri), which occur at the interface of the chaparral and the mixed conifer forest, may already have been selected for O₃ tolerance by seedling establishment (the most sensitive tree age class in conifers) after large fires in the 1950s (Minnich, 1999). Strong measures to suppress fires have largely kept chaparral fires from invading the mixed conifer forests, and stand densification in the mixed conifer zone has increased. High stand density, in turn, may weaken the younger cohorts and increase sensitivity to atmospheric pollution (Minnich, 1999).

Other disturbances that play a potential role in sensitivity to atmospheric pollution include cycles of drought stress. Nearly every decade is marked by one or more years of very low precipitation (Graumlich, 1993). During extended periods of drought, foliar injury is lower than in subsequent years with higher average precipitation (Carroll et al., 2003). In the first several years (1975 to 1977) of a Sierran-wide assessment of O₃ injury to pines, O₃ injury increased, because of greater water availability due to greater stomatal conductance and, presumably, greater O₃ uptake. Trees instrumented with monitors to directly measure canopy transpiration had 20% greater stomatal conductance in mesic microsites (riparian areas, mid-slope seeps) than trees in xeric microsites (rock outcrops) (Grulke et al., 2003a). Although the Sierra Nevada
experienced a prolonged drought between 1987 and 1993, it was less severe than other droughts
and O₃ injury did not significantly decrease (Carroll et al., 2003). The same plots showed only a
slight increase in O₃ injury between 1993 and 2000. While drought stress may make trees more
susceptible to insect and pathogen infestation, serious outbreaks of insect infestation are believed
to be indicators, not a cause, of existing stress in the forest (Wickman, 1992).

AX9.6.2.4 Appalachian Mountains

The southern Appalachian Mountain region experiences some of the highest O₃ exposures
of any natural areas in the eastern United States (Chappelka et al., 1997; Hildebrand et al., 1996;
Mueller, 1994; Samuelson and Kelly, 1997). Since the region is the home of the Shenandoah
and Great Smoky Mountains NPs, which have Class I air quality designations by the 1977
Clean Air Act, there has been considerable study of the region’s dominant forest species to
determine O₃ effects. Visible foliar symptoms of O₃ have been found in natural ecosystems
consisting of sassafras (Sassafras albidum) (Chappelka et al., 1999b), black cherry (Prunus
serotina) (Chappelka et al., 1997, 1999a; Hildebrand et al., 1996; Samuelson and Kelly, 1997),
yellow poplar and white ash (Fraxinus americana) (Chappelka et al., 1999b; Hildebrand et al.,
1996). Visible foliar symptoms induced by O₃ have been recreated on the same species in
chamber studies (Chappelka et al., 1985; Chappelka and Chevone, 1986; Duchelle et al., 1982;
Fredericksen et al., 1995; Samuelson, 1994). No response to O₃ exposure has been found for
other hardwood trees, nor for the three conifer species tested (Neufeld et al., 2000).

Long-term foliar injury symptoms have been correlated with decreased radial growth in
yellow poplar and black cherry (Somers et al., 1998) and with decreased biomass in cherry
(Neufeld et al., 1995). Although climatic conditions (drought) largely explained radial growth
reductions, O₃ exposure may have also contributed (McLaughlin and Downing, 1996). Ozone
exposure may also be affecting the understory vegetation in the region (Chappelka et al., 1997;
2003; Davison et al., 2003; Duchelle et al., 1983; Duchelle and Skelly, 1981) and community
composition (Barbo et al., 1998), through impacts on both growth and reproduction (Chappelka,
2002). Foliar litter from trees exposed to elevated O₃ have lower decomposition rates (Kim
et al., 1998). Other air pollutants are likely to be found in this ecosystem but not at the high
deposition values found in the California studies. A decline in forest health in the northern
Appalachians has been primarily attributed to the effects of acidic fog and rain on soil
acidification, lower Ca\(^{2+}\) availability, reduction in fine root biomass, and modification of cuticular wax. However, fog- and O\(_3\)-exposed red spruce forests in New England also show winter injury (Percy et al., 1992).

**AX9.6.2.5 Plantago Studies in the United Kingdom**

One of the most well-documented studies of population and community response to O\(_3\) effects are the long-term studies of common plantain (*Plantago major*) in native plant communities in the United Kingdom (Davison and Reiling, 1995; Lyons et al., 1997; Reiling and Davison, 1992c). Sensitive populations of *P. major* had significant growth decreases in elevated O\(_3\) (Pearson et al., 1996; Reiling and Davison, 1992a,b; Whitfield et al., 1997) and reduced fitness as determined by decreased reproductive success (Pearson et al., 1996; Reiling and Davison, 1992a). While spatial comparisons of population responses to O\(_3\) are complicated by other environmental factors, rapid changes in O\(_3\) resistance were imposed by ambient levels and variations in O\(_3\) exposure (Davison and Reiling, 1995). Molecular patterns of genetic variation suggest that a change in O\(_3\) resistance over time probably resulted from natural selection in genotypes already present in local populations, rather than through an influx of new *P. major* germplasm (Wolff et al., 2000). At the site of common plantain seed collection the highest correlations occurred between O\(_3\) resistance and ambient O\(_3\) concentrations occurred (Lyons et al., 1997), rather than between O\(_3\) resistance and other climatic variables, as found for aspen (Berrang et al., 1991).

**AX9.6.2.6 Forest Health in the Carpathian Mountains**

The Carpathian Mountains cross five countries (the Czech Republic, the Slovak Republic, Poland, Romania, and the Ukraine) and contain many national parks and several biosphere reserves. The forests were largely cleared in the 15th century and were replanted with Norway spruce. As elevation increases, beech (*Fagus sylvatica*) or beech-fir (*Abies alba*) forests grade into Norway spruce or spruce-fir forests. Near the treeline, Norway spruce mixes with dwarf mountain pine (*Pinus mugo*). Dwarf mountain pine forms an almost pure stand just below the alpine vegetation.

The forests of the Carpathian Mountains have been subjected to anthropogenic stressors (e.g., shepherding, metal mining, wood harvest for structures and paper) for hundreds of years,
as described for the Tatra Mountains in the southern Carpathians (Wezyk and Guzik, 2002). The Carpathians have been subjected to regional air pollution stressors since industrialization. Most of the effects of air pollution on forest health degradation were due to (1) heavy metal deposition, (2) soil acidification by acid deposition, and (3) subsequent pest outbreaks, the combination of which led to the forest decline and dieback between 1970 and 1989 (Dunajski, 2002). Industrial pollutants such as SO$_2$ and heavy metals have significantly declined since the 1980s, but O$_3$ exposure has continued to increase (Bytnerowicz et al., 2002a; Bytnerowicz et al., 2004). The increased ownership and use of private cars in Central Europe, as well as the long-range transport of O$_3$ from western Europe, are believed to be responsible for the continued increase in photooxidants. In 1995, drought resulted in significant forest mortality, as well as an epidemic of bark beetle infestation in subsequent years. A network of air quality monitoring sites was installed across Europe in the late 1980s as part of the International Cooperative Programme on Assessment and Monitoring of Air Pollutant Effects on Forests (ICP Forests). Mean defoliation rates for six important forest species across Europe have increased or remained unchanged from 1989 to 1999 (Percy et al., 2002). Ozone concentrations experienced in the Tatra Mountains, especially along the southern slopes, occasionally reach 190 to 200 ppb as 2-week-long averages, with the highest values experienced in early summer at elevations of 1700 to 2300 m (Bytnerowicz et al., 2004). In other parts of the Carpathian Mountains, peak 2-week average O$_3$ concentrations were lower, at 160 ppb (Bytnerowicz et al., 2002b). For all trees inventoried, about 13% exhibited greater than 25% defoliation during 1997 to 2000. There was no difference between extent of damage for broadleaves or conifers. Trees in Poland and the Czech Republic were the most affected by air pollution, while the least damaged forests were in Romania (Bytnerowicz et al., 2002b).

The extent to which O$_3$ exposure affects forest health degradation, and slows forest degradation, is still unknown in Europe. In many of the published studies, the response to a known O$_3$ gradient is largely confounded by other pollutants and/or climatic gradients (Szaro et al., 2002; Widacki et al., 2002). Current levels of ambient O$_3$ are believed to be high enough to reduce bole radial growth (Percy et al., 2002). Although average O$_3$ concentration alone was not related to bole growth, the peak hourly O$_3$ concentration was negatively correlated to bole growth (Muzika et al., 2004). Recent evidence indicates that canopy health of European white
oak (*Quercus robur*), Norway spruce, maritime pine (*Pinus pinaster*), and beech has significantly declined (Huttunen et al., 2001). However, the canopy health of Scots pine has improved. The network of air quality monitoring stations and forest plots is extensive and active. Subsequent correlative analyses including both meteorological and air quality attributes throughout the European Union (EU) will help to determine the specific role of O₃ exposure in forest decline. Historical effects of anthropogenic disturbance may still be confounding.

**AX9.6.2.7 Field Exposure System (FACE), Rhinelander, Wisconsin**

The Aspen Free-Air CO₂ Enrichment facility was designed to examine the effects of both elevated CO₂ and O₃ on aspen (*Populus tremuloides*), birch (*Betula papyrifera*), and sugar maple in a simple reconstructed plantation characteristic of Great Lakes Aspen-dominated forests (Karnosky et al., 2003b; Karnosky et al., 1999). Instead of using chambers to expose the plants to desired gas concentrations, the gas is piped up vertical delivery tubes in the open air. The vertical delivery pipes surround a 30-m diameter circular plot with five different aspen clones in half of the plot, one quarter of the plot planted in aspen and birch, and one quarter in aspen and maple. The O₃ treatment for the first 5 years was 1.5× ambient, with ambient O₃ exposures averaging 35 to 37 ppb (12 h daytime average over the growing season) compared to elevated O₃ rings averaging 49 to 55 ppb for the same time period (Karnosky et al., 2003b).

Elevated CO₂, elevated O₃, and elevated CO₂ + O₃ have had effects on most system components being measured in the study (Table AX9-26) (Karnosky et al., 2003b). One interesting finding of the project has been the nearly complete offset by elevated O₃ of the enhancements induced by elevated atmospheric CO₂ for the pioneer keystone species aspen (Isebrands et al., 2001) and birch (Percy et al., 2002) even though O₃ exposure alone did not always result in a significant response when compared to controls. They also found evidence that the effects on above- and below-ground growth and physiological processes have cascaded through the ecosystem, even affecting microbial communities (Larson et al., 2002; Phillips et al., 2002). This study also confirmed earlier observations of changes in trophic interactions involving keystone tree species, as well as important insect pests and their natural enemies (Table AX9-26) (Awmack et al., 2003; Holton et al., 2003; Percy et al., 2002).
Table AX9-26. Summary of Responses of *Populus tremuloides* to Elevated CO$_2$ (+200 µmol mol$^{-1}$), O$_3$ (1.5 × ambient), or CO$_2$+O$_3$ Compared with Control During 3 Years of Treatments at the Aspen FACE Project (Modified from Karnosky et al. (2003b))

<table>
<thead>
<tr>
<th>Foliar Gene Expression and Biochemistry</th>
<th>CO$_2$</th>
<th>O$_3$</th>
<th>CO$_2$ + O$_3$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubisco; RbcS$^2$ transcripts</td>
<td>d$^1$</td>
<td>d</td>
<td>dd</td>
<td>Noormets et al. (2001a); Wustman et al. (2001)</td>
</tr>
<tr>
<td>PAL transcripts</td>
<td>d</td>
<td>I</td>
<td>d</td>
<td>Wustman et al. (2001)</td>
</tr>
<tr>
<td>Acc oxidase, catalase</td>
<td>d</td>
<td>I</td>
<td>d</td>
<td>Wustman et al. (2001)</td>
</tr>
<tr>
<td>Ascorbate peroxidase</td>
<td>d</td>
<td>n.s.</td>
<td>d</td>
<td>Wustman et al. (2001)</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>d</td>
<td>I</td>
<td>d</td>
<td>Wustman et al. (2001)</td>
</tr>
<tr>
<td>Phenolic glycosides</td>
<td>I</td>
<td>d</td>
<td>n.s.</td>
<td>Kopper and Lindroth (2003a,b); Lindroth et al. (2001)</td>
</tr>
<tr>
<td>Tannins</td>
<td>n.s.</td>
<td>i</td>
<td>i</td>
<td>Kopper and Lindroth (2003a,b); Lindroth et al. (2001)</td>
</tr>
<tr>
<td>Foliar nitrogen</td>
<td>d</td>
<td>n.s.</td>
<td>d</td>
<td>Kopper and Lindroth (2003a,b); Lindroth et al. (2001)</td>
</tr>
<tr>
<td>C:N ratio of foliage</td>
<td>i</td>
<td>n.s.</td>
<td>ii</td>
<td>Lindroth et al. (2001)</td>
</tr>
<tr>
<td>Starch</td>
<td>d</td>
<td>d</td>
<td>n.s.</td>
<td>Wustman et al. (2001)</td>
</tr>
</tbody>
</table>

**Gas Exchange**

<table>
<thead>
<tr>
<th>A$_{max}$ lower canopy</th>
<th>n.s.</th>
<th>dd</th>
<th>id</th>
<th>Noormets et al. (2001b); Takeuchi et al. (2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A$_{max}$ whole canopy</td>
<td>ii</td>
<td>dd</td>
<td>n.s.</td>
<td>Noormets et al. (2001a); Sharma et al. (2003)</td>
</tr>
<tr>
<td>Stomatal limitation</td>
<td>d</td>
<td>n.s.</td>
<td>d</td>
<td>Noormets et al. (2001b)</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>d</td>
<td>di</td>
<td>d</td>
<td>Noormets et al. (2001b)</td>
</tr>
<tr>
<td>Foliar respiration</td>
<td>n.s.</td>
<td>i</td>
<td>n.s.</td>
<td>Noormets et al. (2001a); Takeuchi et al. (2001)</td>
</tr>
<tr>
<td>Soil respiration</td>
<td>ii</td>
<td>d</td>
<td>n.s.</td>
<td>King et al. (2001)</td>
</tr>
<tr>
<td>Microbial respiration</td>
<td>ii</td>
<td>n.s.</td>
<td>n.s.</td>
<td>Phillips et al. (2002)</td>
</tr>
<tr>
<td>Stomatal density</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>Percy et al. (2002)</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>Wustman et al. (2001)</td>
</tr>
<tr>
<td>Chloroplast structure</td>
<td>i</td>
<td>d</td>
<td>d</td>
<td>Oksanen et al. (2001); Takeuchi et al. (2001); Wustman et al. (2001)</td>
</tr>
<tr>
<td>O$_3$ flux</td>
<td>d</td>
<td>ii</td>
<td>i</td>
<td>Noormets et al. (2001b)</td>
</tr>
</tbody>
</table>

**Growth and Productivity**

<table>
<thead>
<tr>
<th>Leaf thickness</th>
<th>i</th>
<th>n.s.</th>
<th>n.s.</th>
<th>Oksanen et al. (2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf size</td>
<td>i</td>
<td>d</td>
<td>d</td>
<td>Wustman et al. (2001)</td>
</tr>
<tr>
<td>Leaf area</td>
<td>i</td>
<td>d</td>
<td>n.s.</td>
<td>Noormets et al. (2001a)</td>
</tr>
</tbody>
</table>

$^1$Responses are shown as significant increases p < 0.05 (I), significant increases p < 0.01 (ii), significant decreases p < 0.05, significant decreases p < 0.01 (dd), both significant increases and decreases reported (id), nonsignificant effects (n.s.), and no data (n.d.)

AX9-281
AX9.6.3 Landscape Condition

In the SAB framework (Figure AX9-20), landscape condition is assessed using the areal extent, composition of component landscape ecosystems or habitat types, and the pattern or structure of component ecosystems or habitat types (including biocorridors). To date, no publications exist on the impacts of O₃ exposure on landscape condition. The effects of O₃ exposure have only been reported at the community or stand level (see Biotic Conditions, below). The following is a description of current discussions by land stewards and of how difficult it will be to quantitatively assess the effect of O₃ exposure on landscape condition.

Landscapes are identified and preserved, such as national parks, Class I wilderness areas, etc., so that they are protected from the effects of O₃ exposure by law. Efforts to determine whether landscapes have been affected by certain levels of exposure rely on valuation of landscape and ecosystem components. Several different approaches of valuation have been used, including pathological (visible symptoms), biomass and allocation, and biogeochemical.

In the pathological approach, a “critical levels” concept is developed, with varying levels of impact viewed as acceptable, interim targets, or as unacceptable. As an example, land managers of Class I wilderness areas may consider a level acceptable if it resulted in no visible O₃ symptoms to sensitive species. In concrete terms, sensitive species may respond to peak O₃ exposures of 60 ppb (e.g., coneflower, in Great Smoky National Mountains NP; [Davison et al., 2003]), and so the critical exposure level would be < 60 ppb for any hourly value during the growing season. An interim target would be that less than 5% of the sensitive plants would have visible symptoms on <15% of the leaf surface. An unacceptable level of O₃ exposure would be any result more pronounced than the interim target. The advantage of the foliar injury approach is that large crews with relatively simple training can assess individual species within the landscape and “see” the effect of the oxidant exposure. There are several disadvantages, however. Some species (e.g., white fir) exhibit no foliar injury but do have shifts in biomass allocation in response to oxidant exposure (Retzlaff et al., 2000). Other species have shown significant decreases in foliar injury due to needle loss, with retranslocation of nutrients to remaining foliage, and subsequent increased photosynthetic rate (Beyers et al., 1992). In addition, the development of foliar symptoms within a species is related to sunlight and microclimate (Davison et al., 2003).
Figure AX9-20. A conceptual diagram of processes and storage pools in sources and sinks that are affected by O$_3$ exposure. A plus (+) denotes an increase in process rate or pool size, a minus (−) denotes a decrease in process rate or pool size, and a plus-minus (±) denotes that both increases and decreases have been reported in response to O$_3$. Primary effects in the shoots (1°) are distinguished from secondary effects in roots (2°) since the primary site of O$_3$ action occurs in the leaves.

Source: Andersen (2003).

In the biomass approach, O$_3$ exposure resulting in a measurable decline in biomass (usually of a target, sensitive species) is used to evaluate landscape condition. The bulk of the information available is from seedling responses to controlled chamber exposures, reviewed in the previous section. Some information exists for species in natural environments, but teasing out concurrent stressors and finding adequate controls may be intractable. For example, in a long-term gradient of O$_3$ exposure, N deposition, and drought, the site with the highest O$_3$ exposure had the greatest whole tree biomass (pole-sized trees) due to growth stimulation by N deposition (Grulke and Balduman, 1999).
In the biogeochemical approach, changes in biogeochemical cycling are used to assess landscape condition. Ozone-sensitive species have well known responses to $O_3$ exposure, including altered C allocation to below- and above-ground tissues, and altered rates of leaf production, turnover, and decomposition. Changes in turnover rates of ephemeral tissues (leaves, fine roots) also affect nutritional status of the remaining tissue. These shifts can affect overall C and N loss from the ecosystem in terms of respired C, and leached aqueous dissolved organic and inorganic C and N. Instability in C and N pools and their dynamics can affect landscape-level nutrient dynamics even without significant inputs of N deposition. The endpoint assessment is based on changes in water quality from or in the landscape, correlated to a defined oxidant exposure level. These approaches are linkable: visible injury at a particular level could be related to reduction in photosynthate, which would reduce whole plant biomass (and carbon dynamics). If $O_3$-sensitive species dominate the landscape, then changes in C and N dynamics over time would be expected to alter biogeochemical cycles. Examples of forest types that contain geographically extensive, $O_3$-sensitive species that could be used in assessing landscape-level changes include Ponderosa pine in the western United States, yellow poplar or loblolly pine in the eastern United States deciduous forests, and Norway spruce in the Carpathian Mountains of eastern Europe.

Water quantity may also be affected by $O_3$ exposure at the landscape level. Moderately high $O_3$ exposure may affect the mechanism of stomatal opening (McAinsh et al., 2002), resulting in sluggish stomatal opening and closing (Reich and Lassoie, 1984). During moderately high $O_3$ exposure in a drought year, canopy transpiration was greater for yellow poplar than on adjacent days with lower $O_3$ exposure, which could alter water use at the landscape level. Oxidant exposure ($O_3$ and NO$_x$) may decrease the ability of exposed plants to close stomata at night (Grulke et al., 2004), thus increasing water loss from the landscape. Ecosystem models should aid in interpreting $O_3$-exposure effects at the landscape level.

**AX9.6.4 Biotic Condition**

**AX9.6.4.1 Ecosystems and Communities**

The SAB framework described by Young and Sanzone (2002) identifies community extent, community composition, trophic structure, community dynamics, and physical structure as EEAs for assessing ecosystem health.
COMMUNITY EXTENT

Ecosystem function is dependent on areal extent, constituent species composition, trophic structure and its dynamics, and community physical structure. Genetic variation within species, and the dynamics of the interactions that exist among different species and their biotic and abiotic environment, are also involved (Agrawal and Agrawal, 2000). There are no reports of O\textsubscript{3} exposure altering community distribution or extent.

COMMUNITY COMPOSITION

Significant changes in plant community composition resulting directly from O\textsubscript{3} exposure has been demonstrated in two forested areas: the mixed conifer forest of the San Bernardino Mountains, CA and the mixed conifer forest of the Valley of Mexico near Mexico City. It is also likely that community composition has changed in response to O\textsubscript{3} exposure in the coniferous forests of the Carpathian Mountains, but this has not yet been definitively shown.

The first forest communities shown to be affected by O\textsubscript{3} were the *Pinus ponderosa*-dominated stands of the San Bernardino Mountains in southern California (Miller, 1973). Miller suggested that mixed forests of *P. ponderosa*, *Pinus jeffreyi*, and *Abies concolor* were changing to predominantly *A. concolor* because of the greater O\textsubscript{3} sensitivity of the pines. Significantly greater mortality of young mature trees (50 to 99 years old) occurred in sites that also showed higher foliar injury relative to sites that showed slight foliar injury (McBride and Laven, 1999). For *P. ponderosa*, 33% of the trees in the high foliar injury sites died versus 7% of the trees in the low foliar injury sites over the decade-long census. In contrast, 24% of *Abies concolor* died in high foliar injury sites, whereas no trees died in slight injury sites. The authors suggested that certain age classes were especially sensitive to O\textsubscript{3} exposure, because they are emerging into the canopy where higher O\textsubscript{3} concentrations are encountered. Future projections based on past changes in community composition have been conducted for 2024 and 2074 (McBride and Laven, 1999). In their projections, the population of Ponderosa pine nearly disappears in all tree age classes, and the community is dominated by California black oak (*Quercus kelloggii*) in all tree age classes, followed by Incense-cedar (*Calocedrus decurrens*) and sugar pine (*Pinus lambertiana*) by the year 2074. Their projections do not account for potential changes in genetic structure of the more O\textsubscript{3}-sensitive species.
In the Valley of Mexico, the closed forest structure changed to a woodland from high pollutant exposure (Fiscus et al., 2002). Cryptogamic community diversity also significantly declined in response to prolonged, extreme O$_3$ exposure (Zambrano and Nash, 2000). Together, these two examples illustrate the potential for shifts in community composition in response to O$_3$ stress.

**TROPHIC STRUCTURE**

*Above-Ground*

One of the first reports of trophic level interactions in natural communities was the O$_3$-induced predisposition of Ponderosa pine to attack by bark beetles (Cobb et al., 1968; Stark et al., 1968; Stark and Cobb, 1969). Trees exposed to oxidant injury had lower resin production, flow, and exudation pressure. Also, several attributes associated with tree defense against beetle attack including sapwood and phloem moisture content and phloem thickness were compromised by oxidant exposure (Pronos et al., 1999). Another trophic level has been implicated, in that O$_3$-injured Ponderosa pine had the same rate of bark beetle infection, but healthy trees had greater numbers of bark beetle predators and parasitoids (Dahlsten et al., 1997). This suggests that O$_3$ damage rendered the pines inhospitable for the natural enemies of the bark beetles. Similar findings were presented by Percy et al. (2002) for aphids whose abundance was increased in young *Populus tremuloides* stands exposed to elevated O$_3$. In that study, the levels of natural enemies of aphids (ladybirds, lacewings, spiders, and parasitoids) were significantly decreased under elevated O$_3$.

*Below-Ground*

Processing of plant-derived carbon compounds by soil organisms comprising the soil food web is a fundamental property of a functional and stable below-ground ecosystem (De Ruiter et al., 1998; Wolters, 1998). Soil food web organisms are responsible for recycling nutrients and for development of soil properties such as porosity, aggregate structure, water-holding capacity, and cation exchange capacity. A shift in food-web species diversity or functional complexity in response to O$_3$ stress may alter ecosystem processes.

Evidence that soil organisms are affected by O$_3$ indicates the potential for changes in soil food-web structure and function. Since O$_3$ does not penetrate the soil beyond a few centimeters,
the proposed mechanism by which \( \text{O}_3 \) alters soil biota is through a change in C input to soils (Andersen, 2003). Ozone can alter C inputs to soil and hence soil processes through four different pathways: (1) leaf-litter quality and quantity (see Material Cycling, below), (2) C allocation to roots (see Physiological Status, below), (3) interactions among root symbionts, and (4) rhizodeposition. The complex nature of the effects of \( \text{O}_3 \) on trophic interactions and food webs calls for additional basic research and modeling.

There have been no comprehensive studies on the effects of \( \text{O}_3 \) on structural components of soil food webs; however, studies have shown that \( \text{O}_3 \) affects free-living soil organisms of food webs. In the few cases where soil microbial communities have been examined, \( \text{O}_3 \) has led to changes in bacterial and fungal biomass, and, in some cases, changes in soil enzyme activity. Phillips et al. (2002) examined the effects of elevated \( \text{CO}_2 \) and \( \text{O}_3 \) on C flow through heterotrophic microbial communities in soils collected from a FACE study in Wisconsin. Ozone decreased abundance of fungal phospholipid fatty acids in aspen and birch-aspen plots but had few other direct effects on measured soil parameters. The greatest effect of \( \text{O}_3 \) was to eliminate significant increases in microbial respiration resulting from elevated \( \text{CO}_2 \), suggesting an important role for \( \text{O}_3 \) in altering C flow through soils. Shafer (1988) found that \( \text{O}_3 \) tended to increase the number of fungal propagules and bacteria exhibiting phosphatase activity in the rhizosphere of sorghum. Ozone in combination with simulated acid rain stimulated soil arylsufatase activity (Reddy et al., 1991). The response was observed at low concentrations, but was reversed at high concentrations, suggesting a threshold level of \( \text{O}_3 \), possibly involving different mechanisms. Ozone significantly decreased soil microbial biomass in the fall after one season of exposure in a wheat and soybean system (Islam et al., 2000). Other studies have shown shifts in microbial and fungal biomass in response to \( \text{O}_3 \) stress, but responses were variable (Scagel and Andersen, 1997; Yoshida et al., 2001).

Decreased allocation to roots associated with \( \text{O}_3 \) exposure alters N fixation in legumes and actinorrhizal species. Ozone exposure was found to decrease nodulation in a number of species (Manning et al., 1971; Tingey and Blum, 1973). In alder (\textit{Alnus serrulata}), host root cells of nodules showed cytoplasmic breakdown and lacked organelles when seedlings were exposed to \( \text{O}_3 \) for 27 days (Greitner and Winner, 1989).

Ozone has been shown to affect mycorrhizal colonization (Adams and O'Neill, 1991; Edwards and Kelly, 1992; Ho and Trappe, 1984; McCool et al., 1982; Simmons and Kelly, 1989;
Smith and Read, 1997). Although short term in nature, several studies have found enhanced mycorrhizal short-root formation under O\textsubscript{3} stress. White pine (\textit{Pinus strobus}) (Stroo et al., 1988), Norway spruce (Rantanen et al., 1994), Northern red oak (\textit{Quercus rubra}) (Reich et al., 1985), Douglas fir (\textit{Pseudotsuga menziesii}) (Gorissen et al., 1991a), European silver fir (\textit{Abies alba}) (Wollmer and Kottke, 1990), and Scots pine (Kasurinen et al., 1999) all showed some increase in mycorrhizal presence when exposed to O\textsubscript{3}. Others have shown minimal or no effects of O\textsubscript{3} on mycorrhizas (Kainulainen et al., 2000b; Mahoney et al., 1985; Meier et al., 1990). Stroo et al. (1988) found that percent infection increased from 0.02 to 0.06 ppm O\textsubscript{3}, then decreased from 0.06 to 0.14 ppm; the total number of short roots were unaffected, however. In cases where stimulation was observed, the response was often noted shortly after initiation of exposure, often at relatively low concentrations. Good examples of this transitory response can be found in results with Norway spruce and Scots pine (Kasurinen et al., 1999; Rantanen et al., 1994). In these studies, O\textsubscript{3} increased mycorrhizal short roots initially but differences were not evident by the end of the experiment.

Evidence suggests that decreased below-ground allocation associated with O\textsubscript{3} stress alters mycorrhizal host-symbiont compatibility. Edwards and Kelly (1992) found a shift in fungal morphotypes present on loblolly pine roots, even though the number of mycorrhizal short roots per gram fine root was not significantly affected by O\textsubscript{3}. Qui et al. (1993) found increased numbers of morphotypes present in O\textsubscript{3}-sensitive loblolly pine seedlings exposed to O\textsubscript{3}. Roth and Fahey (1998) found an interaction between O\textsubscript{3} and acid precipitation treatments on the composition of fungi forming ectomycorrhizae on red spruce saplings, possibly driven by nutrient availability. Carbohydrate requirements vary among fungal species (Bidartondo et al., 2001), and O\textsubscript{3} may affect species composition by altering carbohydrate availability in roots. A shift in species dominance could lead to a change in successional patterns of mycorrhizal communities.

In the few studies that examined root exudation in response to O\textsubscript{3} exposure, O\textsubscript{3} was found to alter the quantity and quality of root exudates. McCool and Menge (1983) found a significant decrease in exudation of amino acids in tomato exposed to 300 ppb O\textsubscript{3}. McCrady and Andersen (2000) observed increased root exudation in nonmycorrhizal wheat. No apparent change in root exudation was found in labeling studies of ECM Ponderosa pine (Andersen and Rygiewicz, 1995a). Inconsistency in the literature probably results from species differences and
experimental protocols; however, these examples illustrate the potential effects of O$_3$ on rhizosphere C flux.

Decreased C allocation to roots of O$_3$-exposed plants may reduce root longevity and accelerate root turnover, increasing rhizodeposition of C and N. Fine root turnover decreased in mature northern red oak exposed to elevated O$_3$ (seasonal exposure ranging from 152 to 189 ppm-h), whereas seedlings did not show any reduction in turnover (Kelting et al., 1995). King et al. (2001) found a trend toward decreased live root biomass and increased dead root biomass in aspen exposed to O$_3$ in a FACE study, suggesting possible changes in both production and longevity.

Other studies also suggest that O$_3$ alters C flux to soils, resulting in changes in CO$_2$ efflux from soils. Both root respiration and soil CO$_2$ efflux decreased from loblolly pine seedlings exposed to O$_3$ (Edwards, 1991). Soil CO$_2$ efflux increased in response to O$_3$ in Ponderosa pine seedlings (Andersen and Scagel, 1997). No direct assessments of hyphal growth and turnover in response to O$_3$ stress have been conducted. Ozone decreased C allocation to extrametrical hyphae of a Ponderosa pine mycorrhiza, which might be expected to decrease growth and increase hyphal turnover (Andersen and Rygiewicz, 1995b).

COMMUNITY DYNAMICS, PHYSICAL STRUCTURE

One of the best-documented examples of change in long-term forest community dynamics, in dominant overstory trees, occurred in the San Bernardino Mountains between 1968 and 1974 (as reported by Miller [1973] and Miller et al. [1989]). Plots were recently re-inventoried ~25 years after establishment (Arbaugh et al., 2003). Of the six codominant canopy species, white fir showed the greatest change, increasing in both numbers and bole growth for a 286% change in basal area/ha in the San Bernardino Mountains. Sugar pine basal area also increased significantly (by 334%), but this species represents only a small portion (1%) of the total basal area of the forest sampled. The most O$_3$-sensitive species (Miller et al., 1982), Ponderosa and Jeffrey pine, had the lowest increase in basal area/ha (76 and 62%, respectively). These two species represented 72% of the basal area/ha of all stands inventoried. Ponderosa pine had the greatest mortality rate of all canopy species inventoried (46%), followed by white fir and black oak (35% and 33%), Jeffrey pine (29%) and Incense cedar and sugar pine (both 7%). In moist sites (at the western end of the San Bernardino Mountains), there was significant recruitment of
Incense cedar, white fir, and sugar pine. Only one study directly attributed tree mortality to O\textsubscript{3} exposure: it accounted for 7% of mortality in the Sierra and Sequoia NFs (Carroll et al., 2003).

Species diversity in the understory can be quite large, making studies of O\textsubscript{3} effects on understory community dynamics very challenging. However, there have been some attempts to quantify understory responses, ranging from describing relative sensitivity to their visible symptoms (Temple, 1999; Treshow and Stewart, 1973) to very complex measures of community structure and composition (Westman, 1979, 1981). The lowest percentage cover and lowest species diversity in California coastal sage scrub was correlated with the highest O\textsubscript{3} exposures as estimated by extrapolation from the closest air monitoring stations (Westman, 1979). The understory also has the potential to influence responses to O\textsubscript{3} of dominant keystone species, as has been shown in controlled experiments with both Ponderosa pine (Andersen et al., 2001) and Loblloly pine (Barbo et al., 2002). Barbo et al. (1998) exposed an early successional forest community to ambient air, charcoal-filtered air, non-filtered air, and 2× ambient in the Shenandoah NP. They found changes in species performance, canopy structure, species richness, and diversity index consistent with the view that O\textsubscript{3} can induce a shift in vegetation dominance and community structure.

There have been few studies evaluating the effect of O\textsubscript{3} exposure on the physical structure of natural ecosystems. Despite an extensive array of allometric equations for conifers in the western United States (Ter-Mikaelian and Korzukhin, 1997), none appear to predict individual tree shape in a site of moderate O\textsubscript{3} exposure, suggesting that O\textsubscript{3} may effect allometry (Grulke et al., 2003a). Canopy structural changes are also implied by the measure of canopy transparency used in the USDA Forest Service’s Forest Health Monitoring (FHM) assessment. The loss of epiphytic lichens within the canopy is a clear example of plant community structural change occurring along an O\textsubscript{3} gradient (Nash and Sigal, 1999; Zambrano and Nash, 2000).

As of yet, there have been no comprehensive studies on the effects of O\textsubscript{3} on structural or functional below-ground components (Andersen, 2003). Phillips et al. (2002) found evidence for changes in the bacterial and fungal biomass below aspen and aspen/paper birch stands exposed to elevated O\textsubscript{3}. Subsequent study showed that O\textsubscript{3} exposure decreased cellobiohydrolase activity in the soil microorganisms, driving the change in the microbial community (Larson et al., 2002).
AX9.6.4.2 Species and Populations

Ozone can affect species and populations of species found in ecosystems through changes in population size, genetic diversity, population structure and/or dynamics, and habitat suitability (Young and Sanzone, 2002). For example, if individuals of a species are lost due to O₃ exposure, population size declines. Often very young (e.g., conifer seedlings, see Section AX9.6.4.3 below) and old individuals differ in their sensitivity, so that population structure also will be altered by O₃ exposure. If resource allocation to reproductive output is altered by O₃ exposure, population dynamics will be altered. Communities dominated by O₃ sensitive species in the canopy or understory may be altered sufficiently for the habitat to become unsuitable for other species. Genetic selection acts on the individual plant, which represents a certain proportion of the populations’ genetic variation. If an O₃-sensitive individual succumbs through multiple stresses, including O₃ stress, the genetic variation represented in the population generally declines, unless sensitive individuals have low inherent genetic variability (e.g., Staszak et al., 2004).

While the concept of natural selection induced by O₃ exposure and related changes in natural plant communities has been around for a long time (Dunn, 1959; Miller et al., 1972), the concept of evolution of O₃ tolerance is still not widely accepted. The unequivocal demonstration that considerable genetic variation in O₃ resistance exists within and between plant populations, and that ambient levels of O₃ may differentially affect fitness-related traits (i.e., growth, survival, and fecundity), suggests that O₃ may potentially drive the natural selection for resistant individuals. Dunn (1959) presented circumstantial evidence that ambient O₃ in the Los Angeles area was high enough to drive the selection of O₃-resistant Lupinus bicolor genotypes. Since Dunn’s (Dunn, 1959) research on O₃-induced population changes, researchers have demonstrated differences in O₃ tolerance among other plant populations. In the devastating forest decline southeast of Mexico City, the remaining trees (primarily Abies religiosa in the “cemetery forests”) appear to be less affected by foliar injury than trees that were lost, despite continued high-O₃ exposures (Alvarado et al., 1993). However, even the most convincing work in this field (Berrang et al., 1986, 1989, 1991), with Populus tremuloides, where a strong correlation between visible foliar injury after O₃ exposure and maximum O₃ concentration at the origin of the population was shown (Berrang et al., 1991), a change in gene frequency at any one site over time has not yet been demonstrated (Bell et al., 1991; Reiling and Davison, 1992a).
Furthermore, the selection intensity of O$_3$ has been questioned (Bell et al., 1991; Taylor et al., 1994; Taylor and Pitelka, 1992) and the emergence of O$_3$ exposure since the 1950s as an environmental stressor may not have been long enough to affect tree populations with long generation times (Barrett and Bush, 1991).

The loss of O$_3$-sensitive individuals results in natural selection favoring O$_3$-tolerant species (Bradshaw and McNeilly, 1991). Increased levels of mortality of O$_3$-sensitive individuals have occurred for *Pinus jeffreyi* and *P. ponderosa* exposed to ambient O$_3$ along the western slope of the Sierra Nevadas (Miller et al., 1998; Peterson et al., 1987), for *Pinus strobus* exposed to ambient O$_3$ in southern Wisconsin (Karnosky, 1981), and for *P. ponderosa* in the San Bernardino Mountains (Carroll et al., 2003). In these examples, individuals that consistently had greater foliar injury and lower needle retention were lost in repeated surveys. Ozone-induced loss of all individuals except the most tolerant and breeding among the surviving individuals to yield more more tolerant populations has not yet been demonstrated for plants exposed to O$_3$, except for the relatively short-term (2 years) adaptation exhibited in *Trifolium repens* (Heagle et al., 1991) and *Plantago major* (Davison and Reiling, 1995). Heagle et al. (1991) were able to show the adaptation of a *Trifolium repens* population to elevated O$_3$ in just two growing seasons. Similarly, Davison and Reiling (1995) compared the O$_3$ resistance of *P. major* populations grown from seed collected from the same sites over a period of increasing O$_3$. The two independent populations studied exhibited increased O$_3$ resistance, consistent with the idea of selection for O$_3$ tolerance. Using random amplified polymorphic DNA primers, this team also showed that the later populations are subsets of the earlier ones, consistent with in-situ evolution rather than with catastrophic loss and replacement of the populations (Wolff et al., 2000). The problem is determining whether spatial patterns in O$_3$ resistance and changes in time are casually related to O$_3$, because there were very strong correlations with other factors (Davison and Barnes, 1998; Reiling and Davison, 1992c). The potential for the evolution of O$_3$ resistance has been clearly demonstrated by Whitfield et al. (1997) in their study of O$_3$ selection of common plantain, where they showed that within a matter of a few generations, it was possible to increase O$_3$ resistance in an initially O$_3$-sensitive population. Wild radish (*Raphanus sativus*) developed O$_3$ resistance after only one generation of exposure to O$_3$ (Gillespie and Winner, 1989).

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A third independent line of research suggesting O$_3$ may be affecting the genetic diversity of wild plant populations was presented by Paludan-Müller et al. (1999) who showed that northwest European provenances of European beech were more sensitive to O$_3$ than were southeast European provenances that had experienced higher O$_3$ levels. Recent research on the genetic structure of 50-year-old Ponderosa pines in the San Bernardino Mountains suggests that distinct differences in frequency of some alleles and genotypes occurred, with the O$_3$-tolerant trees being more heterozygous (Staszak et al., 2004). While both of these studies were only correlational, they are consistent with previous studies of this type, suggesting O$_3$-induced population changes. Again, other environmental stressors besides O$_3$ exposure could have been involved in effecting change within these populations.

Natural selection for O$_3$ tolerance can also be facilitated by reductions in fitness related to lower seed yields of O$_3$-sensitive species or individuals. The impacts of O$_3$ on reproductive development, recently reviewed by Black et al. (2000) can occur by influencing (1) age of flowering, particularly in long-lived trees that often have long juvenile periods of early growth without flower and seed production; (2) flower bud initiation and development; (3) pollen germination and pollen tube growth; and (4) seed, fruit, or cone yields and seed quality (Table AX9-24). In addition, vegetatively propagated species can have lower numbers of propagules under elevated O$_3$ conditions (Table AX9-24).

Several studies suggest that reproductive structures are clearly sensitive to O$_3$ and that O$_3$ can affect fitness of plants by affecting either the sporophytic or gametic generations. Decreased numbers of flower spikes and seed capsules per plant were found for plantain growing under elevated O$_3$ (Lyons and Barnes, 1998; Pearson et al., 1996; Reiling and Davison, 1992a). Similar responses were seen for *Brassica campestris* plants exposed to a single dose of 100 ppb O$_3$ for 6 h. Stewart et al. (1996) and Bosac et al. (1998) reported an increase of flower bud abortion for oilseed rape (*Brassica napus* L.) similarly exposed to a short duration of elevated O$_3$. Floral initiation period can be delayed in O$_3$-sensitive plants, as was described for dogbane (*Apocynum androsaemifolium*) grown under ambient O$_3$ in the eastern United States. In one of the few comparisons of whole plant O$_3$ sensitivities with that of male gametophytes, Hormaza et al. (1996) found a high correlation of relative O$_3$ sensitivity of pollen tube elongation with that of O$_3$ effects on net photosynthesis and relative growth rates for 6 species of fruit trees.
Clearly, the concept of O$_3$-induced genetic change is an area that needs additional research attention. Repeated collections over time from wild populations receiving high O$_3$ exposures to examine population responses and relative sensitivity changes, the sampling of genetic diversity along known O$_3$ gradients, and the use of modern biotechnological approaches to characterize and quantify genetic diversity are useful approaches to test for O$_3$-induced impacts on diversity in natural ecosystems.

AX9.6.5 Organism Condition

PHYSIOLOGICAL STATUS

The generalized effects of O$_3$ exposure on plants are well known, and have been reviewed from several viewpoints over the last decade (Darrall, 1989; De Kok and Tausz, 2001; Heath and Taylor, 1997; Matyssek et al., 1995; Pell et al., 1997; Reich, 1987; Schraudner et al., 1997; U.S. Environmental Protection Agency, 1996). The topic of individual species response and modification of response by other factors has been addressed thoroughly in Sections AX9.6.3 and AX9.6.4 of this chapter. Here, this subsection will describe the physiological changes in response to O$_3$ that have been hypothesized to lead to changes in ecosystem structure or function.

Above-Ground Responses

The first critical step leading to O$_3$ response is uptake of O$_3$ by the leaves, leading to changes in C and nutrient relations that are thought to alter plant growth and competitiveness (see Section AX9.3). Ozone enters leaves through stomata, reacts with cell walls or membranes, and starts a series of adverse reactions. Cuticular uptake of O$_3$ is believed to be negligible (Coe et al., 1995; Kerstiens and Lendzian, 1989). Once inside the leaf, O$_3$ and its byproducts lead to membrane disruption, chlorophyll breakdown, and decreased Rubisco levels (Schweizer and Arndt, 1990). In turn, photosynthesis is decreased, as is stomatal conductance (Weber et al., 1993). Also, O$_3$ often leads to increased maintenance respiration, decreased foliar nutrient content, and imbalances in tissue nutrient content and retention. When photosynthetic pigments have been damaged, the pigment must be fully broken down (and/or new N and Mg must be taken up and transported to the leaf) for the pigment to be regenerated (Bjorkman and Demmig-Adams, 1995). Ozone exposure alters within-plant priorities for resources: less C is available for allocation to roots and spring regrowth, and less foliar biomass is retained. At the whole-
organism level, O₃ exposure decreases root mass (Grulke et al., 1998a) and radial bole growth (Muzika et al., 2004; Peterson et al., 1991) with little impact on height growth. Visible symptoms of O₃ injury vary between species and genotypes but often include upper leaf surface stipple, chlorotic mottle, or large bifacial blotches of necrotic tissue. Premature senescence is typical of almost all O₃-induced foliar damage. All of these changes can alter the plant’s ability to function in a broader ecosystem context.

The underlying mechanisms of O₃-injury response in conifers, broadleaf deciduous trees, and herbaceous species are assumed to be similar. However, several differences in long-lived species are important at the ecosystem level. Most of the research on O₃ effects has been conducted on herbaceous species (i.e., crops). Although a number of native herbaceous species have been identified as O₃-sensitive, there are no published physiological studies on the effect of O₃ exposure on herbaceous or shrub species in situ. In natural ecosystems, the majority of species are not annuals, unless the system is highly disturbed. Nonetheless, response of crop species to elevated O₃ may be used as an analog for native annual response: phenological staging is accelerated (soybeans; Booker et al. [2004]), thus “avoiding” additional O₃ exposure.

Conifers have roughly half the stomatal conductance of deciduous broadleaf trees (Reich, 1987), leading to proportionally less O₃ uptake at the same O₃ exposure level. Yet, except for species of larch, individual conifer needles are longer-lived and active over a greater portion of the year. Therefore, needle longevity can also work against the tree by increasing cumulative O₃ exposure and exposure to other stressors. Increased needle longevity is not always a disadvantage, for example, conifers are physiologically active in early spring and late fall, during times of lower oxidant concentrations. These periods can contribute significantly to a net positive annual C balance and from the standpoint of nutrient storage, are important in reparation responses to pollutants. Patterson and Rundel (1995) reported that Jeffrey pine had significant stomatal opening (one third that of a typical summer day) in midwinter with snow on the ground. At least pole-sized and larger trees can mitigate reductions in C acquisition due to oxidant exposure in the summer with C assimilation on favorable days in the winter. The interaction of environmental factors, plant phenology (the timing of growth events; birth and mortality of plant parts), physiological status (nutritional or moisture status; dormant or active growth within the year), and tree age (interannual differences in resource acquisition and allocation) all contribute to the complexity of long-lived species (and hence ecosystem) response to O₃ exposure.
One widely observed response to $O_3$ exposure is premature leaf loss. As noted above, premature leaf loss may reduce $O_3$ uptake during high-$O_3$ years, but it has several negative consequences. Early leaf loss results in reduced C uptake through photosynthesis. Premature needle loss also results in less N retranslocation compared to normally senescing leaves, which reduces whole plant N balance (Fenn and Dunn, 1989) and carbohydrate availability for overwinter storage (Grunke et al., 2001). Because of such effects accumulated over several years of $O_3$ exposure, subsequent-year C and N reserves can be affected (Andersen et al., 1991).

Conversely, a series of drought years can decrease $O_3$ uptake, as well as reduce C and nutrient acquisition, altering resource allocation to defenses (e.g., antioxidants) (Grunke et al., 2003b) (or resins) against insect infestation, rendering the tree more susceptible to $O_3$ injury. Conifers have thicker cuticles than either broadleaf deciduous or herbaceous species. Continued $O_3$ exposure may compromise cuticular integrity (Bytnerowicz and Turunen, 1994). Once cuticular integrity is breached, individual leaves (needles) are likely to be excised, thus contributing generally to defoliation and reduced C acquisition.

With the exception of the extensive research conducted on mature tree response to $O_3$ exposure in California forests (Arbaugh et al., 1998; Grulke et al., 1996, 1998b, 2001, 2002b, 2003a,b, 2004; Grulke, 1999; Grulke and Balduman, 1999; Peterson et al., 1987, 1991, 1995; Wieser et al., 2002), the vast majority of studies of $O_3$ effects on forest trees have been conducted on young seedlings (Chappelka and Samuelson, 1998) and little is known about acclimation to $O_3$ (Skärby et al., 1998). Chamber exposure studies can be used to document foliar symptoms and develop response variables for the whole plant. These response variables can then be field tested on mature trees using correlative analyses (e.g., Grulke and Lee [1997]; Grulke et al. [2003b]). Without the initial work in chamber exposure studies, field responses to $O_3$ exposure would be difficult to verify and distinguish from other concurrent stressors.

Predicting mature tree responses to $O_3$ solely from seedling response studies is complex, because seedlings or saplings do not necessarily respond to $O_3$ in the same way as mature trees (Karnosky et al., 2003a; Norby et al., 1999). Although species dependent, $O_3$ has been found to have stronger effects on leaf function in younger rather than older trees (Kolb and Matyssek, 2001). Each component physiological attribute “matures” at a different rate. Gas exchange patterns differ between seedlings and mature trees. For example, leaf respiration of juvenile Ponderosa pine was greater than that of mature trees (Momen et al., 1996). In the conifers
tested, the highest gas exchange rates (and by inference stomatal uptake of $O_3$) are found in seedlings (e.g., in scions of red spruce, Rebbeck et al. [1993]; giant sequoia, Grulke and Miller [1994]; Ponderosa pine, Grulke and Retzlaff [2001]; and Norway spruce, Wieser et al. [2002]). Patterns of biomass (Grulke and Balduman, 1999) and carbohydrate allocation (Grulke et al., 2001) differ between immature and mature trees. Pole-sized trees had greater reduction in root, foliar, and bole carbohydrate concentrations than did old growth trees. Antioxidant defenses vary with both tree age and needle age (Tegischer et al., 2002). Based on all attributes measured in both Ponderosa pine and giant sequoia, the youngest tree age considered representative of mature trees was 20 years old (Grulke et al., 1996; Grulke and Retzlaff, 2001). In some broadleaf deciduous tree species, seedlings are more conservative, and mature trees have greater gas exchange rates, as is the case for *Quercus rubra* (Edwards et al., 1994; Kelting et al., 1995; Samuelson and Kelly, 1996, 1997) and *Fagus sylvatica* (Braun et al., 1999). In another broadleaf deciduous tree species (black cherry), gas-exchange rates of seedlings were faster, but total $O_3$ flux to leaves of seedlings was lower than that of mature trees due to differences in leaf ontogeny (Fredericksen et al., 1995, 1996b).

Nitrogen deposition modifies the effects of oxidant exposure through several offsetting physiological mechanisms (see Section AX9.4.4). Nitrogen deposition, in wet or dry particulate form, ultimately increases site fertility, but increased soil N availability decreases C allocation to roots, further exacerbating the effects of $O_3$ exposure on roots (Grulke et al., 1998a). Increased N availability also increases foliage turnover: fewer needle age classes are retained (Gower et al., 1993). Therefore, the combination of both increased N and $O_3$ exposure increases foliar turnover. Finally, N deposition and increased plant N nutrition can increase stomatal conductance, leading to increased $O_3$ uptake. Alternatively, increased N counteracts the effect of $O_3$ on photosynthesis by increasing photosynthetic pigments and enzymes. Nitrogen deposition may mitigate the degree of foliar injury from oxidant pollution via higher available N for reparation of photosynthetic pigments. Nitrogen amendments also modify the antioxidant defense system in complex ways (Polle, 1998).

Attributes of $O_3$ injury to trees (foliar injury, needle retention, and canopy transparency), as well as presence of pathogens and insect infestation, are routinely inventoried in established sample plots distributed on Federal lands across the United States (Forest Health Protection, USDA Forest Service). Foliar injury to several widespread, herbaceous species nationally...
recognized as sensitive (bioindicators) is also assessed (U.S. National Park Service, 2003). This assessment is part of a larger assessment of forest tree growth and dynamics (the Forest Inventory and Analysis Program) (Smith et al., 2003; Smith, 2002). Risk of O$_3$ injury is then estimated for the dominant forest tree species in the sample plots. For example, 12% of sampled black cherry, 15% of loblolly pine, and 24% of sweetgum (Liquidambar styraciflua) were found to be in the highest risk category in the northeast and mid-Atlantic states (Coulston et al., 2003). In the Carpathian Mountains, 12 to 13% of all trees (broadleaf and coniferous) have greater than 26% crown defoliation (Bytnerowicz et al., 2002b). In general, broadleaves (primarily beech) trees were less affected (8 to 45%) than spruce (up to 37%) and fir (up to 50%) (Bytnerowicz et al., 2002b). Ozone injury was directly correlated with cumulative O$_3$ exposure in the Sierra Nevada Mountains (Arbaugh et al., 1998); with the best correlation being found across sites where >90% of the trees had O$_3$ injury. Although direct links of visible foliar symptoms induced by O$_3$ to adverse effects on biomass are not always found, visible foliar symptoms have been linked to decreased vegetative growth (Karnosky et al., 1996; Peterson et al., 1987; Somers et al., 1998), as well as reproductive function (Black et al., 2000; Chappelka, 2002).

Foliar O$_3$ injury has also been associated with adverse effects on competitive ability and survival in forest communities (Karnosky, 1981; Karnosky et al., 2003a; McDonald et al., 2002). Competition can alter organism condition and affect susceptibility to O$_3$. Ponderosa pine seedlings were more susceptible to O$_3$, as determined by decreased plant biomass, when grown in competition with blue wild-rye grass (Andersen et al., 2001). Similarly, the magnitude of O$_3$ effects on height and diameter growth depended on the competitive status of Populus tremuloides trees (McDonald et al., 2002). These studies show the importance of including competition as a concurrent stressor in assessing whole plant responses to O$_3$. The age of the community (“time since disturbance”) may also affect the ability of individuals to effectively respond to O$_3$. Unfortunately, the vast majority of O$_3$ studies have been conducted on open-grown plants, often grown in pots where competition is absent both above and below ground.

Clearly, age-dependent O$_3$ responsiveness and juvenile-mature correlations remain important research questions in attempting to scale up to ecosystem level responses. Patterns of allocation between root, stem, and leaf differ between immature and mature trees. Tree architecture varies with tree age, and leaf area distribution in space and time may change in
response to elevated \( O_3 \). All of these factors influence gas exchange in the canopy. Furthermore, there may be few generalities that can be made from seedling to mature tree response to \( O_3 \) within a plant functional group (Karnosky, 2003a; Norby et al., 1999). Consequently, modeling ecosystem response is limited to either dealing with monospecific plantations or assigning average responses to a mix of species.

**Below-Ground Responses**

The effect of \( O_3 \) on the soil ecosystem is thought to occur through physiological changes in the root and interactions with soil organisms (Andersen, 2003). Comparatively little is known about how changes in root growth and metabolism are translated through the soil food web, resulting in changes in soil and, hence, ecosystem processes. An overview of physiological changes likely to lead to changes at the ecosystem level is provided below.

Ozone stress decreases C allocation to roots (Cooley and Manning, 1987; Gorissen et al., 1994; Gorissen and Van Veen, 1988; Manning et al., 1971; McCool and Menge, 1983; McLaughlin and McConathy, 1983; Rennenberg et al., 1996; Spence et al., 1990; U.S. Environmental Protection Agency, 1996). Since roots are often dependent on current photosynthate for their structural development (Marshall and Waring, 1985; Ritchie and Dunlap, 1980; Van Den Driessche, 1978, 1991), C-limiting stressor such as \( O_3 \) can have rapid and significant effects on root growth. In many cases, decreased allocation to roots in response to \( O_3 \) occurs quickly, with reductions in root growth occurring within one growing season (Andersen and Rygiewicz, 1995a; Andersen and Rygiewicz, 1991; Gorissen et al., 1991b; Gorissen and Van Veen, 1988; Spence et al., 1990; U.S. Environmental Protection Agency, 1996). Decreased C allocation below ground is often associated with decreased root-shoot ratio, but observed responses in root-shoot ratio are highly variable owing to several factors including intra- and interspecies variation, culture conditions, and ontogenetic drift (Reich, 2002). Root-shoot ratio is a point-in-time measurement that does not include C lost to exudation, respiration, or turnover. Therefore, biomass and ratios of biomass (such as root-shoot ratio) do not necessarily reveal physiological changes in response to \( O_3 \) stress.

Decreased C acquisition leads to reduced carbohydrate levels and storage pools in \( O_3 \)-exposed plants (Andersen and Scagel, 1997; Cooley and Manning, 1987; Gorissen et al., 1994; Ito et al., 1985; McLaughlin et al., 1982; Rebbeck et al., 1988; Tingey et al., 1976b).
Although it is difficult to quantify changes in the field, Grulke et al. (1998a) found decreased medium and fine root biomass with increased pollutant load across an O$_3$ gradient in southern California. Coarse and fine root starch concentrations also were lowest in mature trees at the most polluted site (Grulke et al., 2001). The effects of O$_3$ could not be completely separated from other known stresses across the pollutant gradient, but it appeared that O$_3$ was an important factor in the patterns observed.

Decreased storage pools can lead to carry-over effects on root growth that are compounded over time. Decreased carbohydrate storage pools were associated with decreased root growth during the spring following exposure to O$_3$, even in the absence of additional O$_3$ exposure (Andersen et al., 1991, 1997). Decreased spring root growth was attributed to decreased stored C reserves as well as to premature loss of older foliage age classes during the previous fall. Aside from the loss of photosynthetic surface area associated with premature senescence, early loss of foliage in the fall occurs when allocation to roots is at a maximum in many species (Kozlowski and Pallardy, 1997). Older needle age classes preferentially allocate photosynthate basipetally to stems and roots (Gordon and Larson, 1970; Rangnekar et al., 1969); and the loss of older needles in the fall during allocation to root growth and storage; and in the spring during periods of root growth, preferentially impacts roots and root processes.

Ozone has also been shown to affect root metabolism as evidenced by changes in root respiration. Edwards (1991) found decreased root and soil CO$_2$ efflux during a 2-year exposure of loblolly pine to O$_3$. Fine root respiration increased in mature red oak exposed to O$_3$, while total soil CO$_2$ efflux increased in the spring and decreased in the summer and fall (Kelting et al., 1995). The authors attributed increased root respiration to increased nutrient uptake in support of increased demands in the shoot. Ozone decreased root system respiration in aspen after 12 weeks of exposure, but the decrease was closely associated with decreased root biomass and probably not metabolic processes (Coleman et al., 1996). Whether other metabolic shifts occur in the roots of plants exposed to O$_3$ needs to be examined.

Measurable effects on roots may occur before effects on shoots are observed because shoots have immediate access to C for repair and compensation, whereas roots must compete with shoots for C. Mortensen (1998) found decreased root, but not shoot, growth in Betula pubescens at O$_3$ exposures of 42 nMol mol-L (applied 12 h day$^{-1}$), whereas both root and shoot growth were reduced at higher exposures. Chromosomal aberrations were found in root tips of
Norway spruce exposed to O₃, even in the absence of biochemical changes in needles (Wonisch et al., 1998, 1999). Using relatively high O₃ concentrations (0.15 ppm O₃ 6 h day⁻¹), Hofstra et al. (1981) found metabolic changes in Phaseolus vulgaris root tips prior to the development of leaf injury. Morphological changes in root tips occurred within 2 to 3 days, and metabolism declined within 4 to 5 days of initiation of O₃ exposure.

Feedback signals from roots can influence the degree of O₃ response. Stolzy et al. (1964) exposed tomato roots to periods of anaerobic conditions and tracked a change in leaf susceptibility to O₃. An exposure of roots to low oxygen conditions for 3 h did not alter photosynthesis, but foliar damage was decreased when the roots were subsequently exposed to O₃. In this case, a signal originating in the root appeared to alter leaf sensitivity to O₃, the signal possibly being hydraulic in nature and leading to decreased O₃ uptake.

**SYMPTOMS OF DISEASE OR TRAUMA, SIGNS OF DISEASE**

Although insects and diseases are dynamic components of forest ecosystems, trees can be especially susceptible to outbreaks due to the presence of multiple stressors such as drought and pollutant exposure. Ozone can have direct effects on insect or disease organisms, indirect effects on the insect or pathogen through changes to the host, and direct or indirect effects on natural enemies of the insect or pathogen (Pronos et al., 1999). A full discussion of O₃ effects on insect and pathogen interactions can be found in Section AX9.4.

Although the multitude of interacting factors makes it difficult to identify causative factors in the field, some recent examples suggest a role for O₃ in the timing or magnitude of disease attacks in the field. After periods of drought stress (such as 1995 in central Europe), the incidence of bark beetle (Ips spp.) appears to increase. In 1998 and 1999, the mean daily capture of Ips was lowest in plots with low O₃ exposure; the converse was also true (Grodzki et al., 2004). Elevation confounded the relationships, but the differences in Ips frequency in relation to O₃ concentrations were highly significant at lower elevations. In the Valley of Mexico, a 1982 to 1983 drought was documented, but not described as precipitating a bark beetle attack in the early 1980s. However, a link between air pollutant exposure and bark beetle attacks were implicated, because attacked trees were already O₃-stressed at the time of the bark beetle attack (Alvarado et al., 1993).
An early study showed that oxidant exposure predisposed Ponderosa pine to the root pathogen *Fomes annosus* (James et al., 1980). Both root diseases (Pronos et al., 1999) and O$_3$ exposure (Grulke and Balduman, 1999) can each reduce root biomass, leading to increased drought stress, insect attack, and subsequent windthrow or death. Trees may take several years to die, and the patterns of precipitation and annual total rainfall interact to drive the level of drought stress experienced by the tree (Pronos et al., 1999). Additional research is necessary to fully understand the complex interactions occurring between O$_3$ stress and other biotic stresses.

**AX9.6.6 Ecosystem, Chemical, and Physical Characteristics (water, soil)**

**AX9.6.6.1 Nutrient Concentrations, Trace Inorganic, and Organic Chemicals**

Ozone exposure reduces the nutritional content of tissues, as well as causing elemental imbalances. Foliar nutrient content may be too high (toxic) or too low (deficient), but the relative amounts and ratios among all nutrients can also result in imbalances.

Although N deposition and foliar N content increased with O$_3$ exposure in the San Bernardino Mountains, K, Mg, Fe, and Al were all also higher in Ponderosa pine at sites more exposed to air pollution (Poth and Fenn, 1998). Trees with greater foliar injury (due to O$_3$ exposure) had higher current-year needle concentrations of P, K, Zn, and Fe than trees at the same site that were less injured. In drought-stressed Ponderosa pine with O$_3$ exposure, foliar N was also elevated and retained in the remaining needles (Temple et al., 1992). At a relatively clean site in the eastern San Bernardino Mountains, N, P, and K were efficiently readorsbed, but the amount of P remaining in the foliage was relatively high compared to defined thresholds. The fact that other elements were modified besides the N being deposited emphasizes the degree of chemical imbalance in the tissue. Foliar micronutrients were within the normal ranges reported for Ponderosa pine (Poth and Fenn, 1998; Powers, 1981). Because both N deposition and O$_3$ exposure reduce root biomass, it was unlikely that the foliar nutrient content was higher due to greater uptake. Instead, it appears that retranslocation from senescing tissue was responsible. Across a pollution gradient in the Carpathian Mountains in eastern Europe, only S/N (expected due to high S deposition) and Fe/Mn ratios were out of balance relative to established norms. The S was relatively high due to SO$_2$ deposition, and the Fe was relatively high due to smelter plumes. No imbalances could be directly attributed to O$_3$ exposure (Mankovska et al., 2004).
AX9.6.7 Ecological Processes

AX9.6.7.1 Energy Flow

All green plants generate and use energy-containing C compounds through the processes of photosynthesis and respiration. Whole-plant C uptake is dependent on photosynthesis rates, leaf area, and leaf phenology. The effects of O₃ at the site of action in the leaf are discussed in Section AX9.2. Here, the main focus is on whole-plant carbon dynamics resulting from changes in C acquisition or use under O₃ stress.

In natural ecosystems, O₃ has been shown to depress photosynthesis in sensitive tree species including Ponderosa pine (Grulke et al., 2002b; Miller et al., 1969; Takemoto et al., 1997; Weber et al., 1993) and aspen (Coleman et al., 1995a; Noormets et al., 2001a,b; Sharma et al., 2003; Yun and Laurence, 1999a). In a study of mature Jeffrey pine, trees in mesic microsites had greater O₃ uptake over the growing season in comparison to trees in xeric microsites (Grulke et al., 2003a) and greater O₃ uptake was correlated with lower mid-canopy needle retention, lower branch diameters, and lower foliar N content (Grulke et al., 2003a). Chamber studies have also shown negative effects of O₃ on tree seedling canopy structure (Dickson et al., 2001) and leaf area (Neufeld et al., 1995; Wiltshire et al., 1994). It is well known from chamber and field studies that O₃ exposure is correlated with lower foliar retention (Grulke and Lee, 1997; Karnosky et al., 1996; Miller et al., 1963, 1972; Pell et al., 1999; Topa et al., 2001).

In contrast to the relatively consistent findings for photosynthesis, O₃ effects on respiration have been more variable. Stem respiration was unaffected by O₃ exposure (Matyssek et al., 2002), suggesting that construction costs of new stems are not affected by O₃. However, the bole represents a relatively large storage pool of carbohydrates in mature trees, and the timing of phenological events among individual trees may help to confound the ability to statistically detect differences in stem respiration across pollutant gradients (Grulke et al., 2001). Belowground respiration has been found to both increase and decrease in response to O₃, depending on the approach and timing of CO₂ measures (Andersen and Scagel, 1997; Coleman et al., 1996; King et al., 2001; Scagel and Andersen, 1997). The decreased soil respiration is thought to be due to reduced root growth under O₃ exposure, but could also be partially explained by decreased microbial respiration in response to O₃. Additional research is necessary to identify
the role of O₃ in affecting root versus heterotrophic respiration, particularly over long time intervals.

Carbohydrate availability and use influence the degree to which plants respond to O₃ exposure. A model simulation of the effect of O₃ exposure on bole growth of Ponderosa pine showed a 15% reduction in mass (Weber and Grulke, 1995), largely influenced by differences in carbohydrates allocated and partitioned in repair processes elsewhere in the tree. Foliar respiration is thought to increase under elevated O₃ because maintenance costs (energy needs) of leaves damaged by O₃ are higher than normal (Grulke and Balduman, 1999; Noormets et al., 2001b). However, differences in foliar respiration are subtle and difficult to detect statistically. Foliar carbohydrate studies also suggest that more C is used under O₃ stress for repair processes (Grulke et al., 2001; Topa et al., 2001) which would result in increased respiration. Ozone exposure also reduced enzymatic activities of carbohydrate metabolism related to the breakdown of sucrose (Einig et al., 1997). Changes in soil respiration in response to O₃, even though O₃ does not penetrate into the soil, illustrates the tight coupling of plant C balance and soil biota and illustrates the potential role O₃ plays in altering ecosystem C balances (Andersen, 2000).

Ozone can affect plant allometry through changes in energy use, potentially affecting net primary production (NPP) at larger scales. The net effect of O₃ impacts on photosynthesis and respiration for sensitive components of natural ecosystems is that height growth (Isebrands et al., 2001; Oksanen, 2003a) and radial growth (Isebrands et al., 2001; Oksanen, 2003b; Peterson et al., 1987, 1991) can be negatively affected by O₃. This has been extrapolated to decreased NPP (Hogsett et al., 1997; Laurence et al., 2000).

Energy flow in plant communities can be altered by O₃ through changes in C allocation. It is well known that elevated O₃ affects C allocation to roots (Andersen et al., 1997; Coleman et al., 1995b; Grulke et al., 1998a, 2001; Grulke and Balduman, 1999) by decreasing or inhibiting phloem loading of carbohydrates (Grantz and Farrar, 1999; Landolt et al., 1997), or of carbohydrate metabolism (Einig et al., 1997). This leads to depressed root growth (Andersen et al., 1991; Coleman et al., 1996; Grulke et al., 1998a) and the potential for plant communities to have an increased susceptibility to drought through altered root-shoot balance. Furthermore, it can negatively affect below-ground food webs (Phillips et al., 2002; Scagel and Andersen, 1997).
Another energetically costly response to O₃ exposure is that the production of defense compounds, such as antioxidants, tend to increase under elevated O₃ conditions (Sheng et al., 1997; Tausz et al., 1999c, 2002). Antioxidants help the plant scavenge free radicals before they can cause damage to membranes or cell walls, but they demand C for production such that growth can be adversely affected. In mature Jeffrey pine, stomatal uptake of O₃ elicited one complex of antioxidant defenses in mesic microsites, while endogenously generated free radicals in the chloroplast elicited a second complex of antioxidant defenses in xeric microsites (Grulke et al., 2003b).

**AX9.6.7.2 Material Flow**

Plants as producers are responsible for using inorganic atmospheric C and reducing it into organic forms used by consumers, thus driving nutrient processes in ecosystems. Ozone has the potential to disrupt material flow through organic C cycling and changes in nutrient cycling, particularly N and P cycling. Although there is indirect evidence that O₃ is disrupting C and nutrient cycling at the ecosystem level, there is little direct evidence that O₃ alters nutrient processing at ecosystem scales.

The greatest annual nutrient and C input to ecosystems is from foliar and root turnover. Excision of plant parts and whole plant mortality are potentially much larger, but syncopated, ecosystem inputs. Ozone exposure alters C cycling in the ecosystem by affecting the within-plant C allocation and partitioning in dominant, O₃-sensitive plants, and through chemical composition and rate of decomposition of sloughed plant parts (roots, branches, leaves) (Figure AX9-19).

In addition to O₃-induced changes in the quantity of C and nutrient inputs into ecosystems, O₃ also can alter the nutrient quality of inputs. Ozone exposure alters nutrient levels in the foliage (Boerner and Rebbeck, 1995; Fenn and Poth, 1998; Lindroth et al., 2001; Momen et al., 2002) and affects the C:N ratio (Andersen et al., 2001; Grulke et al., 2003b; Grulke and Lee, 1997; Lindroth et al., 2001). Concentrations of compounds such as tannins, lignin, and phenolics (Baumgarten et al., 2000; Findlay et al., 1996; Kim et al., 1998; Saleem et al., 2001) are also affected by O₃ exposure, which in turn alters decomposability (Fenn and Dunn, 1989) and litter buildup in the ecosystem.
There are several possible pathways by which O3 may affect litter quality and, hence, litter decomposition, thus altering nutrient flow in ecosystems. These include altered C quality, altered nutrient quality, and alteration of leaf surface organisms important in decomposition pathways. For example, yellow poplar and black cherry litter exposed to O3 showed greater N loss during decomposition than charcoal-filtered controls, although mass loss did not vary among O3 treatments (Boerner and Rebbeck, 1995). Subsequent studies showed that although foliar N was not affected by O3 exposure in yellow poplar leaves, foliage decomposed more slowly (Scherzer et al., 1998). Other studies have also shown a change in foliar N concentration in response to O3 treatment, affecting the C:N ratio and possible litter quality (Andersen et al., 2001).

In some cases, it appears that N remobilization from foliage into the plant is not complete at the time of foliage abscission in O3-exposed plants (Findlay and Jones, 1990; Matyssek et al., 1993; Patterson and Rundel, 1995; Stow et al., 1992). Greater N content of senesced litter could increase rates of decomposition. When O3-exposed cottonwood (Populus deltoides) leaves abscissed at the same time as control leaves, they decomposed at similar rates; however, prematurely senesced foliage from O3-exposed cottonwood decomposed more slowly than controls despite their higher N content (Findlay et al., 1991; Findlay and Jones, 1990). Higher N in senesced leaves appeared to be related to organic complexes formed by bound phenolics in O3-exposed leaves, making the litter less palatable to decomposers, and slowing decomposition rates (Findlay et al., 1996; Jones et al., 1994). Increased phenolics also have been found in European silver birch (Betula pendula) exposed to O3 (Saleem et al., 2001).

Carbon quality in leaf litter also changes in O3-exposed foliage. Compositional changes in leaf structural characteristics, such as lignin content, would be expected to alter rates of litter decomposition (Fogel and Cromack, 1977; Kim et al., 1998; Meentemeyer, 1978). Blackberry litter exposed to elevated O3 had greater permanganate lignin than the control treatment, a difference that was inversely related to mass-loss rates in decomposition studies (Kim et al., 1998).

Ozone may affect early stages of decomposition by altering populations of leaf surface organisms before or after senescence. Magan et al. (1995) found a shift in phyllosphere fungi on Scots pine, Sitka spruce (Picea sitchensis), and Norway spruce exposed to O3, but the potential effect of these changes on subsequent litter decomposition was uncertain. The slowest
decomposition rates of preexposed blackberry leaves were found when senesced foliage was exposed to O$_3$ during decomposition, suggesting a possible direct effect of O$_3$ on microorganisms in decomposing litter (Kim et al., 1998). Whether O$_3$ concentrations at the soil surface influence initial stages of litter decomposition remains to be addressed.

Ozone exposure also reduces nutritional content of foliage because of the degradation of chlorophyll. Resynthesis of chlorophyll may be limited by nutritionally poor soils or low soil moisture, as well as alteration of root uptake by O$_3$ exposure and other stressors. Foliar exposure to O$_3$ may also increase leaching of nutrients (Kerstiens and Lendzian, 1989). Ozone exposure promotes early senescence of foliage (Heath and Taylor, 1997; Miller and Elderman, 1977), with higher nutrient content than if excised later in the growing season (Poth and Fenn, 1998).

Since O$_3$ can slow decomposition through changes in leaf quality and quantity, leaf litter can accumulate (Fenn and Dunn, 1989). The accumulation of soil organic matter from increased leaf litter, even in the absence of acidic deposition, can lower soil pH (Binkley, 1992). Lower soil pH can promote loss of nutrients, particularly cations, from the system, further reducing nutrient availability to the plant. Complex organic compounds in decomposing litter may also tie up nutrients, rendering them less available to plants.

Other factors such as nutrient deposition also affect the degree to which O$_3$ influences C and nutrient flow through ecosystems. In high-pollution sites, the effect of N deposition is difficult to separate from O$_3$ exposure, and reviews of the effect of acidic (and N) deposition on ecosystem nutrient dynamics are important to consider (see Binkley [1992]; Fenn et al. [1998, 2003b]). For example, at moderately high pollution sites, foliar content of N is higher than that at lower pollution sites, but so are P, Mg, and Fe contents (Poth and Fenn, 1998). Although significant changes in foliar tissue chemistry have occurred in response to long-term pollutant deposition in the Carpathians (Fenn et al., 2002; Mankovska et al., 2004), much of this response is correlated to heavy metal and N and S deposition. At this point, the contribution of O$_3$ exposure alone cannot be isolated without careful between-site comparisons of plant response and understanding the depositional velocities of constituent atmospheric species. The significant effects of plant response along known pollution gradients are important to consider, because heavy metal and N and S deposition has significantly declined over the last decade, while O$_3$ exposures remain high and declines in forest health have been sustained.
Models provide a means to track material flows through ecosystems. Two biogeochemical models were parameterized to capture long-term effects of O₃ exposure, N deposition, and climate on a Ponderosa pine-dominated site in the eastern San Bernardino Mountains. Simulated O₃ exposure resulted in faster production and turnover of foliage and a shift in C from the canopy (15% reduction) to the forest floor (increase of 50 to 60%) (Arbaugh et al., 1999). When O₃ exposure was combined with that of N deposition, litter mass increased exponentially.

The direct effect of O₃ exposure on below-ground nutrient dynamics and ecosystem material flow is poorly understood. Additional research will be necessary to understand spatial and temporal dynamics of nutrient and C flow in ecosystems and to separate the effects of O₃ from those attributable to N deposition.

**AX9.6.8 Hydrological and Geomorphological**

At present, there are no publications on the effects of O₃ exposure that are carried through at the ecosystem level to changes in mass water flow, channel morphology, riparian habitat complexity, or sediment movement. It is possible that processes occurring at smaller scales are affecting geomorphological processes in ecosystems; however, difficulty in scaling these responses spatially and temporally have made it difficult to show experimentally. It is possible that O₃ exposure affects water quality through changes in energy and material flows, as discussed previously.

**AX9.6.9 Natural Disturbance Regimes**

There has been little research on how natural disturbances interact with O₃ to affect performance of plants, communities, and ecosystems. The frequency, intensity, extent, and duration of natural disturbances are variable and unpredictable. However, there have been enough ecophysiological studies to suggest that O₃ could predispose plant communities to certain natural stresses, e.g., drought stress or extreme low-temperature stress during the winter.

While several studies have shown that drought stress reduces O₃ uptake through stomatal closure, evidence also suggests that O₃ can alter plant water use and susceptibility to drought. In controlled studies, Reich and Lassoie (1984) showed that relatively low O₃ concentrations could diminish stomatal control and alter water use efficiency. Ash trees (*Fraxinus excelsior*) exposed to elevated O₃ had greater water use early in the growing season, but less water use late in the
growing season when exposed to elevated O$_3$ (Wiltshire et al., 1994). Under moderate drought stress, Norway spruce trees grown under elevated O$_3$ consumed water faster and showed higher stomatal conductances than controls (Karlsson et al., 1995). Pearson and Mansfield (1993) showed that successive O$_3$ episodes disrupted stomatal function, making beech seedlings more susceptible to drought. Previous year O$_3$ exposure was shown to have a carry-over effect in the following growing season for beech (Pearson and Mansfield, 1994).

Few studies showing the effects of O$_3$ on water relations of field-grown trees are found in the literature. However, Grulke et al. (2003a) examined the effects of O$_3$ on canopy transpiration of Jeffrey pine from mesic and xeric microsites and found that trees from mesic sites had 20% more O$_3$ uptake than those in the xeric sites. The authors also concluded that the mesic trees had greater O$_3$ injury as evidenced by lower needle retention, whereas trees in xeric microsites had greater chlorotic mottle. Chlorotic mottle induced by stomatal uptake of O$_3$ is indistinguishable from that of endogenously produced oxidants resulting from partially closed stomata, a reduction of CO$_2$ inside the leaf, and production of strong oxidizers within the chloroplast when excited electrons are passed to O$_2$ instead of CO$_2$ under high light levels.

Trees living near the limits of their freezing-tolerance range may be especially susceptible to predisposition of freezing injury by O$_3$ (Sheppard et al., 1989). However, Aleppo pine exposed to elevated O$_3$ had enhanced winter hardiness (Wellburn and Wellburn, 1994). As with the seasonal carryover of drought susceptibility, the influence of elevated O$_3$ on freezing tolerance is carried over from summer to winter. Such effects have been demonstrated for Sitka spruce (Lucas et al., 1988) and for red spruce (Waite et al., 1994). Sorting out the role of elevated O$_3$ in contributing to frost or low-temperature damage in forests remains difficult due to the presence of other factors that may affect senescence.

**AX9.6.10 Scaling to Ecosystem Levels**

The vast majority of literature describing O$_3$ effects comes from short-duration herbaceous plant or tree seedling studies under controlled conditions. Scaling results from these studies requires extrapolation over both space and time in order to understand the full extent of changes in ecosystems. In addition to spatial, temporal, and age-related complexities, ecosystems are composed of organisms whose lifetimes range from hours to centuries (Laurence and Andersen, 2003). Forested ecosystems are affected by environmental conditions such as water and nutrient
availability, as well as by intra- and interspecies competition. Therefore, direct experimentation
to determine the response of forested ecosystems is not simply a matter of determining the effect
of O₃ on individual mature trees. In addition, even if an experiment can be conducted,
extrapolation of the results across landscapes and regions remains challenging. Nonetheless,
models provide a means to explore possible long-term changes and to identify important
research uncertainties.

Approaches to scaling fall roughly into two categories: (1) process-based modeling to
extrapolate physiological responses to O₃ based on seedling studies and (2) field assessments
using surveys and growth correlations, often in association with stand-level models to address
ecosystem complexity. Comparatively good information is available on process level effects
of O₃ in seedlings and, therefore, some models offer the opportunity to use this information to
scale O₃ effects at the stand and regional scales (Chappelka and Samuelson, 1998; Fuhrer et al.,
1997; Hogsett et al., 1997; Laurence et al., 2000). Field assessments offer the opportunity to
examine larger plot sizes, older trees, and trees growing under realistic competition. These two
approaches are discussed in more detail below.

AX9.6.10.1 Scaling from Seedlings to Mature Trees

A number of investigators have used simulation models based on physiological processes
to integrate available data and predict the effects of O₃ on mature trees. Such models predict tree
growth by simulating fundamental mechanisms, rather than through a statistical analysis of
empirical data. For instance, the process of photosynthesis is simulated based on environmental
conditions and physiological characteristics, and then the fixed C is allocated to plant growth
using principles of plant physiology. Models based on mechanisms should be applicable across
wide areas if the important functional relationships are represented accurately in the models and
if the environmental conditions are accurately identified. The ability of six models (TREGRO,
CARBON, ECOPHYS, PGSM, TREE-BGC, and W91) to simulate the effects of climatic
change and O₃ have been reviewed by Constable and Friend (2000). Of these models, only
PGSM and TREGRO explicitly simulated the effects of O₃ on foliar processes.

The TREGRO model was used to simulate C allocation and tissue growth in seedlings and
mature red oak trees based on the experimental data discussed above (Weinstein et al., 1998).
For seedlings at 2×-ambient O₃, only the total nonstructural carbohydrate (TNC) storage pool
was predicted to be affected. For mature trees, large decreases were predicted for TNC, leaves, stem, branch, and both fine and coarse roots. Most predicted effects in mature trees were consistent with observations in the field, but the simulations overestimated the effect of 2×-ambient O$_3$ on root TNC and growth. The authors suggested that this discrepancy may have been due to trees reducing respiration in response to O$_3$ stress, a response not simulated in the model.

For *Abies concolor*, TREGRO was parameterized and simulated growth of a mature tree for 3 years to test for effects of O$_3$ exposure and drought stress (Retzlaff et al., 2000). Reductions in O$_3$ exposure-mediated carbon assimilation were translated to losses in whole tree biomass that probably would not be detectable in the field. However, TNC levels in branch tissue were simulated to be lowered by over 50%, and branch growth was reduced in a moderately polluted site relative to a clean site. Low O$_3$ exposure (sufficient to decrease C assimilation by 2.5%) and drought stress (25% reduction in annual precipitation, which is common on a decadal scale) acted synergistically to reduce C gain of whole tree biomass of *A. concolor*. Simulated results of the tests were comparable to effects found in OTCs for seedlings and pole-sized trees in clean and moderately polluted sites.

Models such as TREGRO are usually parameterized from many different sources of data, including chamber experiments and plantations, from seedlings to mature trees, making it difficult to validate that they reproduce changes that occur as trees develop from seedlings to maturity. To address this issue, physiological and growth data were collected from a natural stand of *P. ponderosa* and used to parameterize the TREGRO model (Grulke and Retzlaff, 2001). Representative trees of each of five tree age classes were selected based on population means of morphological, physiological, and nearest neighbor attributes. Seedlings were observed to differ significantly from pole-sized and older trees in most physiological traits. The changes in biomass with tree age predicted from the model closely matched those of trees in the natural stand.

The PGSM model was used to simulate *Pinus ponderosa* seedling growth responses to O$_3$ exposure and drought stress (Chen et al., 1994). Drought stress was predicted to reduce the effect of O$_3$ on growth, as was observed in the experimental data. The TREGRO model was used to simulate responses of Pacific Coast and interior varieties of *P. ponderosa* to five simulated O$_3$ exposures between subambient and 3×-ambient (Constable and Taylor, 1997).
Simulated growth of var. ponderosa was reduced more than that in var. scopulorum with all O₃ exposures. Drought was protective of O₃ exposure. Similar results were also found in a relatively moist Ponderosa pine plantation (Panek and Goldstein, 2001), whereas drought was synergistically deleterious with cumulative O₃ exposure in a natural stand (Grulke et al., 2002b).

For Pinus taeda, the Plant Growth Stress Model (PGSM) was calibrated with seedling data and then used to simulate the growth of mature trees over a 55 year period in the Duke Forest, NC, using estimates of historical O₃ concentrations (Chen et al., 1998). Simulated stem diameter and tree height were comparable to observed values. In another simulation using TREGRO, loblolly pine was more sensitive (greatest reduction in C gain) to a peak O₃ episode in July (Constable and Retzlaff, 1997), whereas mature yellow poplar (Liriodendron tulipifera) was more sensitive to a peak O₃ episode in August.

For Populus tremuloides, the ECOPHYS model was used to simulate the relative above-ground growth response of an O₃-sensitive clone (259) exposed to square-wave variation in O₃ concentration (Martin et al., 2001). The model adequately simulated several effects of O₃, including a greater effect on stem diameter than on stem height, earlier leaf abscission, and reduced stem and leaf dry matter production at the end of the growing season. For Acer saccharum, the TREGRO model was used to predict effects of a 10-year O₃ exposure on root and stem growth of a simulated 160-year-old tree (Retzlaff et al., 1996). Twice-ambient O₃ exposure (for Ithaca, NY) was predicted to deplete the TNC pools and reduce fine root production.

**AX9.6.10.2 Surveys, Growth Correlations, and Stand-Level Modeling**

Stand-level studies have included surveys of O₃ symptoms, correlations of radial growth with O₃ and other environmental factors, and regional-scale modeling. In addition, open air O₃ exposure systems, such as those being used on Populus tremuloides-mixed stands in northern Wisconsin (Karnosky et al., 2003b) and on Fagus sylvatica and Picea abies in Germany (Nunn et al., 2002) offer an opportunity to examine larger plot sizes, older trees, and trees growing under realistic competition. Plots along natural O₃ gradients, as have been used very effectively in southern California forest studies (Miller and McBride, 1999b) and with P. tremuloides stands in the Great Lakes region (Karnosky et al., 1999), offer additional insights into ecosystem level responses. Undoubtedly, however, simulation modeling will have to become an integral component of research in order to predict adequately ecosystem responses to O₃ (Laurence and
Andersen, 2003). Results of these approaches are discussed below, organized into three U.S. regions: (1) northern states (including the upper Midwest and the Northeast), (2) southeastern states, and (3) western states (primarily California). A fourth section contains selected information from Europe.

**Northern and Midwestern United States.** In recent years, the USDA Forest Service has conducted systematic O$_3$ biomonitoring surveys in most north-central and northeastern states (Coulston et al., 2003; Smith et al., 2003). Plots are located on a systematic grid, and trained field crews evaluate up to 30 plants of up to six species that have foliar injury symptoms diagnostic of O$_3$ damage. For the United States as a whole, injury has been found more often in eastern than in interior or west-coast states. As expected, O$_3$ injury is more common and more severe in areas with higher O$_3$ concentrations. Of sampled Prunus serotina plots, ~12% were estimated to be at high risk for injury based on a injury index derived from the survey data (Coulston et al., 2003). *P. serotina* was estimated to be at risk for injury on the Allegheny Plateau and the Allegheny Mountains (in Pennsylvania, West Virginia, and Maryland), as well as in the coastal plain of Maryland and Virginia.

Ozone concentration, foliar injury, and physiological traits were measured on *P. serotina* trees of different sizes in Pennsylvania (Fredericksen et al., 1995). The proportion of foliage injured was 46% for seedlings, 15% for saplings, and 20% for canopy trees. Cumulative O$_3$ flux was the most useful O$_3$ metric for predicting injury. Injury was negatively correlated ($r^2 = 0.82$) with net photosynthetic rates, but was not related to stomatal conductance. *P. serotina* is discussed further below, because detailed surveys have been conducted in the Shenandoah and Great Smoky Mountains NPs in the southeastern United States.

Over the past several decades, some surveys of white pine (*Pinus strobus*) have reported significant associations between foliar injury and reduced growth (Anderson et al., 1988; Benoit et al., 1982). However, a review of 93 surveys conducted from 1900 through the late 1980s concluded that methodological problems were pervasive, including such issues as proximity to roads, lack of peer review, lack of random sampling, small sample sizes, and lack of quantitative methods to estimate severity (Bennett et al., 1994). Because of these problems, along with evidence of adequate growth rates for *P. strobus* regionally and contradictory evidence from numerous studies of symptom production in response to controlled O$_3$ exposure, these authors
concluded that there was no clear evidence of decline in *P. strobus* (Bennett et al., 1994). A more recent study in Acadia National Park (ANP) in Maine found no association between O₃ exposure in OTCs and symptom development in *P. strobus*, calling into question whether symptoms previously ascribed to O₃ may be caused by some other stress (Kohut et al., 2000). However, another ANP study found significant correlations between O₃ exposure and the radial growth of trees during 10 years in 7 of 8 stands examined (102 trees total; (Bartholomay et al., 1997). Taken together, these results suggest that there may not be an association between growth of *P. strobus* trees and putative O₃ symptoms, but there may be an association between O₃ exposure and radial growth of mature trees in the field.

The study of O₃ effects was undertaken from 1990 to 1993 in the ANP in Maine, because this location experiences elevated O₃ exposures due to transport from urban areas located upwind (Kohut et al., 2000). Thirty-two species of plants found in the park were propagated and exposed to O₃ in OTCs. In addition, ambient O₃ concentrations were monitored at the study site at 15 m above sea level and near the top of Cadillac Mountain at 470 m above sea level. At the study site, the maximum 1-h O₃ concentration was 140 ppb, which occurred in both 1990 and 1991. Daytime 12-h O₃ concentrations were 35, 41, 36, and 37 ppb during the four years; and O₃ concentrations were consistently higher at the high-elevation site. Species showing foliar injury at ambient O₃ concentrations included black cherry (*Prunus serotina*), quaking aspen (*Populus tremuloides*), white ash (*Fraxinus americana*), jack pine (*Pinus banksiana*), big-leaf aster (*Aster macrophyllus*), and spreading dogbane (*Apocynum androsaemifolium*). Species showing foliar injury at 1.5 × ambient O₃ concentrations included grey birch (*Betula populifolia*), small sundrops (*Oenothera perennis*), and bunchberry (*Cornus canadensis*). Species remaining uninjured at 2 × ambient O₃ concentrations included paper birch (*Betula papyrifera*), white pine (*Pinus strobus*), pitch pine (*Pinus rigida*), red spruce (*Picea rubens*), Eastern white cedar (*Thuja occidentalis*), English oak (*Quercus robur*), Canada bluejoint grass (*Calamagrostis canadensis*), wild radish (*Raphanus raphanistrum*), and Canada mayflower (*Maianthemum canadense*). Because of their O₃ sensitivity and diagnostic symptoms, big-leaf aster, spreading dogbane, quaking aspen, white ash, and black cherry were recommended as bioindicators for the ANP.

The PnET-II model was applied to 64 locations across the northeastern United States to simulate the effects of ambient O₃ on mature hardwood forests (Ollinger et al., 1997). In this model, O₃ effects on each of several layers of the forest canopy were represented by a single
linear equation relating predicted $O_3$ uptake to decreased net photosynthetic rate. Wood growth was predicted to decrease between 3 to 22%, with greatest reductions in southern portions of the region where $O_3$ levels were highest and on soils with high water-holding capacity where drought stress was absent. Little variation was predicted among years, because high $O_3$ often coincided with hot, dry weather conditions that reduced predicted stomatal conductance and $O_3$ uptake.

In order to estimate the impact of $O_3$ on forests, effects must be evaluated not only on individuals, but also on mixtures of species and the composition of forest stands. The PnET model described above evaluated the effects of $O_3$ on broad forest types (an evergreen/deciduous mix), but did not address specific forest species composition. In order to address competition among species, the TREGRO model was linked to the ZELIG forest stand growth model and a geographic information system was created to predict the effects of $O_3$ across the north-central and northeastern United States (Laurence et al., 2000). ZELIG is a gap-succession model used to simulate succession in mixed stands typical of eastern and northern forests. Ambient $O_3$ generally caused a reduction of 2 to 4% in the growth of *Quercus rubra* across the region during the 100-year simulation. The response followed the pattern of $O_3$ exposure, with little effect in the northwest part of the region, but with greater effect in southern locations. The $O_3$ response of *Acer saccharum* to $O_3$ varied widely, but the overall growth response was always positive, indicating that the evergreen/deciduous mix was able to take advantage of the decrease in the growth of *Q. robur* and other species caused by $O_3$. In the northernmost part of the region, *A. saccharum* growth increased by up to 3%, but in the southern part of the region, its growth increased by up to 12%. The authors ascribed this enhanced growth to a combination of warmer temperatures and reduction in the growth of *Prunus serotina*, a minor component of the simulated stand that was very sensitive to $O_3$.

**Southeast United States.** In a survey of the Great Smoky Mountains NP, foliar injury attributed to $O_3$ was found on 47% of the more than 1,600 plants examined (Chappelka et al., 1997). In subsequent surveys of injury in the park, injury was found on mature trees of the following species: *Sassafras albidum*, *Prunus serotina*, and *Liriodendron tulipfera* (Chappelka et al., 1999a,b). In a similar study in Shenandoah National Park, injury was found on *Fraxinus americana*, *P. serotina*, and *L. tulipfera* (Hildebrand et al., 1996).
For *Prunus serotina* seedlings grown in soil in OTCs and exposed to relatively low ambient levels of O₃ in Pennsylvania, there was no correspondence between visible foliar stipple, leaf gas exchange, and seedling growth between two families previously shown to differ in O₃ symptoms (Kouterick et al., 2000). However, significant exposure-response relationships were found for foliar injury in the Great Smoky Mountains and Shenandoah National Parks. In each park, foliar injury was evaluated on mature *P. serotina* trees on three plots at different elevations near O₃ monitors during 1991 to 1993 (Chappelka et al., 1999a,b; Hildebrand et al., 1996). In 1991, incidence was 60% and 45% for the two parks and 33% in both parks during 1992 and 1993. Symptoms were greater at the highest elevations where O₃ concentrations were highest.

In another study, radial growth rates were measured in 44 *P. serotina* trees ranging in age from 19 to 56 years old with and without O₃ symptoms, at three sites in the Great Smoky Mountains National Park. Trees with O₃ symptoms were compared to similar-sized trees with few symptoms. There was no evidence that trees with O₃ symptoms had lower growth rates (p = 0.6) (Somers et al., 1998).

In the Great Smoky Mountains National Park, radial growth rates were measured for 44 *L. tulipfera* trees ranging in age from 30 to 58 years old, with and without O₃ symptoms, at three sites at different elevations (Somers et al., 1998). Trees with O₃ symptoms averaged 30% lower growth rates over ten years (p = 0.0005). Seedlings of *Liriodendron tulipfera* were exposed for two seasons to 2×-ambient O₃ exposures in OTCs in Delaware, OH (seasonal SUM00 exposures of 107 and 197 ppmh) (Rebeck, 1996). Foliar O₃ symptoms were observed, but growth was not reduced.

In order to evaluate the influence of interspecies competition on O₃ effects, the linked TREGRO and ZELIG modeling system was used to predict the effects of O₃ over 100 years on the basal area of species in a *Liriodendron tulipfera*-dominated forest in the Great Smoky Mountains NP (Weinstein et al., 2001). Ambient O₃ was predicted to reduce the basal area of *L. tulipfera* by 10%, whereas a 1.5×-ambient exposure was predicted to cause a 30% reduction. Basal area of *Acer rubrum* and *Prunus serotina* was predicted to increase for some years, but then decrease by up to 30%, with few changes in the total basal area of all species by the end of the simulation.

In order to evaluate the influence of interspecies competition on O₃ effects, the linked TREGRO and ZELIG modeling system was used to predict the effects of O₃ on the basal area of
*Pinus taeda* and *Liriodendron tulipfera* growth throughout their ranges (Laurence et al., 2003). The models were parameterized using biological and meteorological data from three sites in the southeastern United States (in Alabama, Louisiana, and North Carolina). Forest stand response to five O₃-exposure regimes with annual SUM06 values ranging from 0 to 100 ppmh per year was simulated for 100 years. The simulated basal area of the two species was generated within the context of four other tree species common in southeastern forests. Basal area of *P. taeda* was highly responsive to precipitation and O₃ exposure, with the greatest increases under high-precipitation, low O₃-exposure scenarios and the greatest decreases under low-precipitation, high O₃-exposure scenarios. The basal area of *L. tulipifera* did not significantly differ (+10%) from simulations using a “base case” (ambient O₃, average precipitation).

Systematic biomonitoring surveys found that approximately 24% of sampled sweetgum and 15% of sampled *Pinus taeda* plots were estimated to be at high risk for foliar injury on the coastal plain of Maryland and Virginia (Coulston et al., 2003; Smith et al., 2003). In a study in Tennessee, the effect of ambient (uncontrolled) O₃ on 28 mature canopy-dominant 50 to 90-year-old *P. taeda* trees in five stands was measured over a 5-year period (McLaughlin and Downing, 1995, 1996). Of many O₃ metrics, a 3-day average of hourly O₃ values ≥ 40 ppb (AOT40) was found to best explain short-term variation in stem expansion as measured with dendrometer bands. Interactions between O₃, temperature, and drought stress (as indicated by the weekly moisture stress index) accounted for 63% of the short-term variation in stem growth rates. Because there are interactions among O₃, drought stress, and temperature that may differ with the averaging time (days to years), this type of study cannot provide conclusive proof of cause and effect (Reams et al., 1995). However, the results do suggest that the effects of O₃ measured on loblolly seedlings may also be occurring in mature trees in both wet and dry sites. The magnitude of effects of O₃ on growth, including interactions with other variables in this study, ranged from 0 to 15% over 5 years, with an average of 5.5%.

**Western United States.** The USDA Forest Service conducted O₃ biomonitoring surveys in Washington, Oregon, and California during one year (1998), and in the Sierra Nevada and Sequoia NFs every other year for several decades (Campbell et al., 2000). Overall, only one plot showed any symptoms of O₃ injury outside of the Sierra Nevada and Sequoia National Forests. In the Sierra Nevada National Forest, between 30 and 40% of trees showed injury from 1989
through 1997. In the Sequoia National Forest, between 40 and 50% of the trees surveyed showed injury from 1990 through 1998.

For *Pinus ponderosa* along a well-studied gradient of O$_3$ exposure in the San Bernardino mountains, chlorotic mottle was highest on foliage at the most polluted site, as has been found previously (Grulke and Balduman, 1999). Based on whole-tree harvests, root biomass was lowest at the most polluted sites, confirming previous studies with seedlings under controlled conditions, as discussed above. Ozone responses in highly polluted environments such as Southern California may not be predicted adequately by extrapolating effects from single-factor experiments. Instead, combined approaches utilizing field experiments and modeling efforts may be required to properly account for a combination of stressors including O$_3$, N deposition, and drought. Furthermore, the available studies underscore the lack of correlation between O$_3$ symptoms and mature tree effects. If a field survey fails to find a correlation between mature tree growth and O$_3$, this result may be due to the dominant effect of another factor such as N deposition and may not be evidence that O$_3$ does not reduce the growth of mature trees.

The TREGRO model was used to evaluate how projected future temperature and CO$_2$ concentrations might affect the response of individual Ponderosa pine to O$_3$ at seven sites in California, Oregon, and Washington (Tingey et al., 2001). As expected, growth decreased with increasing O$_3$ exposure. Differences in O$_3$ response among sites appeared to be due primarily to differences in precipitation.

Often air quality standards do not translate directly into measurable improvements in tree growth or productivity. To evaluate whether past improvements in air quality have improved Ponderosa pine growth, TREGRO was used to simulate growth at sites in the San Bernardino Mountains in California (Tingey et al., 2004). Ozone and meteorological data from the past 37 years was used to run the simulations. Despite variation in precipitation and temperature, O$_3$ was found to reduce simulated tree growth. The authors were able to simulate growth improvements as air quality improved during the 1980s and 1990s, suggesting that improvements in emission control strategies benefitted Ponderosa pine. The model simulations were qualitatively consistent with improvements in canopy condition that were observed at sites where O$_3$ reductions were the greatest.
**Studies in Europe.** In a 4-year study of *Fagus sylvatica* in Switzerland at 57 forest sites ranging in age from 65 to 173 years, stem increment was found to decrease with increasing maximum O$_3$ exposure (Braun et al., 1999). In this study, O$_3$ concentration was estimated by interpolation among monitoring stations, and other site conditions such as soil water status and temperature were interpolated from weather stations. Other factors such as N deposition, tree diameter, and canopy dominance were also found to be significantly associated with stem increment. The maximum annual O$_3$ dose (expressed as AOT40) was found to be more strongly associated with decreased stem increment than was the average O$_3$ dose over the 4 years. A growth reduction of 22.5% (confidence interval 14.3 to 28.6%) was associated with each 10 ppmh increment of O$_3$ (expressed as AOT40). This decrease was steeper than the 6.1% growth reduction summarized previously from several OTC studies with *F. sylvatica* seedlings (Fuhrer et al., 1997). However, the authors suggest that this difference may be explained largely by the 4 years of exposure in their forest survey study as compared to the 1-year exposures for seedlings. As with any forest survey, these results must be interpreted with caution because O$_3$ exposure was correlated with other variables, such as tree age and the deposition of NO$_2$ and SO$_2$.

**AX9.6.11 Summary of Ecological Effects of Ozone Exposure on Natural Ecosystems**

In this chapter, an effort has been made to discuss the adverse effects of O$_3$ on natural ecosystems within the context of the SAB framework for assessing and reporting ecological conditions (Young and Sanzone, 2002) (Figure AX9-21). Using this framework, there is evidence that tropospheric O$_3$ is an important stressor of natural ecosystems, with well-documented impacts on the biotic condition, ecological processes, and chemical/physical nature of natural ecosystems. In turn, the effects of O$_3$ on individual plants and processes are scaled up through the ecosystem affecting processes such as energy and material flow, intra- and interspecies competition, and NPP. Thus, effects on individual keystone species and their associated microflora and fauna may cascade through the ecosystem to the landscape level. This suggests that by affecting water balance, cold hardiness, tolerance to wind, and by predisposing plants to insect and disease pests, O$_3$ may even influence the occurrence and impact of natural disturbance. Despite the probable occurrence of such effects, however, there are essentially no
instances where highly integrated ecosystem-level studies have conclusively shown that $O_3$ is indeed altering ecosystem structure and/or function.

Systematic injury surveys demonstrate that foliar injury occurs on $O_3$-sensitive species in many regions of the United States. However, the frequent lack of correspondence between foliar symptoms and growth effects means that other methods must be used to estimate the regional
effects of O\textsubscript{3} on tree growth rates. Investigations of the radial growth of mature trees, in combination with data from many controlled studies with seedlings, as well as a few studies with mature trees suggest that ambient O\textsubscript{3} is reducing the growth of mature trees in some locations. Studies using models based on tree physiology and forest stand dynamics suggest that modest effects of O\textsubscript{3} on growth may accumulate over time and may interact with other stresses. For mixed-species stands, such models predict that overall stand growth rate is generally not likely to be affected. However, competitive interactions among species may change as a result of growth reductions of sensitive species. These results suggest that O\textsubscript{3} exposure over decades may be altering the species composition of forests in some regions.

**RESEARCH NEEDS**

The knowledge base for examining the range of ecological effects of O\textsubscript{3} on natural ecosystems is growing, but significant uncertainties remain regarding O\textsubscript{3} effects at the ecosystem level. For example, there is a need for information on the following ecosystem-level responses:

- **Ecosystem Processes.** Little is known about the effects of O\textsubscript{3} on water, C, and nutrient cycling, particularly at the stand and community levels. Effects on belowground ecosystem processes in response to O\textsubscript{3} exposure alone and in combination with other stressors are critical to projections at the watershed and landscape scales. Little is yet known about the effects of O\textsubscript{3} on structural or functional components of soil food webs, or how these impacts could affect plant species diversity (Andersen, 2003).

- **Biodiversity and Genetic Diversity.** The study of genetic aspects of O\textsubscript{3} impacts on natural ecosystems has been largely correlational in nature and it remains to be shown conclusively whether O\textsubscript{3} affects biodiversity or genetic diversity (Davison and Barnes, 1998; Pitelka, 1988; Winner et al., 1991). Studies of competitive interactions under elevated O\textsubscript{3} levels are needed (Laurence and Andersen, 2003), and reexamination via new sampling of population studies to bring in a time component to previous studies showing spatial variability in population responses to O\textsubscript{3} are needed. These studies could be strengthened by modern molecular methods to quantify impacts on diversity.

- **Natural Ecosystem Interactions with the Atmosphere.** Little is known about feedbacks between O\textsubscript{3} and climate change on volatile organic compound (VOC) production, which in turn, could affect O\textsubscript{3} production (Fuentes et al., 2001). At moderate-to-high O\textsubscript{3} exposure sites, aberrations in stomatal behavior could significantly affect individual tree water balance of sensitive trees, and if the sensitive tree species is dominant, hydrologic balance at the watershed and landscape level could be affected. This has not been addressed in any model because O\textsubscript{3} exposure effects, if included in the modeling effort have assumed a linear relationship between assimilation and stomatal conductance.
• **Below-Ground Interactions.** While the negative effects of \( \text{O}_3 \) on below ground growth are well characterized, interactions of roots with the soil or microorganisms are not.

• **Other Interactive Effects.** Interaction studies with other components of global change (e.g., warming, increasing atmospheric \( \text{CO}_2 \), \( \text{N} \) deposition, etc.) or with various biotic stressors are needed to better predict complex interactions likely in the future (Laurence and Andersen, 2003). Whether \( \text{O}_3 \) will negate the positive effects of an elevated \( \text{CO}_2 \) environment on plant carbon and water balances is not yet known; nor is it known if these effects will scale up through the ecosystem. How might \( \text{O}_3 \) affect the progress of pest epidemics and insect outbreaks as concentrations increase is unclear (Ball et al., 1998).

• **Reproduction Effects.** Information concerning the impact of \( \text{O}_3 \) on reproductive processes and reproductive development under realistic field or forest conditions are needed as well as examination of reproductive effects under interacting pollutants (Black et al., 2000).

• **Comparative Extrapolation.** The vast majority of \( \text{O}_3 \) studies of trees have been conducted with young, immature trees and in trees that have not yet formed a closed canopy. Questions remain as to the comparability of \( \text{O}_3 \) effects on juvenile and mature trees and on trees grown in the open versus those in a closed forest canopy in a competitive environment (Chappelka and Samuelson, 1998; Kolb and Matyssek, 2001; Samuelson and Kelly, 2001).

• **Scaling-Up Issues.** Scaling the effects of \( \text{O}_3 \) from the responses of single or a few plants to effects on communities and ecosystems is a complicated matter that will require a combination of manipulative experiments with model ecosystems, community and ecosystem studies along natural \( \text{O}_3 \) gradients, and extensive modeling efforts to project landscape-level, regional, national and international impacts of \( \text{O}_3 \). Linking these various studies via impacts on common research quantification across various scales using measures of such factors as leaf area index or spectral reflective data, which could eventually be remotely sensed (Kraft et al., 1996; Panek et al., 2003), would provide powerful new tools for ecologists.

• **Comparative Risk Assessment Methodologies.** Methodologies to determine the important values of services and benefits derived from natural ecosystems such that these could be used in comprehensive risk assessment for \( \text{O}_3 \) effects on natural ecosystems (Heck et al., 1998).
AX9.7 ECONOMIC EVALUATION OF OZONE EFFECTS ON AGRICULTURE, FORESTRY, AND NATURAL ECOSYSTEMS

AX9.7.1 Introduction

The adverse consequences of ambient air pollutant exposures on vegetation, ecosystems, and components of the material environment have been documented since the beginning of the industrial revolution. Attempts to quantify the monetary damage and injury resulting from tropospheric O\(_3\) exposures to managed agriculture, forests, and natural ecosystems date back at least to the 1950s.

Both methodological and data problems plagued early efforts to assess the monetary damages of air pollution to crops and natural vegetation. Adams and Crocker (1989) discussed the methodological issues, e.g., a lack of reliable data on effects from air pollutants on crop yields or the failure to develop and apply appropriate economic models. Some of these problems were remedied by the EPA’s National Crop Loss Assessment Network (NCLAN) in the 1980s. The EPA’s NCLAN facilitated the performance of economic assessments by providing O\(_3\)-crop yield data with which to estimate O\(_3\)-crop yield response functions (see Heagle [1988] for a review of NCLAN procedures and findings). NCLAN also funded a series of economic assessments that, along with subsequent economic assessments, documented substantial economic damages to agriculture. (See Spash [1997] for a detailed review of economic assessments, many of which used NCLAN data.)

Since the completion of the NCLAN program in the late 1980s, the number of economic assessments of air pollution studies focusing on terrestrial ecosystems in general, and agriculture in particular, has declined. For example, for the period of 1980 to 1990, 33 economic studies of O\(_3\) and other air pollutant effects on U.S. crops were published in peer-reviewed journal outlets (Spash, 1997). However, in preparing this section of the current O\(_3\) AQCD, only four peer-reviewed economic assessments were found for the decade of 1991 to 2000 that addressed vegetation in the United States. In addition, one peer-reviewed article (Kuik et al., 2000) was found dealing with agriculture in the Netherlands. Recent interest in global climate change, and the potential effects of global warming on O\(_3\) and other photochemical oxidants, has renewed interest in the effects of air pollution on both managed and unmanaged terrestrial ecosystems (Adams et al., 1998). In addition, concern is growing regarding the effects of air pollutants on natural ecosystems and on the services they provide (Daily, 1997). Unfortunately, this interest
has not yet translated into additional peer-reviewed publications addressing \( \text{O}_3 \) or other air pollutant effects on ecosystems.

This section of the current \( \text{O}_3 \) criteria document first discusses the availability of economic information and its usefulness in forming environmental policy. Next, economic assessments of air pollution effects and findings from the 1996 AQCD (U.S. Environmental Protection Agency, 1996) are discussed, followed by a synthesis of the limited literature available since the 1996 AQCD with respect to \( \text{O}_3 \) effects on agriculture, forestry, and ecosystems. Finally, limitations and continuing uncertainties are reviewed. The most fundamental of these is the lack of measurements of the economic effects of air pollution on natural ecosystems. Other issues include the variability of performance in both managed and natural ecosystems under increased climatic and air pollution variability as well as the challenges related to spatial and temporal scales used in performing economic assessments. To date, this set of effects has been sparsely addressed.

**AX9.7.2 The Measurement of Economic Information**

Economic science is an exercise in deductive logic in which testable hypotheses about the behavior of economic agents (i.e., farmers, consumers, resource owners) and markets are deduced from a body of theory. That body of theory is based on a series of premises proposed by economists and philosophers dating back over two centuries to Adam Smith. These premises gradually evolved into a theoretical foundation primarily dealing with microeconomics and culminating in structural relationships that define the operation of markets. This foundation was first laid out in a comprehensive and rigorous fashion by Alfred Marshall in 1920. Samuelson (1948) formalized these theoretical relationships, resulting in what is sometimes referred to as modern, or neoclassical, economics.

The insights gained from the theoretical foundation of economics helped shape the nature of applied economic, or policy-relevant research. An example of such applied research is when economists seek to measure the economic consequences of air pollution on agriculture. Such an application is described in Adams and Horst (2003), who provided a graphical representation of the measurement of the effects of air pollution on the well-being of producers and consumers. Economic theory is applied to real-world problems when the methods of economics and the need
for data from other disciplines come into play. When estimating the economic effects of an environmental change, economists need an economic model or method that is theoretically consistent, i.e., defensible, as well as data with which to estimate both environmental science and economic relationships for use in the model. Among accepted economic assessment methods, the actual choice is frequently determined by the nature of the problem to be addressed. It should be noted that the choice of assessment method can affect the type of economic information that is obtained. Even with a given assessment method, results are sensitive to specific data treatment or assumptions (Adams, 1999). For example, some methods only measure effects on a particular group e.g., farmers. Other methods may measure effects across several groups. Thus, one should not expect the magnitudes of damage or benefits to be identical across economic assessments. One should, however, expect that the direction of the effects will be similar.

Once it is established that an assessment meets basic economic criteria, e.g., including human behavioral responses, the selection of the specific economic assessment method is often a relatively minor issue in terms of estimating benefits of air pollution control (or disadvantages of increases in air pollution). Although results differ across approaches, the differences are largely attributable to specific features of the assessment (e.g., whether the natural science data include a particular effect or relationship, whether effects on consumers are measured, and so forth). The nature and quality of the air quality forecasts used in the assessments can greatly influence the sensitivity of the assessments (Adams and Crocker, 1989). This is particularly noticeable when dealing with forecasts of seasonal air pollution changes (Adams et al., 1988). From the standpoint of providing policy guidance, the differences in economic estimates attributable to the assessment methods are often swamped by uncertainty in the natural and physical science forecasts. This has also been noted in recent economic assessments of climate change (Adams et al., 1998). In many settings, the quality of economic assessments of air pollution is likely to be improved more by refining the physical and natural science data used in the assessments than by intensive efforts to fine-tune the assessment techniques (Adams, 1999).
Evidence from the plant science literature cited in the 1996 O₃ AQCD (U.S. Environmental Protection Agency, 1996) is unambiguous with respect to the adverse effects of tropospheric O₃ on some types of vegetation. For example, the 1996 AQCD noted that findings from the EPA multiyear NCLAN program in the 1980s provided rigorous corroboration of at least two decades of previous research and a century of anecdotal observations which showed that O₃ at ambient levels caused physical damage to plants in general and to important crop species in particular. Specifically, NCLAN established that ambient O₃ levels resulted in statistically significant reductions in yields for some crop species (Heagle et al., 1988). The 1996 AQCD also assessed the results of studies regarding O₃ effects on crops, forests, and natural vegetation in more detail. More recent reviews, such as the comprehensive survey of the economic literature on agricultural effects by Spash (1997), corroborate the synthesis of results reported in the 1996 AQCD.

The number and quality of assessments of the economic consequences of O₃ exposures on vegetation reported in the 1996 AQCD are primarily a function of the state of evidence obtained from scientific studies in each vegetation category. For example, the plant science evidence reviewed in the 1996 AQCD concerning effects of O₃ exposures on agricultural crops was reported to be more valid than for individual tree species or plant communities (ecosystems). As a result, most economic assessments discussed in the 1996 AQCD focused on the data obtained from studies of agricultural crops. The economic literature dealing with O₃ effects on forest productivity in the 1996 AQCD was sparse. The few economic assessments of tree or forest effects reported in the 1996 AQCD were confined to evaluations of assumed or hypothetical changes in output, such as board feet of lumber (e.g., Haynes and Adams, 1992). As noted in the 1996 AQCD, O₃ effects on ecosystems and their services had not been measured in any systematic fashion and no peer-reviewed economic assessments were yet reported.

This section first briefly reviews economic assessments drawn from the review in the 1996 criteria document. This review is the benchmark against which recent articles are then discussed in the subsequent section. As was the case in 1996, the discussion of economic valuation of ecosystem effects is generally limited to conceptual and methodological issues, given the continued lack of empirical analyses in this category.
AX9.7.3.1 Agriculture

In view of the importance of U.S. agriculture for both domestic and world consumption of food and fiber, reductions in U.S. crop yields could adversely affect human welfare. The plausibility of this premise has resulted in numerous attempts to assess, in monetary terms, the losses from ambient O\textsubscript{3} exposures, or the benefits of O\textsubscript{3} control, to agriculture. Twenty-three assessments of the economic effects of O\textsubscript{3} exposures on agriculture were reviewed in the 1996 AQCD, highlighting key issues in the validity of these assessments (U.S. Environmental Protection Agency, 1996). First, the evidence should reflect how crop yields will respond under actual field conditions to O\textsubscript{3} exposures. Second, the air quality data used to frame current or hypothetical effects of O\textsubscript{3} on crops should represent actual exposures sustained by crops at individual sites or production areas. Finally, the assessment methodology into which such data are entered should (1) capture the economic behavior of producers and consumers as they adjust to changes in crop yields and prices that may accompany changes in O\textsubscript{3} air quality; (2) accurately reflect institutional considerations, such as regulatory programs and income support policies (e.g., provisions of federal Farm Bill legislation), that may result in market distortions; and (3) use measures of well-being that are consistent with economic principles.

Assessments of O\textsubscript{3} damage to agricultural crops reported in the 1996 AQCD employed procedures for calculating economic losses that met the conditions described above. More specifically, the assessments provided 23 quantitative estimates of the economic consequences of exposures to O\textsubscript{3} and other air pollutants to agriculture that reflect producer-consumer decision-making processes, associated market adjustments, and some measure of distributional consequences between affected parties. Many of the economic assessments reviewed in previous O\textsubscript{3} documents also focused on O\textsubscript{3} effects in specific regions, primarily California and the Corn Belt (e.g., Garcia et al., 1986). This regional emphasis in the earlier literature may be attributed to the relative abundance of data on crop response and air quality for selected U.S. regions, as well as the importance of some agricultural regions (such as California) for the U.S. agricultural economy.

Two U.S. national studies described in previous O\textsubscript{3} criteria documents that are worthy of additional comment are Kopp et al. (1985) and Adams et al. (1986). These were judged to be “adequate” in terms of the three critical areas of data inputs in the 1996 AQCD. Together, it was argued, they provide a reasonably comprehensive estimate of the economic consequences of
changes in ambient air O₃ levels on agriculture. Because of their central role in the 1996 criteria document, these two studies are reviewed briefly below.

The Kopp et al. (1985) and Adams et al. (1986) studies indicated that ambient levels of O₃ were imposing substantial economic costs of ~$3.4 billion (in 2000 U.S. dollars) on agriculture. Both were judged to suffer from several sources of uncertainty, but the document concluded that these possible improvements in future assessments were not likely to greatly alter the range of agricultural benefit estimates arising from O₃ reductions for several reasons. First, the studies covered about 75 to 80% of U.S. agricultural crops (by value). For inclusion of the other 20% to significantly change the estimates would require that their sensitivities to O₃ be much greater than for the crops that have been included to date. Second, model-sensitivity analyses reported in past studies indicate that changes in plant exposure-response relationships must be substantial to translate into major changes in economic benefits estimates. For example, it was assumed unlikely that use of different exposure measures, or inclusion of interaction effects, would greatly alter the magnitude of the economic estimates. Third, it was believed that countervailing effects would mitigate against large swings in the estimates, e.g., longer O₃-exposure periods may predict greater yield losses, but O₃-water stress interactions tend to reduce the yield estimates.

Other national assessments reported in the 1996 AQCD provided general corroboration of the results of Kopp et al. (1985) and Adams et al. (1986). An evaluation of these studies, in terms of the adequacy of information from plant exposure studies and aerometric and economic data, was presented in the 1996 AQCD, along with estimates of benefits or damages associated with changes in O₃. Most of the studies added onto either Kopp et al. (1985) or Adams et al. (1986). A relevant question was whether subsequent studies provided any “surprises” in terms of magnitude of economic effects.

Common themes or findings from these and earlier O₃ and other air pollution studies were summarized in two synthesis papers, those of Adams and Crocker (1989) and Segerson (1991). The major conclusion is that the agricultural effects of tropospheric O₃ at ambient levels impose economic costs to society or, conversely, that reductions in ambient O₃ should result in societal benefits.
Several studies contained in the 1996 AQCD still are of interest. For example, one finding pertains to the relationship between federal farm programs and air pollution regulations (McGartland, 1987). In each case, the inclusion of farm programs in the economic models resulted in modest reductions in the economic benefits of O₃ control due to increased farm program costs. As Segerson (1987) noted, however, it is not clear that these increased costs should be charged against the potential benefits of an O₃-regulatory standard but, rather, considered as an additional cost associated with the inefficiencies of the farm program. It should also be noted that the nature of federal farm programs was changed dramatically by Congress in 1996 in an attempt to reduce the federal government’s role in agriculture. Although more recent federal legislation, such as the 2002 Farm Bill (U.S. Congress, 2002), appears to be restoring the federal government’s role in the farming sector, this issue currently is not as important as suggested by earlier studies, due to the declining reliance on deficiency payments to farmers, which tend to distort resource allocation.

Another national study (Adams et al., 1988) analyzed economic benefits under a regulatory alternative involving a seasonal (crop growing season) O₃-exposure index measured as a 12-h mean, instead of hourly levels or percent changes from ambient as reported in earlier studies. Specifically, a seasonal average of 50 ppb O₃ (measured as a 12-h seasonal mean), with a 95% compliance level, was reported in Adams et al. (1988). The result (a $2.9 billion benefit in 2000 dollars) is similar to the assumed 25% reduction across all regions reported by Adams et al. (1986). At least one study also combined environmental stressors (e.g., O₃, UV-B radiation) in performing economic assessments. Adams and Rowe (1990), using the same model as Adams et al. (1986), reported that a 15% depletion of stratospheric O₃ (resulting in a 13% increase in tropospheric O₃) would cause an economic loss of ~$1.4 billion in 2000 dollars attributed to the tropospheric O₃ increase. Reducing VOCs/NOₓ motor vehicle emissions by 10% would result in a benefit of ~$0.3 billion, while a complete elimination of motor vehicle emissions would yield a benefit of ~$3.4 billion (1990 dollars). The range of these numbers is consistent with values reported in Adams et al. (1986), Kopp et al. (1985), and other national-level analyses, i.e., estimates of from $1.0 to 2.0 billion for reductions in ambient O₃ of 25 to 40%.
The long-term nature of air pollution effects on perennial species such as trees creates challenges to plant scientists in attempts to sort out the effects of specific individual stressors such as $O_3$ from among the many other potential causal factors (Skelly, 1988). It also creates problems in terms of measuring the impacts on direct economic value of goods, such as reductions in board-feet of lumber produced per unit of time.

Most of the literature in the 1996 AQCD dealing with forest species reported the effects of $O_3$ exposures in terms of foliar injury (Davis and Skelly, 1992; Freer-Smith and Taylor, 1992; Simini et al., 1992; Taylor and Hanson, 1992). This emphasis on foliar effects in the forest effects literature (rather than marketable yield) is similar to the state of science for agricultural crops prior to 1980. More recent studies address the effects of air pollutants on forest tree species diversity (Bringmark and Bringmark, 1995; Vacek et al., 1999; Weiner et al., 1997). However, such information is of limited use in economic assessments. The exception is in measuring the economic value of aesthetic changes in a forest stock, where changes in species composition may affect recreational values (Crocker, 1985).

The data concerning changes in marketed output, such as board-feet of lumber or changes in growth rates in managed forests or effects on the growth of almond, peach, apricot, pear and plum trees in orchards, cited in the 1996 document (U.S. Environmental Protection Agency, 1996) have not been quantified. In addition, the economic impact of reductions in growth of seedlings of evergreen trees, e.g., slash pine, presented in the same document have not been valued. The few studies which attempted to measure economic losses arising from exposures to $O_3$ or other pollutants circumvented the lack of plant science data by assuming (often arbitrary) reductions in forest species growth or harvest rates (Adams, 1986; Callaway et al., 1986; Crocker and Forster, 1986; Haynes and Adams, 1992). Although the economic estimates reported in the 1996 AQCD are comparable to those reported for agriculture (e.g., $2.6 billion for eastern Canada forests, $2.9 billion for eastern U.S. forests in 2000 dollars), the lack of yield and/or growth effects data makes these studies only suggestive at best, of the economic consequences of forest effects directly attributable to $O_3$ exposures questionable. Recent developments in forestry economic modeling capabilities, in support of climate change research, have enhanced the ability to measure the effects of environmental stressors on this sector (Adams et al., 1996; McCarl et al., 1998). However, these models need data on changes in either
timber production or growth rates, both of which are lacking for forest species under alternative O₃ levels.

AX9.7.4 Studies Since 1996 of Ozone Exposure Effects on the Economic Value of Agriculture, Forests, and Ecosystems

Of the few current (post-1996) economic studies addressing agricultural effects, none offer new insights of value in determining the economic cost of O₃ exposures. These post-1996 studies used variants of the economic methods from earlier assessments and measure yield changes from response functions arising from the NCLAN or similar data. For example, Kim et al. (1998) used a mathematical programming model of the San Joaquin Valley agricultural sector in California, combined with crop yield response functions, to assess the economic effects of O₃ on California crops. Their results showed net benefits from reductions in ambient O₃ levels, a finding consistent with all previous economic assessments. In another study, Westenbarger and Frisvold (1994) measured agricultural exposures to O₃ (and acid precipitation) in the United States. Though not an economic analyses of the costs of ambient exposures, they identified areas of the United States of greatest potential economic damage based on the interface between regional pollution levels and the value of crop production in each region.

A study by Murphy et al. (1999) of the economic effects of tropospheric O₃ on U.S. agriculture is of note here, because it confirms the general magnitude of economic effects reported by the two key studies performed a decade earlier (Adams, 1986; Adams et al., 1985). Specifically, Murphy et al. (1999) evaluated benefits to eight major crops associated with several scenarios concerning the reduction or elimination of O₃ precursor emissions from motor vehicles in the United States. Their analysis reported a $2.8 to 5.8 billion (1990 dollars) benefit from complete elimination of O₃ exposures from all sources, i.e., ambient O₃ reduced to a background level assumed to be 0.025 to 0.027 ppm. While the analytical framework is similar to Adams et al. (1986) in the use of NCLAN-based yield response functions and a mathematical programming-based economic optimization model, the study is novel in its focus on the role of motor vehicle emissions of VOCs/NOₓ in total anthropogenic O₃ levels. The study is also notable in its careful attention to federal farm program effects, particularly the deficiency payment component.
In addition to these studies in peer-reviewed journals, a number of site-specific effects studies have been performed, primarily by consulting companies for state public utility commissions. Although perhaps of use to public utility commissioners concerned with effects from single power plants or other localized sources, these regional studies generally contribute little to the assessment of air pollution effects at the national level. Also, such reports are not peer reviewed. Therefore, they are not discussed here.

There have been a number of recent studies of air pollutant effects on tree species in the literature. Some have reported changes in total biomass and focused on European species (Kurczynska et al., 1997). Other studies have assessed changes in composition of forest species (biodiversity) or forest health due to exposure to air pollutants (Bringmark and Bringmark, 1995; McLaughlin and Percy, 1999; Vacek et al., 1999). As noted previously, changes in forest biomass and composition are more difficult to value than marketable products. However, measures of forest composition or health have implications for an area of increasing policy concern, that being the effect of air pollutants and other environmental stressors on unmanaged (natural) ecosystems and the services they provide (Goulder and Kennedy, 1997; Pimentel et al., 1997). Considerable discussion has occurred among ecologists and economists as to the appropriate means for valuing these services (Anderson, 1990; Carpenter and Dixon, 1985; Common and Perrings, 1992). A number of conceptual articles have been published on this issue in both economic and ecological journals (Bergstrom, 1990; Castle, 1993; Pearce, 1993; Suter, 1990).

A continuing empirical challenge concerns the lack of information on how changes in biodiversity affect ecosystem performance resulting from O3 stresses and the problem of establishing economic values for such changes (Cairnes and Lackey, 1992; Norton, 1987; Pimm, 1997; Polasky, 2001; Randall, 1991). As noted in the 1996 AQCD, and more recently by Daily (1999, 2000) and Polasky (2001), there continues to be a lack of empirical studies that actually assess the economic value of changes in biodiversity or in service flows due to any environmental stressors. While some studies report monetary estimates, the estimates are generally for expository purposes and those would not be as defensible as the agricultural studies described earlier. For example, Costanza et al. (1997) assigned a value to the world’s ecosystems, but the procedures used render this an exploratory study at best. As assessed by Polasky (2001), “In general, the field of valuation of ecosystem services is in its infancy.”
He attributed the lack of empirical studies due to both a lack of the understanding of ecology of ecosystem services and to the absence of reliable methods to estimate the value of these services.

In summary, the studies of crop and forest responses in the economic literature indicate that O$_3$ reduces crop yields and imposes economic costs. The economic literature also indicates that O$_3$ adversely influences the physiological performance of tree species and demonstrates, as expected, that changes in growth have economic consequences. However, the economic data and literature available on ecosystem effects of O$_3$ exposures are not sufficient to determine the economic costs.

**AX9.7.5  Limitations of Scientific Studies and Economic Information**

The 1996 O$_3$ AQCD discussed the need for additional research on both ecological functions and economic methodology in order to better understand the economic implications of air pollutants on ecosystem services. As noted by Daily (1999, 2000), Polasky (2001), and others, this research agenda continues to be important. Despite the large number of discussion and survey articles published since 1996, there still are not sufficient data by which to estimate confidently the magnitude of economic effects of O$_3$ to forests and natural ecosystems. Nor is it apparent that ongoing research is adequate to answer this question in the next criteria document cycle. Specifically, there do not appear to be any comprehensive, ecological studies underway that attempt to measure changes in ecosystem outputs under alternative O$_3$ or other air pollutant levels. Thus, in the near term, ecosystem services can only be discussed in qualitative terms. However plausible the likelihood of economic damages to ecosystems, the available scientific and economic information does not provide specific guidance on the magnitudes of these effects.

Besides the need to improve our understanding of the effects of O$_3$ exposures on natural ecosystems, a number of areas of research could help assess the full economic consequences of such pollutants on managed ecosystems. The first of these is the relationship between O$_3$ exposure levels and the variability of crop yields or changes in forest biodiversity. Most assessments are based on the average or expected yield response of a crop to air pollution exposure. However, the variability in yields (the spread or dispersion around the mean) appears to be affected by the nature of plant exposure to pollutants (Hogsett et al., 1985a; Musselman et al., 1983). Plants exposed to the same mean dose but with different second moments of the distribution of exposure may have different mean yields (Altshuller and Lefohn, 1996; Lefohn
and Benedict, 1982). In addition, the variability of the yield of the plant may also be increased by greater variability in exposures (e.g., a higher frequency of extreme events). The economic significance of higher yield varieties is such that variability may impose additional economic costs, because most farmers have been reported to be averse to risk and prefer less variability for a given yield (or profit). To date, no economic assessments of O\textsubscript{3} damages to agriculture or vegetation in general include risk-averse behavior (studies cited here assumed risk neutrality). To assess the economic consequences of a relationship between farmers’ risk preferences and O\textsubscript{3}-induced changes in yield variability will require more information on the potential effects of changes in O\textsubscript{3} on crop yield variability. While no economic studies were found on the effects of O\textsubscript{3}-induced changes on yield variability, a number of recent studies of climate change effects on crop yields have indicated increased economic costs in the presence of increased climatic variability (e.g., Mearns et al., 1997; Dixon and Segerson, 1999). Analogous economic costs would be expected for changes in air pollution distributions; and these effects need to be examined and quantified.

Another research area concerns the need improvement in our understanding of temporal (dynamic) and spatial characteristics of O\textsubscript{3} exposures and their implications for crop yields, production and producer profits. Most economic studies are static, in that they compare two states of the world (e.g., economic activity at one O\textsubscript{3} level versus at an alternative level). In addition, most national-level studies (the type needed to evaluate Secondary National Ambient Air Quality Standards [SNAAQS]) display coarse regional-level resolution in terms of crop response, O\textsubscript{3} exposure, and economic behavior. The responses of producers to changes in yields due to changes in O\textsubscript{3} levels are generally assumed to be similar over geographical areas up to several states in size. However, the changes between air quality scenarios are more likely to be characterized by transient changes in exposure levels, which means the producer responses are also likely to be gradual, rather than abrupt. Similarly, the lack of finer scale (regional-level) data and modeling capabilities suggests that important micro-level physical and economic effects are ignored. To what extent these abstractions influence net economic effects is an empirical question. Research on these types of abstractions and assumptions within other economic settings, such as climatic change, have shown that they have implications for economic measurements (Adams, 2002).
Another issue is the natural or background level of O\textsubscript{3} (or other pollutants of interest) assumed in economic studies. While many economic studies focus on changes in pollution levels from current conditions, some studies have measured the economic damages between an assumed, or pristine, level and current levels in agricultural areas. Such an analysis, it is reasoned, will provide a measure of the net damages due to anthropogenic sources. The challenge here is to have a correct measure of the background level of the pollutant. Recent research by Lefohn et al. (2001) has suggested that background levels may be considerably higher than assumed in some of the previous economic assessments reported in the 1996 AQCD (25 to 30 ppb in most studies). For example, Lefohn et al. (Lefohn et al., 2001) detected hourly readings of from 40 to 80 ppb during winter and spring months in remote areas of the United States. If background O\textsubscript{3} levels are in the range, then the economic damages estimated in studies with lower background levels will be overstated. The issue of the background O\textsubscript{3} level is important to all assessments of vegetative damages due to O\textsubscript{3}.

In terms of expanding economic methods for future assessment, analysts should consider using more “reduced form” estimation methods, particularly in situations where the availability of dose-response functions is limited. This estimation approach is exemplified by Garcia et al. (1986). Specifically, their econometric study of the impact of O\textsubscript{3} on producer profits used such a reduced-form approach. In this approach, farmer actions are modeled as a function of ambient O\textsubscript{3} levels (along with other explanatory variables) without the direct use of dose response functions. The advantage of this procedure is that one source of modeling uncertainty, the need for dose-response functions, including time-consuming crop experiments to generate data, is reduced. Actual responses of farmers’ profits across air pollution gradients of ambient pollution are observed instead of hypothesized. Although this procedure has not been widely used in air pollution economic assessments, it has been used in a number of relatively recent climate change studies (e.g., Mendelsohn et al., 1994). The reduced-form method suffers from the fact that if proposed O\textsubscript{3} levels are lower than those observed in the estimation sample, then the prediction accuracy of the method deteriorates. Also, some dose-response information is needed, if only to establish the plausibility of the economic estimates.

Another area that may improve economic assessments is incorporating consideration of livestock issues. To date, most agricultural economic assessments of O\textsubscript{3} impacts have ignored the livestock sector. Presumably this is because ambient O\textsubscript{3} levels do not noticeably affect meat
yields. However, if feed prices and pasture conditions are affected by ambient O₃ levels, then a more accurate estimate of economic impacts would be forthcoming by including this link to livestock in the assessment. These types of feed production and feed price effects are included in the mathematical programming model used in Adams et al. (1986), but not in most other O₃ effects assessments. The significance of livestock-feed linkages are demonstrated in a recent study in the Netherlands by Kuik et al. (2000). Using a mathematical programming model similar to that in Adams et al. (1986), Kuik et al. (2000) found that livestock effects were prominent, due mainly to improved pasture yields under reduced ambient O₃ levels.

**AX9.7.6 Conclusions**

Substantial progress has been made over the past two decades in our understanding of the effects of O₃ and other oxidants on vegetation, particularly for agriculturally important plant species. The physical and economic effects on agriculture are well documented and provide useful information for the setting of SNAAQS. Effects on forests and natural ecosystems remain problematic, due to limitations in natural science data and economic methods. The problem is most acute for valuing natural ecosystem service flows.

The current limitations surrounding forests and natural ecosystems present a rich research agenda. However, not all research needs are likely to lead to better policies. Thus, areas of greatest potential value in terms of regional policymaking need to be prioritized. Such priority setting can be assisted by sensitivity analyses with existing economic models. By measuring the changes in economic effects arising from changes in key parameters, research data gaps most likely to affect economic values can be identified.
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